Peptide-based targeting of connexins and pannexins for therapeutic purposes

Caufriez, Anne; Böck, Denise; Martin, Charlotte; Ballet, Steven; Vinken, Mathieu

Published in:
Expert Opinion on Drug Discovery

DOI:
10.1080/17460441.2020.1773787

Publication date:
2020

Peptide-based Targeting of Connexins and Pannexins for Therapeutic Purposes

Abstract

Introduction: Connexin and pannexin (hemi)channels play an important role in paracrine and autocrine signalling pathways. The opening of these cellular pores is linked to a wide range of diseases. Therefore, pharmacological closing of connexin and pannexin (hemi)channels seems a promising therapeutic strategy. However, available inhibitors of connexin and pannexin (hemi)channels cope with recurring problems concerning selectivity, specificity, stability and/or solubility.

Areas covered: A number of peptides that mimic specific regions in the native sequence of connexins and pannexins have the potential to overcome some of these hurdles. In this paper, an overview is provided on these peptide-based inhibitors of connexin and pannexin (hemi)channels for therapeutic purposes.

Expert opinion: Peptide mimetics can become valuable tools in the treatment of connexin-related and pannexin-related diseases, provided that available peptides are optimised, and new peptide mimetics are designed based on knowledge of the mechanisms underlying the gating control of connexin and pannexin (hemi)channels.

Key words: connexins, (hemi)channel inhibition, pannexins, peptide mimetics.

1. Introduction

In human, and in vertebrates in general, intercellular communication is mediated by gap junctions, which facilitate transport of small molecules and ions, such as glutamate, adenosine triphosphate (ATP) and calcium, between the cytoplasm of opposing cells [1]. Gap junctions are formed by the docking of 2 connexin (Cx) hemichannels, each consisting of 6 transmembrane proteins of the Cx family (Figure 1) [2]. Over the years, it has become clear that Cx hemichannels are not merely building blocks of gap junctions but can also function as single membrane channels. Cx hemichannels enable a similar exchange of small molecules and ions as gap junctions, though between the cytosol of an individual cell and its extracellular environment (Figure 1) [3]. Today, 21 Cx family members have been identified in human, named according to their molecular weight. All Cx subtypes share a common topology consisting of 4 transmembrane domains, 2 extracellular loops, 1 cytoplasmic loop and a cytoplasmic *N*- and *C*-terminal tail (Figure 2), and undergo post-translational modifications such as phosphorylation, *S*-nitrosylation and ubiquitination [2], [4]. These post-translational modifications are key mechanisms in Cx hemichannel regulation. In this context, phosphorylation of the *C*-terminal tail by protein kinase A and C alters Cx hemichannel permeability [5], [6]. Undocked Cx hemichannels are, in contrast to gap junctions, generally closed. However, their activation can be triggered by pathological stimuli, such as oxidative and mechanical stress, a decrease in extracellular calcium concentration or an elevation of intracellular calcium concentration [7], [8].

In 2000, a new protein family with a similar topological structure as Cx was identified in vertebrates, called pannexin (Panx) proteins (Figure 3). The Panx family comprises of 3 subtypes, namely Panx1, Panx2 and Panx3, named according to the timeline of their discovery. Like Cxs, Panx proteins also oligomerise into multimeric channels (Figure 1) [9]. However, extensive glycosylation at the second extracellular loop prevents the formation of gap junctions

[10]. Hence, they are called Panx channels, and not Panx hemichannels. Post-translational modifications such as *N*-glycosylation, *S*-nitrosylation and phosphorylation regulate Panx trafficking and opening [4]. Panx channels open under both physiological and pathophysiological conditions [11], [12]. In physiological conditions, Panx-mediated release of ATP and potassium contributes to normal homeostatic cell function. In certain pathological pathways, interactions between Panx channels and purinergic receptors can lead to high levels of extracellular ATP and potassium, and an influx of calcium resulting in inflammasome activation [12], [13].

Both Cx and Panx (hemi)channels contribute to paracrine and autocrine signalling pathways during inflammation and cell death through the release of ATP and other signalling molecules [14]–[19]. These channel types are therefore considered emerging targets in the treatment of various diseases (Table 1) [20]–[22]. Several chemical-based, RNA-based as well as antibody-based blockers are at hand. However, most of these compounds show non-specific and/or non-selective characteristics by inhibiting multiple Cx species or Cx hemichannels, gap junctions, Panx channels and/or other targets at once, which in some cases can lead to increased cell death [23], [24]. A group of peptide-based inhibitors was proven to be an exception, as a number of peptide mimetics showed specific and selective channel inhibition [25]. These peptides mimic channel-specific regions of the native protein sequence and can thereby potentially alter Cx or Panx (hemi)channel properties. Cx43 and Panx1 have been the main focus of peptide mimicry due to their high abundance and expression in a wide range of human tissues [26], [27]. In this paper, an overview is provided on peptide mimetics targeting Cx and Panx (hemi)channels and their potential use in the treatment of a Cx-related and Panx-related diseases, with particular attention being paid to Cx43 and Panx1.

2. Connexin peptide mimetics

2.1. Peptides mimicking sequences of the extracellular loops

The extracellular loop of Cx hemichannels appeared to be the most favourable target for the design of more selective inhibitors due to their accessibility in contrast to their full channel counterparts. Nevertheless, peptides containing the conserved motives QPG and SHVR of the first extracellular loop and the SRPTEK motif of the second extracellular loop still interfered with the formation of gap junctions [28]. This led to the development of currently known peptide mimetics ^{43}Gap26, ^{37,40}Gap26, ^{32}Gap27, ^{40}Gap27, ^{43}Gap27and ^{43}Peptide5 [28]. Of note, the superscript in the nomenclature of these peptide analogues refers to the Cx subtype they are targeting.

The conserved SHVR motif of the first extracellular loop is incorporated in the sequence of Gap26 peptide mimetics. The 2 slightly different sequences are both categorised under the Gap26 code, one targeting Cx37 and Cx40, and the other targeting only Cx43 (Table 2 and Figure 2) [29], [30]. Cells treated with either of these Gap26 peptides showed Cx hemichannel inhibition within minutes. However, upon longer exposure times, decreased gap junction activity was equally observed [28], [31]. This effect may occur due to the high turn-over rate of Cx proteins, through which peptides can bind to the extracellular loops of a new Cx hemichannel before assembling into gap junctions [29]. Gap26 has been suggested to inhibit Cx hemichannels by shifting the voltage dependent opening to higher voltages, yet solid substantiating data are lacking [31].

There are 3 Gap27 peptides, each targeting different Cx types, namely Cx32, Cx40 and Cx43 [28], [30], [32]. These peptides mimic the conserved SRPTEK motif of the second extracellular loop, but have the same time-dependent effect on gap junction activity as Gap26 (Table 2 and

Figure 2) [31]. ^{43}Gap27 and ^{40}Gap27 were shown to decrease osteoclastic activity and angiogenesis in mouse retina, respectively [31], [33].

Like Gap27, Peptide5 contains the SRPTEK motif. However, the mimicked sequence of Peptide5 is shifted in the direction of the *N*-terminal tail in comparison to that of Gap27 (Table 2 and Figure 2) [28]. Peptide5 inhibits Cx43 hemichannels at a concentration of 5-10 µM, yet incubation at higher concentration (100 µM or higher) also leads to inhibition of gap junctions [34]. *In vivo* tests in mice have shown that Peptide5 reduces tissue damage after spinal cord injury and attenuates the permeability of vessels after retinal ischemia/reperfusion [35]. The exact mechanism of Cx43 hemichannel inhibition by Peptide5 remains unravelled, but the peptide is currently being tested in preclinical trials as Peptagon™ (OcuNexus Therapeutics Inc.) for use in the treatment of diabetic retinopathy [35].

