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Review

Innexin and pannexin channels and their signaling

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ABSTRACT

Innexins are bifunctional membrane proteins in invertebrates, forming gap junctions as well as non-junctional membrane channels (innexons). Their vertebrate analogues, the pannexins, have not only lost the ability to form gap junctions but are also prevented from it by glycosylation. Pannexins appear to form only non-junctional membrane channels (pannexons). The membrane channels formed by pannexins and innexins are similar in their biophysical and pharmacological properties. Innexons and pannexons are permeable to ATP, are present in glial cells, and are involved in activation of microglia by calcium waves in glia. Directional movement and accumulation of microglia following nerve injury, which has been studied in the leech which has unusually large glial cells, involves at least 3 signals: ATP is the "go" signal, NO is the "where" signal and arachidonic acid is a "stop" signal.

1. Pannexons and innexons

It was only recently that the pannexin/innexin membrane channels were discovered [1–3], and yet these "new" channels mediate several ancient forms of important intercellular communication in animals ranging from primitive invertebrates to humans. The list of the channels' functions and properties has shifted and become refined each year to the point that a review seems timely, with emphasis not only on diverse functions but on common features of the channels, such as serving as conduits for release of ATP from cells.

The channels also present a problem of evolutionary interest. When vertebrates evolved from invertebrates, a major step was the appearance of connexins, new gap junctional molecules that functioned much like the old innexins in invertebrates. This created a divergence that remains puzzling and has highlighted the need to understand the various roles of innexins. The vertebrates retained the innexins as pannexins; in evolving, they stopped forming gap-junctions between cells but continued as membrane channels to the extracellular space, which we now know to be one of the original functions of innexins, as described below.

2. Calcium waves, ATP release, and pannexins/innexins

Soon after the discovery of calcium waves and their spreading through gap junctions [4], it was discovered that the waves could jump between cells and cell layers that were not in junctional communication or even in direct contact [5]. This observation proved that gap junctions were not the exclusive pathway for wave propagation and that there had to be an extracellular signal molecule mediating wave transmission to non-contiguous cells. That signal was eventually identified as ATP [5,6]. It also became apparent that some part of ATP release was sensitive to gap junction channel inhibitors [7], leading to a plethora of papers using such pharmacological evidence to conclude that connexin "hemichannels" are involved. As of late 2013, a PubMed search with the terms "hemichannels" and "ATP", for example, yielded 209 publications. The complication of the term "connexin hemichannels" is not only in its misleading name, but also its physiological relevance. The term is derived from the fact that gap junction channels, which span the two membranes of contacting cells, are composed of two parts, each one residing in a plasma membrane of apposing cells (hemi-gap junction channels) that join to form the complete gap junction channel. The physiological relevance comes into question when the conditions are considered under which connexin "hemichannel" activity is observed [8–10]. Extremely low extracellular calcium concentrations and high positive potentials are required to see channel activity.

When pannexins were discovered, on the basis of homology to innexins in vertebrates through a database search [3], they were initially considered to represent a redundant system to the connexin gap junctions. However, gap junction formation by pannexins is unlikely for a number of reasons [11–13], and to date there is no evidence that pannexin-based gap