2.2. Peptides mimicking sequences of intracellular regions

The interaction between the cytoplasmic loop and *C*-terminal tail mediates the gating mechanism of Cx hemichannels and gap junctions. Gap junctions are in an open state when there is no interaction between the *C*-terminal tail and cytoplasmic loop, while such interaction is critical for Cx hemichannel opening [36]. Experiments in HeLa cells transfected with Cx43 showed that Cx hemichannels, which lack their *C*-terminal tails, can be reopened when exposed to a peptide named CT10 peptide, also known as Cx43CT (Table 2 and Figure 2). CT10 reproduces the last 10 amino acids of the *C*-terminal tail of Cx43. Inhibition of Cx43-mediated ATP release by a peptide mimetic, called TAT-L2, pinpointed its mimicked L2 region (amino acid 119 to 144) as an essential sequence of the cytoplasmic loop in the interaction with the *C*-terminal tail (Table 2 and Figure 2). Moreover, amino acids H126 and I130 were proven to be essential for the binding of the L2 region to the *C*-terminal tail, as peptides mutated at these amino acid positions lack the inhibitory capacity of TAT-L2 [36]. This interaction is of utmost importance for the regulation of Cx43 hemichannel opening, but an additional trigger is

required, such as mechanical stress, decrease of extracellular calcium concentration or moderate elevations in intracellular calcium concentrations [7], [8], [37]. To date, 2 peptides mimicking the L2 region are available, namely ^{43}Gap19 and ^{32}Gap24 (Table 2 and Figure 2).

^{43}Gap19 inhibits Cx43 hemichannel currents by binding to the *C*-terminal tail, thereby preventing the cytoplasmic loop/*C*-terminal tail interaction, although not to the full extent. Nonetheless, ^{43}Gap19 has the advantage of being a selective inhibitor, as it does not affect gap junction or Panx1 channel activity [38], [39]. *In vitro* and *in vivo* tests showed that ^{43}Gap19 is capable of inhibiting Cx43 hemichannels in acute and chronic liver disease [39]–[41]. It also protects against myocardial ischemia/reperfusion injury [42].

^{32}Gap24 is a peptide that mimics a 13 amino acid long stretch of the L2 region of Cx32 (Table 2 and Figure 2) [8]. *In vitro* studies showed that Cx32 hemichannel-mediated ATP release is inhibited by ^{32}Gap24 at concentrations of 17 µM without affecting gap junctions [8]. However, ^{32}Gap24 does inhibit Panx1 channels at higher concentrations [43]. *In vivo* administration of ^{32}Gap24 to mice resulted in alleviation of non-alcoholic steatohepatitis, liver fibrosis and acetaminophen-induced liver injury [39]–[41]. Its mode-of-action is still unknown but might involve a mechanism similar to that of ^{43}Gap19.

Peptides targeting intracellular regions of Cxs need to access the intracellular environment. Cell-penetrating peptides (CPP), such as the TAT-peptide, an oligoarginine tag and Xentry peptide, have been anchored to Cx-derived peptide sequences in order to enhance the uptake into the cell *via* endocytosis [44], [45]. ^{43}Gap19 can enter the cell on its own due to the KKFK cell-translocation motif of the L2 region. Nonetheless, comparison of the IC_{50} (half maximal inhibitory concentration) of ^{43}Gap19 itself (47 µM) and TAT-^{43}Gap19 (7 µM) for the inhibition of ATP release in glioma cells showed that the entry of ^{43}Gap19 into the cell, and its effect accordingly, might be improved by linking to a TAT-tag. These results emphasise the importance of CPPs in the design of peptide mimetics targeting intracellular domains [42].

Importantly, the CPP itself can have effects on the target as suggested for the TAT-tag [46]. *In vivo* tests showed increased toxicity of TAT-Gap19 when administrated in higher concentrations [46].

The length of the *C*-terminal tail differs strongly among the different Cx subtypes. In this context, Cx26 has a *C*-terminal tail merely consisting of 10 amino acids, while Cx62 has a *C*-terminal tail of more than 250 amino acids [47], [48]. Mimicry of the *C*-terminal region can therefore result in more specific peptides. However, other proteins, such as tubulin, zonula-occludens 1 (ZO-1) or β-Catenin, are known to interact with the *C*-terminal tail of Cx43. The interference of the peptide mimetics with the interaction between the *C*-terminal tail and the other proteins might consequently alter other cellular pathways [49].

All 5 available peptides mimicking the *C*-terminal tail are derived from Cx43. CT10 and CT9 mimic the last 10 or 9 amino acids of the *C*-terminal tail, respectively, while the Src homology 3 binding domain (SH3) of Cx43 is reproduced by a peptide named ΔSH3 (Table 2 and Figure 2) [36], [50]. CT10, CT9 as well as ΔSH3 promote a "ready-to-open" conformational state of Cx hemichannels and are thus not suitable as therapeutics. Nevertheless, they are interesting tools to investigate the function of Cx hemichannels and gap junctions [36], [50].

The juxtamembrane 2 (JM2) peptide, which mimics a sequence close to the fourth transmembrane domain, inhibits trafficking of Cx43 to the cell surface and hence reduces Cx hemichannel-mediated transport (Table 2 and Figure 2) [51]. However, a decrease in Cx hemichannel abundance at the cell surface also affects gap junction formation and might therefore lower cell viability [24], [51].

αCT1 mimics the last 9 amino acids of Cx43 and is linked to an antennapedia sequence that facilitates cellular internalisation of the peptide mimetic (Table 2 and Figure 2). The interaction between Cx43 and the PDZ-domain of ZO-1, a region that is suggested to be involved in the

regulation of Cx trafficking and gap junction assembly, is disrupted by αCT1 [52], [53]. This interference leads to increased gap junction plaque formation and decreased Cx hemichannel activity. αCT1 is currently in clinical trials as Granexin™ (FirstString Research Inc.) for the treatment of diabetic foot ulcer closure, since the peptide mimetic was reported to accelerate wound healing, while reducing inflammation and scarring [54].

3. Pannexin peptide mimetics

3.1. Peptides mimicking sequences of the extracellular loops

Panx-related research mainly focuses on Panx1. In 2006, a series of peptides, containing 8 to 21 consecutive amino acids of the native Panx1 sequence, were tested *in vitro*. Through measurements of ATP-evoked dye uptake and current measurements, ^{10}Panx1 was identified as a potent Panx1 inhibitor (Table 3 and Figure 3) [13]. Since then, the 10 amino acid long stretch of the first extracellular loop has been used to prove that inhibition of Panx1 channels prevents inflammation-induced neuron cell death in the enteric nervous system, hereby identifying Panx1 as a potential new target for inflammatory bowel disease treatments [55]. Results of animal studies also confirmed the significance of Panx1 inhibitors, such as ^{10}Panx1, in the treatment of drug-induced liver toxicity [56].

Another set of 5 peptides, namely PanxE1a, PanxE1b, PanxE1c, PanxE2a and PanxE2b (Table 3 and Figure 3), mimicking sequences of the 2 extracellular loops of Panx1, was synthesised and screened *in vitro*. In spite of the differences in sequence (a longer sequence that is shifted towards the *N*-terminal end), PanxE1b inhibited Panx1 channel currents to the same extent as ^{10}Panx1. The efficiency of the scrambled versions of ^{10}Panx1 and PanxE1b was tested against that of the native sequences. The observed decrease in Panx1 channel inhibition of the scrambled version in comparison to the native sequence suggests a sequence specific inhibition, which substantiates the value of peptide mimetics of the native sequence. Regardless of this

sequence-specific inhibition, both ^{10}Panx1 and PanxE1b inhibit Cx46 hemichannels as well [43].

Targeting the 2 extracellular loops is the most straightforward approach for the design of Panx1 inhibitory peptide mimetics. Over the years, research has proven the involvement of several amino acids of the extracellular loops in the activation and inhibition mechanisms of Panx1 channels. Peptide mimetics can also reach these target sequences more easily due their extracellular localisation [57]–[61]. Apart from post-translational modification sites, such as the glycosylation site at residue N254, the extracellular loops also contain interesting target sites for peptide mimetics [10]. An example is the binding site of extracellular ATP, which is known to block Panx1 channels at high concentrations [58].

3.2. Peptides mimicking sequences of the intracellular regions

Although mimicry of the extracellular loops seems promising, efforts have also been made to design peptides based on the intracellular regions of Panx1. To identify the essential amino acid motifs in α1 adrenoreceptor (α1DAR)-mediated activation of Panx1 channels, 2 novel peptides were synthesised, called IL1 and IL2 (Table 3 and Figure 3). These peptides mimic sequences of the intracellular loop of mouse Panx1, which are to a great extent conserved in human. Only IL2 significantly decreased phenylephrine-induced contractile responses and inhibited ATP release in murine pressurised thoracodorsal arteries. Thereafter, clusters of 3 to 4 consecutive amino acids of the IL2 sequence were substituted by alanines. This so-called “Ala-scan” was used to determine which amino acids are important for activation and thus of relevance for inhibition. Only the cells co-transfected with α1DAR and a Panx1 mutated at the YLK motif showed no phenylephrine stimulation of Panx1 channel currents, identifying this 3 amino acid long stretch as a possible target for inhibition of α1DAR-mediated activation of Panx1 channels [62].

A TAT-like sequence was anchored onto the sequences of both IL1 and IL2 to facilitate entry into the cell. In order to exclude the tag's influence on the channel inhibition, the sequence on its own was tested and showed no significant effect on ATP release and other phenylephrine-induced responses. There was also no significant effect observed for the scrambled version of IL2, which confirms sequence-specific inhibition, as was seen for ^{10}Panx1 and PanxE1b [43], [62]. In comparison with ^{10}Panx1, the intracellular loop-derived peptide was less effective in reducing the phenylephrine-induced atrial constriction. Nonetheless, these data suggest that inhibitors, such as IL2 or other Panx1 peptide mimetics targeting the intracellular YLK sequence, should be considered as potential therapeutics for blood pressure disorders [62].

The *C*-terminal tail contains multiple interesting target sequences, such as an auto-inhibitory region near the caspase cleavage site [63]. The discovery that the cleavage of the *C*-terminus by caspases is sufficient to activate Panx1 channels gave rise to the hypothesis of a ball-and-chain mechanism, in which the *C*-terminal tail functions as a Panx1 channel blocker by interacting with the pore. The removal of a group of 12 specific residues immediately downstream of the cleavage site appeared to be critical for the activation by caspase 3. Hence, the question arose whether this sequence on its own could block Panx1 channels. First, a peptide mimicking only the auto-inhibitory region was tested using whole-cell recordings of Panx1channel currents, but no inhibitory effect was detected (Table 3). Subsequently, 2 larger Panx1-derived constructs were synthesised. One mimicked the complete *C*-terminal tail (hPanx1(Ct)) and was linked to an enhanced green fluorescent protein to ensure entry into the cell (Table 3 and Figure 3). The other was a FLAG-tagged construct of the *C*-terminal tail up to amino acid 391 (hPanx1(Ct)Δ391) (Table 3 and Figure 3). Inhibition of Panx1 currents by both constructs indicates that there are important activation regions located more upstream in the sequence. Additional tests, using the purified *C*-terminal region in a cell-free system, substantiated channel blocking by the intracellular *C*-terminal tail. Although amino acid

stretches of more than 50 residues are typically not categorised as peptides, these results still highlight the potential of developing *C*-terminal-derived peptides for the treatment of diseases in which Panx1-mediated signalling is implicated, such as cancer and auto-immune disorders [63].

Another interesting target of the Panx1 *C*-terminal tail is the Src Family Kinase (SFK) consensus-like sequence containing a putative phosphorylation site at residue Y308. As phosphorylation is one of the mechanisms of Panx1 activation and SFK activity increases in hippocampal neurons during ischemia, it is likely that anoxia-induced Panx1 channel opening in neurons is regulated by this family of kinases. $Panx_{305-318}$ was synthesised and anchored to a TAT-tag in order to test if peptides containing the putative phosphorylation site could prevent anoxia-induced Panx1 channel opening (Table 3 and Figure 3). Bath application of TAT-$Panx_{305-318}$ showed decreased anoxic depolarisation of pyramidal neurons, thereby confirming the hypothesis. To exclude effects on the entire brain slice and to ascertain specificity of the phosphorylation site in single neurons, the peptide was also tested in whole-cell patch-clamp recordings. Measurements in these hippocampal neurons showed extensive blocking of the anoxic depolarisation currents. Inclusion of the TAT-tag itself in the patch pipettes in turn excluded interference of the sequence on the reported inhibition by TAT-$Panx_{305-318}$. Inhibition of Panx1 channels through targeting of the phosphorylation site was put forward as a potential new treatment of pathological neuronal depolarisation. Peptides, such as the interfering peptide TAT-$Panx_{305-318}$, could therefore be promising new tools in the prevention of cell death and ischemic core expansions after a stroke. [64].

4. Conclusion and Expert opinion

Cx and Panx (hemi)channels have a central role in the underlying mechanisms of various diseases associated with inflammation and cell death (Table 1). Many efforts have been made over the past 2 decades to identify potent inhibitors of these cellular pores, yet undesirable

inhibitor characteristics such as non-selective and/or non-specific inhibition often impede therapeutic development [64]. Cx mimetic peptides Gap26 and Gap27 not only inhibit Cx hemichannels, but also inhibit gap junctions in a time-dependent way [28], [31]. Peptide5, although also reported to affect gap junction activity, has reached the preclinical stage for the treatment of diabetic retinopathy as Peptagon™ (OcuNexus Therapeutics Inc.) [34]. As this peptide only inhibits gap junctions at higher concentrations, intravitreal injection was selected as the route of administration to have better control over the final concentration of the peptide in the target tissue. Despite its non-selective properties, Peptide5 still qualifies as a potential therapeutic in the treatment of Cx hemichannel-related disease [35].

Nevertheless, peptide mimetics of the intracellular regions seem more promising for several reasons. The amino acid sequences of the cytoplasmic loop and the *C*-terminal tail are not as conserved between the different Cx family members compared to the amino acid sequences of the extracellular loops. These regions differ considerably between the different Cx subtypes and are therefore potential targets in the design of more Cx-specific peptide mimetics [47], [48]. Furthermore, specific intramolecular interactions between the cytoplasmic loop and the *C*-terminal tail have been reported to control opening of Cx hemichannels, while the opposite holds true for gap junctions [36]. Targeting the interaction regions might therefore provide a potential solution for the selectivity problems of currently available inhibitors [36]. ^{32}Gap24 and ^{43}Gap19 have been reported to inhibit Cx hemichannels in several cell types without affecting gap junctions by interacting with the last 10 amino acids of the *C*-terminal tail. Nonetheless, ^{43}Gap19 is not able to completely inhibit Cx43 hemichannels, which can be possibly linked to the involvement of the SH3 binding domain that binds to the cytoplasmic loop [50]. This highlights the complexity of the cytoplasmic loop/*C*-terminal tail gating mechanism and its inhibition. Nevertheless, mimicking the *C*-terminal tail has proven to be promising in the case of the αCT1 peptide, which is currently being tested in clinical trials under

the name of Granexin™ (FirstString Research Inc.) for the treatment of diabetic foot ulcers [54]. A disadvantage of mimicry of the *C*-terminal tail lies with its interaction with several Cx-binding proteins [49]. Interference of Cx-based peptides with these interactions and their associated pathways must be excessively assessed to avoid possible side and/or adverse effects.

The optimisation of Cx peptide mimetics of the intracellular regions also deserves further scrutiny. Selecting suitable CPPs that facilitate entry into the cell is an important aspect in this respect, as it can potentially boost the effects of peptides due to an increased accessibility of target sites [42], [44]. However, a possible effect of the CPP itself at the cellular level should always be taken into account during the selection process, since increased toxicity of compounds have been reported when linked to this kind of tags [46]. To avoid using a CPP, it might be feasible to design peptide mimetics that can enter the cell through open Cx hemichannels. This mechanism of internalisation was suggested for ^{32}Gap24, as it enters the cell without a cell-translocation motif in its sequence or CPP [8].

With Cx mimetic peptides already in clinical trials, it is clear that research on Panx channel inhibitors still has a long way to go. Peptides that mimic intracellular and extracellular Panx sequences have been reported to inhibit Panx1 channels, but specificity problems persist. New insights into the gating mechanisms of Panx1 channels enables a more rational design of peptide mimetics in the future by targeting amino acids or motifs essential for the channel opening and/or closing. For the extracellular loops, great promise lies in targeting the amino acids that are part of the extracellular binding site of ATP [58]. Part of this area was already covered by peptides such as ^{10}Panx1 and PanxEb1. At the time of their introduction, this knowledge was lacking, but now can be used to optimise ^{10}Panx1 to create a more specific inhibitor by shifting or expanding the sequence. Another potential approach can rely on the design of peptide mimetics that are able to interact with possible allosteric binding sites within the extracellular loops and/or transmembrane regions of Panx1, including the binding site of extracellular ATP

[65]. These Panx1 peptide mimetics could decrease Panx1 channel activity by allosteric modulation.

In summary, Cx and Panx peptide mimetics have the potential to overcome some of the hurdles other Cx and Panx (hemi)channel inhibitors are facing, provided that available peptides are optimised and new peptide mimetics are designed based on knowledge of the mechanisms underlying the gating control of Cx and Panx (hemi)channels. At present, most of these mechanisms are still unclear. The pharmacological characterisation of peptide mimetics is also crucial for further optimisation, yet experiments determining the pharmacological properties of these compounds are scarce. This equally applies to the crystal structures of the different Cx and Panx (hemi)channels. The crystal structure of Cx26 was published more than a decade ago, yet still is the only one in its kind thus far [66]. Recently, the cryo-EM structure of Panx1 was characterised [67], [68]. The structure of Cx26 shows that the *N*-terminal tails of the Cx proteins form the pore funnel of the resulting channel. Therefore, no peptide memetics have been developed to mimic this intracellular region [66]. The *N*-terminal tail of Panx1, on the other hand, is of significant importance for its gating mechanism, but until today this area remains unexplored in the field of peptide mimicry [69]. Cx and Panx research mainly focused on Cx32, Cx43 and Panx1. Nevertheless, Panx3 has been identified as a possible target for the treatment of osteoarthritis, hence peptide-based inhibitors mimicking Panx3 could represent promising therapeutics (Table 1) [70]. The same might apply for Cx37 and Cx26 peptide mimetics, which represent potential drugs for the treatment of atherosclerosis, and skin disease and deafness, respectively (Table 1) [21], [71]. It can be anticipated that further exploration of these areas will open great perspectives for large patient populations worldwide in the years to come.

Highlights box: Priorities to be set regarding future development of peptide-based inhibitors of Cx and Panx (hemi)channels.

- Optimisation of existing peptides to improve stability and increase potency and selectivity
- Incorporation of amino acids and/or motifs important in gating mechanisms of Cxs and Panxs into the sequence of new peptide mimetics to obtain a more rational design approach
- Elucidation of gating mechanisms
- Pharmacological characterisation of peptide mimetics
- Determination of crystal and/or Cryo-EM structures

Acknowledgments

This work was financially supported by the grants of the University Hospital of the Vrije Universiteit Brussel-Belgium (‘‘Willy Gepts Fonds’’ UZ-VUB) and the Fund for Scientific Research-Flanders (FWO Grants G009514N and G010214N).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

References

1. Loewenstein WR. Junctional intercellular communication: the cell-to-cell membrane channel. Physiological reviews 1981;61(4):829–913
2. Yeager M, Nicholson BJ. Structure of gap junction intercellular channels. Current Opinion in Structural Biology 1996;6(2):183–92
3. Kar R, Batra N, Riquelme MA, Jiang JX. Biological role of connexin intercellular channels and hemichannels. Archives of Biochemistry and Biophysics 2012;524(1):2–15

4. Johnstone SR, Billaud M, Lohman AW, Taddeo EP, Isakson BE. Posttranslational modifications in connexins and pannexins. The Journal of membrane biology 2012;245(5-6):319–32
5. Bao X, Reuss L, Altenberg GA. Regulation of purified and reconstituted connexin 43 hemichannels by protein kinase C-mediated phosphorylation of Serine 368. Journal of Biological Chemistry 2004;279(19):20058–66
6. Mitropoulou G, Bruzzone R. Modulation of perch connexin35 hemi-channels by cyclic AMP requires a protein kinase A phosphorylation site. Journal of Neuroscience Research 2003;72(2):147–57
7. Srinivas M, Calderon DP, Kronengold J, Verselis VK. Regulation of connexin hemichannels by monovalent cations. The Journal of general physiology 2006;127(1):67–75
8. Vuyst E de, Decrock E, Cabooter L, Dubyak GR, Naus CC, Evans WH, Leybaert L. Intracellular calcium changes trigger connexin 32 hemichannel opening. EMBO Journal 2006;25(1):34–44
9. Sosinsky GE, Boassa D, Dermietzel R, Duffy HS, Laird DW, Macvicar B, et al. Pannexin channels are not gap junction hemichannels. Channels (Austin, Tex.) 2011;5(3):193–7
10. Boassa D, Ambrosi C, Qiu F, Dahl G, Gaietta G, Sosinsky G. Pannexin1 Channels Contain a Glycosylation Site That Targets the Hexamer to the Plasma Membrane. Journal of Biological Chemistry 2007;282(43):31733–43
11. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, et al. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 2010;467(7317):863–7

12. Makarenkova HP, Shah SB, Shestopalov VI. The two faces of pannexins: new roles in inflammation and repair. Journal of Inflammation Research 2018;11:273–88
13. Pelegrin P, Surprenant A. Pannexin-1 mediates large pore formation and interleukin-1β release by the ATP-gated P2X7 receptor. The EMBO Journal 2006;25(21):5071–82
14. Vanden Abeele F, Bidaux G, Gordienko D, Beck B, Panchin YV, Baranova AV, et al. Functional implications of calcium permeability of the channel formed by pannexin 1. The Journal of cell biology 2006;174(4):535–46
15. Gossman DG, Zhao HB. Hemichannel-mediated inositol 1,4,5-trisphosphate (IP3) release in the cochlea: a novel mechanism of IP3 intercellular signaling. Cell communication & adhesion 2008;15(4):305–15
16. Kang J, Kang N, Lovatt D, Torres A, Zhao Z, Lin J, Nedergaard M. Connexin 43 hemichannels are permeable to ATP. Journal of Neuroscience 2008;28(18):4702–11
17. Wei L, Sheng H, Chen L, Hao B, Shi X, Chen Y. Effect of pannexin-1 on the release of glutamate and cytokines in astrocytes. Journal of Clinical Neuroscience 2016;23:135–41
18. Schalper KA, Sánchez HA, Lee SC, Altenberg GA, Nathanson MH, Sáez JC. Connexin 43 hemichannels mediate the Ca2+ influx induced by extracellular alkalinization. American Journal of Physiology - Cell Physiology 2010;299(6):1504–15
19. Dahl G. ATP release through pannexon channels. Philosophical Transactions of the Royal Society B: Biological Sciences 2015;370(1672):1–11
20. Vejar S, Oyarzún JE, Retamal MA, Ortiz FC, Orellana JA. Connexin and pannexin-based channels in oligodendrocytes: Implications in brain health and disease. Frontiers in Cellular Neuroscience 2019;13:1–10
21. Molica F, Figueroa XF, Kwak BR, Isakson BE, Gibbins JM. Connexins and pannexins in vascular function and disease. International Journal of Molecular Sciences 2018;19(6)

22. Cooreman A, van Campenhout R, Ballet S, Annaert P, van den Bossche B, Colle I, et al. Connexin and Pannexin (Hemi)Channels: Emerging Targets in the Treatment of Liver Disease. Hepatology (Baltimore, Md.) 2019;69(3):1317–23
23. Kar R, Riquelme MA, Werner S, Jiang JX. Connexin 43 channels protect osteocytes against oxidative stress-induced cell death. Journal of bone and mineral research the official journal of the American Society for Bone and Mineral Research 2013;28(7):1611–21
24. Ozog MA, Siushansian R, Naus CCG. Blocked gap junctional coupling increases glutamate-induced neurotoxicity in neuron-astrocyte co-cultures. Journal of neuropathology and experimental neurology 2002;61(2):132–41
25. Willebrords J, Maes M, Crespo Yanguas S, Vinken M. Inhibitors of connexin and pannexin channels as potential therapeutics. Pharmacology & therapeutics 2017;180:144–60
26. Baranova A, Ivanov D, Petrash N, Pestova A, Skoblov M, Kelmanson I, et al. The mammalian pannexin family is homologous to the invertebrate innexin gap junction proteins. Genomics 2004;83(4):706–16
27. Söhl G, Willecke K. An update on connexin genes and their nomenclature in mouse and man. Cell communication & adhesion 2003;10(4-6):173–80
28. Warner A, Clements DK, Parikh S, Evans WH, DeHaan RL. Specific motifs in the external loops of connexin proteins can determine gap junction formation between chick heart myocytes. Journal of Physiology 1995;488(3):721–8
29. Braet K, Vandamme W, Martin PEM, Evans WH, Leybaert L. Photoliberating inositol-1,4,5-trisphosphate triggers ATP release that is blocked by the connexin mimetic peptide gap 26. 01434160 2003;33(1):37–48

30. Eugeni EA, Sa CG, Sa JC, Fisiolo DDC, Cato PU, Claudia G. Gap junctional communication coordinates vasopressin-induced glycogenolysis in rat hepatocytes. American Journal of Physiology-Gastrointestinal and Liver Physiology 1998;274(6):1109–16
31. Wang N, Bock M de, Antoons G, Gadicherla AK, Bol M, Decrock E, et al. Connexin mimetic peptides inhibit Cx43 hemichannel opening triggered by voltage and intracellular Ca2+ elevation. Basic Research in Cardiology 2012;107(6):304
32. Vinken M, Decrock E, Vuyst E de, Bock M de, Vandenbroucke RE, Geest BG de, et al. Connexin32 hemichannels contribute to the apoptotic-to-necrotic transition during Fas-mediated hepatocyte cell death. Cellular and molecular life sciences CMLS 2010;67(6):907–18
33. Haefliger JA, Allagnat F, Hamard L, Le Gal L, Meda P, Nardelli-Haefliger D, et al. Targeting Cx40 (Connexin40) expression or function reduces angiogenesis in the developing mouse retina. Arteriosclerosis, Thrombosis, and Vascular Biology 2017;37(11):2136–46
34. O'Carroll SJ, Alkadhi M, Nicholson LFB, Green CR. Connexin43 mimetic peptides reduce swelling, astrogliosis, and neuronal cell death after spinal cord injury. Cell Communication and Adhesion 2008;15(1-2):27–42
35. Chen Y-S, Green CR, Teague R, Perrett J, Danesh-Meyer HV, Toth I, Rupenthal ID. Intravitreal injection of lipoamino acid-modified connexin43 mimetic peptide enhances neuroprotection after retinal ischemia. Drug delivery and translational research 2015;5(5):480–8
36. Ponsaerts R, Vuyst E de, Retamal M, D'Hondt C, Vermeire D, Wang N, et al. Intramolecular loop/tail interactions are essential for connexin 43-hemichannel activity. FASEB Journal 2010;24(11):4378–95

37. Luckprom P, Kanjanamekanant K, Pavasant P. Role of connexin43 hemichannels in mechanical stress-induced ATP release in human periodontal ligament cells. Journal of periodontal research 2011;46(5):607–15
38. Wang N, Bock M de, Decrock E, Bol M, Gadicherla A, Bultynck G, Leybaert L. Connexin targeting peptides as inhibitors of voltage- and intracellular Ca2+-triggered Cx43 hemichannel opening. Neuropharmacology 2013;75:506–16
39. Maes M, Crespo Yanguas S, Willebrords J, Weemhoff JL, da Silva TC, Decrock E, et al. Connexin hemichannel inhibition reduces acetaminophen-induced liver injury in mice. Toxicology Letters 2017;278:30–7
40. Crespo Yanguas S, da Silva TC, Pereira IVA, Willebrords J, Maes M, Sayuri Nogueira M, et al. TAT-Gap19 and Carbenoxolone Alleviate Liver Fibrosis in Mice. International Journal of Molecular Sciences 2018;19(3):817
41. Willebrords J, Cogliati B, Pereira IVA, da Silva TC, Yanguas SC, Maes M, et al. Inhibition of connexin hemichannels alleviates non-alcoholic steatohepatitis in mice. Scientific Reports 2017;7(1):8268
42. Wang N, Vuyst E de, Ponsaerts R, Boengler K, Palacios-Prado N, Wauman J, et al. Selective inhibition of Cx43 hemichannels by Gap19 and its impact on myocardial ischemia/reperfusion injury. Basic Research in Cardiology 2013;108(1):309
43. Wang J, Ma M, Locovei S, Keane RW, Dahl G. Modulation of membrane channel currents by gap junction protein mimetic peptides: Size matters. American Journal of Physiology - Cell Physiology 2007;293(3):C1112–C1119
44. Milletti F. Cell-penetrating peptides: Classes, origin, and current landscape. Drug Discovery Today 2012;17(15-16):850–60

45. Montrose K, Yang Y, Sun X, Wiles S, Krissansen GW. Xentry, a new class of cell-penetrating peptide uniquely equipped for delivery of drugs. Scientific Reports 2013;3:1–7
46. Li W, Bao G, Chen W, Qiang X, Zhu S, Wang S, et al. Connexin 43 Hemichannel as a Novel Mediator of Sterile and Infectious Inflammatory Diseases. Scientific Reports 2018;8(1):166
47. GJA10 - Gap junction alpha-10 protein - Homo sapiens (Human) - GJA10 gene & protein, 26.2.2020. Available at: https://www.uniprot.org/uniprot/Q969M2 [Last accessed 28 February 2020]
48. GJB2 - Connexin 26 - Homo sapiens (Human) - GJB2 gene & protein, 11/12/2019. Available at: https://www.uniprot.org/uniprot/A8DU55 [Last accessed 17 March 2020]
49. Leithe E, Mesnil M, Aasen T. The connexin 43 C-terminus: A tail of many tales. Biochimica et Biophysica Acta - Biomembranes 2018;1860(1):48–64
50. Iyyathurai J, Wang N, D'Hondt C, Jiang JX, Leybaert L, Bultynck G. The SH3-binding domain of Cx43 participates in loop/tail interactions critical for Cx43-hemichannel activity. Cellular and Molecular Life Sciences 2018;75(11):2059–73
51. Rhett JM, Calder BW, Fann SA, Bainbridge H, Gourdie RG, Yost MJ. Mechanism of action of the anti-inflammatory connexin43 mimetic peptide JM2. American Journal of Physiology - Cell Physiology 2017;313:C314-C326
52. Thomas MA, Huang S, Cokoja A, Riccio O, Staub O, Suter S, Chanson M. Interaction of connexins with protein partners in the control of channel turnover and gating. Biology of the Cell 2002;94(7-8):445–56
53. Hunter AW, Barker RJ, Zhu C, Gourdie RG. Zonula Occludens-1 Alters Connexin43 Gap Junction Size and Organization by Influencing Channel Accretion. Molecular Biology of the Cell 2005;16:5686–98

54. Obert E, Strauss R, Brandon C, Grek C, Ghatnekar G, Gourdie R, Rohrer B. Targeting the tight junction protein, zonula occludens-1, with the connexin43 mimetic peptide, αCT1, reduces VEGF-dependent RPE pathophysiology. Journal of Molecular Medicine 2017;95(5):535–52
55. Gulbransen BD, Bashashati M, Hirota SA, Gui X, Roberts JA, MacDonald JA, et al. Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. Nature Medicine 2012;18(4):600–4
56. Maes M, McGill MR, da Silva TC, Abels C, Lebofsky M, Weemhoff JL, et al. Inhibition of pannexin1 channels alleviates acetaminophen-induced hepatotoxicity. Archives of Toxicology 2017;91(5):2245–61
57. Bunse S, Schmidt M, Hoffmann S, Engelhardt K, Zoidl G, Dermietzel R. Single cysteines in the extracellular and transmembrane regions modulate Pannexin 1 channel function. Journal of Membrane Biology 2011;244(1):21–33
58. Qiu F, Wang J, Dahl G. Alanine substitution scanning of pannexin1 reveals amino acid residues mediating ATP sensitivity. Purinergic Signalling 2012;8:81–90
59. Michalski K, Kawate T. Carbenoxolone inhibits Pannexin1 channels through interactions in the first extracellular loop. Journal of General Physiology 2016;147(2):165–74
60. Chiu YH, Schappe MS, Desai BN, Bayliss DA. Revisiting multimodal activation and channel properties of Pannexin 1. Journal of General Physiology 2018;150(1):19–39
61. Boyce AKJ, Epp AL, Nagarajan A, Swayne LA. Transcriptional and post-translational regulation of pannexins. Biochimica et Biophysica Acta - Biomembranes 2018;1860(1):72–82
62. Billaud M, Chiu Y-H, Lohman AW, Parpaite T, Butcher JT, Mutchler SM, et al. A molecular signature in the pannexin1 intracellular loop confers channel activation by the α1 adrenoreceptor in smooth muscle cells. Science Signaling 2015;8(364):ra17

63. Sandilos JK, Chiu Y-H, Chekeni FB, Armstrong AJ, Walk SF, Ravichandran KS, Bayliss DA. Pannexin 1, an ATP Release Channel, Is Activated by Caspase Cleavage of Its Pore-associated C-terminal Autoinhibitory Region. THE JOURNAL OF BIOLOGICAL CHEMISTRY 2012;287(14):11303–11

64. Weilinger NL, Tang PL, Thompson RJ. Anoxia-induced NMDA receptor activation opens Pannexin channels via Src family kinases. Journal of Neuroscience 2012;32(36):12579–88

65. Dubyak GR. Both sides now: Multiple interactions of ATP with pannexin-1 hemichannels. Focus on "a permeant regulating its permeation pore: inhibition of pannexin 1 channels by ATP". American Journal of Physiology - Cell Physiology 2009;296(2):C235-C241 *

Reveals potential target for allosteric modulation of pannexin 1

66. Maeda S, Nakagawa S, Suga M, Yamashita E, Oshima A, Fujiyoshi Y, Tsukihara T. Structure of the connexin 26 gap junction channel at 3.5 Å resolution. Nature 2009;458(7238):597–602

67. Michalski K, Syrjanen JL, Henze E, Kumpf J, Furukawa H, Kawate T. The cryo-EM structure of a pannexin 1 reveals unique motifs for ion selection and inhibition. eLife 2020;9 **

Structure showing heptameric oligomerization of pannexin 1 instead of hexameric

68. Qu R, Dong L, Zhang J, Yu X, Wang L, Zhu S. Cryo-EM structure of human heptameric Pannexin 1 channel. Cell research 2020 **

Structure showing heptameric oligomerization of pannexin 1 instead of hexameric

69. Michalski K, Kawate T. Subtle Modifications of the Pannexin-1 N-Terminus Results in Altered Channel Activity. Biophysical Journal 2018;114(3):495a

70. Moon PM, Penuela S, Barr K, Khan S, Pin CL, Welch I, et al. Deletion of Panx3 Prevents the Development of Surgically Induced Osteoarthritis. Journal of Molecular Medicine 2015;93(8):845–56
71. Lee JR, White TW. Connexin-26 mutations in deafness and skin disease. Expert reviews in molecular medicine 2009;11:e35
72. Orellana JA, Froger N, Ezan P, Jiang JX, Bennett MVL, Naus CC, et al. ATP and glutamate released via astroglial connexin43 hemichannels mediate neuronal death through activation of pannexin 1 hemichannels. J Neurochem 2011;118(5):826–40
73. Hainz N, Becker P, Rapp D, Wagenpfeil S, Wonnenberg B, Beisswenger C, et al. Probenecid-treatment reduces demyelination induced by cuprizone feeding. Journal of Chemical Neuroanatomy 2017;85:21–6
74. Santiago MF, Veliskova J, Patel NK, Lutz SE, Caille D, Charollais A, et al. Targeting Pannexin1 Improves Seizure Outcome. PLoS ONE 2011;6(9):25178
75. Maier N, Güldenagel M, Söhl G, Siegmund H, Willecke K, Draguhn A. Reduction of high-frequency network oscillations (ripples) and pathological network discharges in hippocampal slices from connexin 36-deficient mice. Journal of Physiology 2002;541(2):521–8
76. Lutz SE, González-Fernández E, Ventura JCC, Pérez-Samartín A, Tarassishin L, Negoro H, et al. Contribution of Pannexin1 to Experimental Autoimmune Encephalomyelitis. PLoS ONE 2013;8(6)
77. Lai CPK, Bechberger JF, Thompson RJ, Macvicar BA, Bruzzone R, Naus CC. Tumor-Suppressive Effects of Pannexin 1 in C6 Glioma Cells 2007;67(4):1545–54
78. Lai C, Bechberger JF, Naus CC. Pannexin2 as a novel growth regulator in C6 glioma cells. Oncogene 2009;28:4402–8

79. Nakase T, Söhl G, Theis M, Willecke K, Naus CCG. Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin43 in astrocytes. American Journal of Pathology 2004;164(6):2067–75
80. Karatas H, Erdener SE, Gursoy-Ozdemir Y, Lule S, Eren-Koçak E, Sen ZD, Dalkara T. Spreading depression triggers headache by activating neuronal Panx1 channels. 10959203 2013;339(6123):1092–5
81. Timóteo MA, Carneiro I, Silva I, Noronha-Matos JB, Ferreirinha F, Silva-Ramos M, Correia-De-Sá P. ATP released via pannexin-1 hemichannels mediates bladder overactivity triggered by urothelial P2Y6 receptors. Biochemical Pharmacology 2014;87(2):371–9
82. Bond SR, Naus CC. The pannexins: Past and present. Frontiers in Physiology 2014(5):1–24
83. Lu D, Soleymani S, Madakshire R, Insel PA. ATP released from cardiac fibroblasts via connexin hemichannels activates profibrotic P2Y2 receptors. FASEB journal official publication of the Federation of American Societies for Experimental Biology 2012;26(6):2580–91
84. Calder BW, Matthew Rhett J, Bainbridge H, Fann SA, Gourdie RG, Yost MJ. Inhibition of connexin 43 hemichannel-mediated ATP release attenuates early inflammation during the foreign body response. Tissue engineering. Part A 2015;21(11-12):1752–62
85. Guttman JA, Lin AE-J, Li Y, Bechberger J, Naus CC, Vogl AW, Finlay BB. Gap junction hemichannels contribute to the generation of diarrhoea during infectious enteric disease. Gut 2010;59(2):218–26
86. Skals M, Jorgensen NR, Leipziger J, Praetorius HA. Alpha-hemolysin from Escherichia coli uses endogenous amplification through P2X receptor activation to induce hemolysis.

Proceedings of the National Academy of Sciences of the United States of America 2009;106(10):4030–5

87. Paoletti A, Raza SQ, Voisin L, Law F, Caillet M, Martins I, et al. Pannexin-1-the hidden gatekeeper for HIV-1. Journal of Leukocyte Biology 2013;94(3):390–2
88. Orellana JA, Sáez JC, Bennett MVL, Berman JW, Morgello S, Eugenin EA. HIV increases the release of dickkopf-1 protein from human astrocytes by a Cx43 hemichannel-dependent mechanism. Journal of Neurochemistry 2014;128(5):752–63
89. Masaki K, Suzuki SO, Matsushita T, Matsuoka T, Imamura S, Yamasaki R, et al. Connexin 43 astrocytopathy linked to rapidly progressive multiple sclerosis and neuromyelitis optica. PLoS ONE 2013;8(8):e72919
90. Willebrords J, Maes M, Pereira IVA, da Silva TC, Govoni VM, Lopes VV, et al. Protective effect of genetic deletion of pannexin1 in experimental mouse models of acute and chronic liver disease. Biochimica et biophysica acta. Molecular basis of disease 2018;1864(3):819–30
91. Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology 2011;54(1):133–44
92. Freeman TJ, Sayedyahossein S, Johnston D, Sanchez-Pupo RE, O'Donnell B, Huang K, et al. Inhibition of pannexin 1 reduces the tumorigenic properties of human melanoma cells. Cancers 2019;11(1):1–24
93. Pollok S, Pfeiffer AC, Lobmann R, Wright CS, Moll I, Martin PEM, Brandner JM. Connexin 43 mimetic peptide Gap27 reveals potential differences in the role of Cx43 in wound repair between diabetic and non-diabetic cells. Journal of cellular and molecular medicine 2011;15(4):861–73

94. Ebong EE, Kim S, DePaola N. Flow regulates intercellular communication in HAEC by assembling functional Cx40 and Cx37 gap junctional channels. American Journal of Physiology - Heart and Circulatory Physiology 2006;290(5):2015–23
95. Romanov RA, Rogachevskaja OA, Bystrova MF, Jiang P, Margolskee RF, Kolesnikov SS. Afferent neurotransmission mediated by hemichannels in mammalian taste cells. EMBO Journal 2007;26(3):657–67
96. Ilvesaro J, Tavi P, Tuukkanen J. Connexin-mimetic peptide Gap 27 decreases osteoclastic activity. BMC Musculoskeletal Disorders 2001;2:1–6
97. Wright CS, Van Steensel, Maurice A M, Hodgins MB, Martin PEM. Connexin mimetic peptides improve cell migration rates of human epidermal keratinocytes and dermal fibroblasts in vitro. Wound Repair and Regeneration 2009;17(2):240–9
98. Dahl G, Nonner W, Werner R. Attempts to define functional domains of gap junction proteins with synthetic peptides. Biophysical Journal 1994;67(5):1816–22
99. Bol M, Wang N, Bock M de, Wacquier B, Decrock E, Gadicherla A, et al. At the cross-point of connexins, calcium, and ATP: Blocking hemichannels inhibits vasoconstriction of rat small mesenteric arteries. Cardiovascular Research 2017;113(2):195–206
100. Pelegrin P, Surprenant A. Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1β release through a dye uptake-independent pathway. Journal of Biological Chemistry 2006;282(4):2386–94

Table 1: Diseases linked to the opening of Cx and Panx (hemi)channels

Disease	Cx/Panx	Reference
Alzheimer's disease	Cx43, Panx1	[72]
Demyelination	Panx1	[73]
Epilepsy	Cx36, Panx1	[74], [75]
Encephalomyelitis	Panx1	[76]
Glioma	Panx1, Panx2	[77], [78]
Ischemia/stroke	Panx1, Panx2, Cx43	[21], [79]
Migraine	Panx1	[80]
Overactive bladder	Panx1	[81]
Glaucoma	Panx1	[82]
Crohn's disease/colitis	Panx1	[55]
Atherosclerosis	Cx37, Panx1	[21]
Cardiomyopathy	Cx43	[83]
Hypertension	Panx1	[21]
Myocardial ischemia/reperfusion	Cx43	[21]
Inflammation	Cx32, Cx43	[42], [84]
Microbial infection	Cx43, Panx1	[85], [86]
HIV/AIDS	Cx43, Panx1	[87], [88]
Multiple sclerosis	Cx43	[89]
Acute liver failure	Cx32, C43, Panx1	[39], [56]
Liver fibrosis and cirrhosis	Cx43	[40]
Non-alcoholic steatohepatitis	Cx32, Cx43, Panx1	[41], [90], [91]
Diabetes	Cx43, Panx1	[72]
Osteoarthritis	Panx3	[70]
Melanoma	Panx1	[92]
Non-syndromic deafness	Cx26	[71]
Syndromic deafness associated with skin disorders	Cx26	[71]

Cx, connexin; Panx, pannexin

Table 2: Cx peptide mimetics

Peptide	Target sequence	Cx	Target	Inhibits	Cell type	Reference
^{37,40}Gap26	VCYDQAFPISHIR	Cx37 Cx40	EL1	HC/GJ	Rat hepatocytes, HAEC	[30], [93]
^{43}Gap26	VCYDKSFPISHVR	Cx43	EL1	HC/GJ	EVC304 human epithelial bladder cancer cells, chick corneal epithelial cells, chick neural retina cells, COS-1, chick myocytes, HAEC	[28], [29], [94], [95]
^{32}Gap27	SRPTEKTVFT	Cx32	EL2	HC/GJ	Chick myocytes, xenopus oocytes	[28], [32]
^{40}Gap27	SRPTEKNVFIV	Cx40	EL2	HC/GJ	HAEC, human endothelial cells	[33], [93]
^{43}Gap27	SRPTEKTIFII	Cx43	EL2	HC/GJ	Rat osteoclasts, mice dendritic cells, A7r5 rat aortic smooth muscle cells, ECV304 human epithelial bladder cancer cells	[8], [96]–[98]
^{43}Peptide5	VDCFLSRPTEKT	Cx43	EL2	HC/GJ	Rat neural cells, rat astrocytes, chick myocytes	[28], [34], [99]
^{43}L2	DGANVDMHLKQIEIK KFKYGIEEHGK	Cx43	CL	HC	BCEC	[36]
^{43}Gap19	KQIEIKKFK	Cx43	CL	HC	Rat C6 cells, pig cardiomyocytes, mouse hepatocytes	[39]–[42]
^{32}Gap24	GHGDPLHLEEVKC	Cx32	CL	HC	ECV304 human epithelial bladder cancer cells, rat hepatocytes, Xenopus oocytes	[8], [39], [41], [43]
^{43}CT10 (Cx43CT)	SRPRPDDLEI	Cx43	CT	GJ	Rat C6 cells	[42]
^{43}CT9	RPRPDDLEI	Cx43	CT	GJ	Rat smooth muscle cells, BCEC, HeLa cells	[50], [99]
^{43}ΔSH3	SSPTAPLSPMSPPG	Cx43	CT	GJ	BCEC, HeLa cells	[50]
^{43}JM2	VFFKGVKDRVKGRSD	Cx43	CT	HC/GJ	HeLa cells	[51]
^{43}αCT1	RQPKOWFPNRRKPWK KRPRPDDLEI	Cx43	CT	HC	Rat cardiomyocytes, HeLa cells	[53], [54]

Cx, Connexin; HC, hemichannel; GJ, gap junction; EL, extracellular loop; CL, cytoplasmic loop; CT, C-terminal tail; BCEC, bovine corneal endothelial cells; HAEC, human aortic endothelial cells; COS-1, CV-1 origin carrying SV40; HeLa, Henrietta Lacks

Table 3: Panx1 peptide mimetics

Peptide	Target sequence	Target	Inhibits Panx1 channels	Cell type	Reference
^{10}Panx1	WRQAAFVDSY	EL1	Yes	HEK293 cells, rat CA1 pyramidal neurons, xenopus oocytes, mice smooth muscle cells	[13], [43], [62], [64], [92], [100]
Panx1-1	GTQISCFSPS	EL1	No	HEK293 cells	[100]
Panx1-2	CFSPSSFSWRQAA	EL1	No	HEK293 cells	[100]
Panx1-3	QKNSLQSESGNLP	EL1	No	HEK293 cells	[100]
Panx1-4	YCWAAVQQKNSLQSESGNLP	EL1	No	HEK293 cells	[100]
Panx1-5	LRNDSTVPDQFQ	EL2	No	HEK293 cells	[100]
^{14}Panx1	SGILRNDSTVPDQF	EL2	No	HEK293 cells	[13], [100]
PanxE1a	AQEISIGTQIS	EL1	No	Xenopus oocytes	[43]
PanxE1b	SSFSWRQAAFVDS	EL1	Yes	Xenopus oocytes	[43]
PanxE1c	SESGNLPLWLHK	EL1	No	Xenopus oocytes	[43]
PanxE2a	SSLSDEFVCSIKS	EL2	No	Xenopus oocytes	[43]
PanxE2b	KSGILRNDSTVPDQ	EL2	No	Xenopus oocytes	[43]
IL1	VGQSLWEISE	CL	No	Mice smooth muscle cells	[62]
IL2	KYPIVEQYLK	CL	Yes	HEK293 cells/ mice smooth muscle cells	[62]
Panx1 auto-inhibitory region	GKTPMSAEMREE	CT	No	HEK293 cells	[63]
hPanx1(Ct)	F299-C426	CT	Yes	HEK293 cells	[63]
hPanx1(Ct) Δ391	F299-E391	CT	Yes	HEK293 cells	[63]
Panx$_{305-318}$	LKVYEILPTFDVLH	CT	Yes	Rat CA1 pyramidal neurons	[64]

Panx, pannexin; EL, extracellular loop; IL, intracellular loop; Ct, C-terminal tail; CL; cytoplasmic loop, HEK, human embryonic kidney; CA1, cornu ammonis1

Figure 1: Conformation of Cx hemichannels, gap junctions and Panx channels: Gap junctions are formed by the assembly of 2 Cx hemichannels, which in turn consist of 6 Cx proteins. Like Cxs, Panxs also oligomerise into multimeric structures. Both Cx and Panx(hemi)channels function as single membrane channels that facilitate transport between the intracellular and extracellular environment, while gap junctions connect the cytoplasm of opposing cells.

Figure 2: Cx topology and mimetic peptides: Cxs consist of 4 transmembrane domains, 2 extracellular loops, 1 cytoplasmic loop and a cytosolic N- and C-terminal tail. The first extracellular loops of Cx37, Cx40 as well Cx43 are mimicked by peptides under the Gap26 code. Gap27 and Peptide5, on the other hand, mimic regions of the second extracellular loop. Gap27 targets Cx32, Cx40 and Cx43, while Peptide5 is used for Cx43 inhibition. JM2, ΔSH3, CT9, CT10, αCT mimic the C-terminal tail of Cx43 and Gap24 reproduces a sequence of the cytoplasmic loop of Cx32. Both L2 and Gap19 also mimic the cytoplasmic loop but of Cx43.

Figure 3: Panx topology and mimetic peptides of Panx1: Panxs consist of 4 transmembrane domains, 2 extracellular loops, 1 cytoplasmic loop and a cytosolic N- and C-terminal tail. Peptide mimetics [10]Panx1 and PanxE1b reproduce sequences of the first extracellular loop of Panx1. There is only 1 peptide that mimics Panx1's cytoplasmic loop, namely IL2 and $Panx_{305-318}$, hPanx(Ct) as well as hPanx1(Ct)Δ391 mimic sequences of its C-terminal tail.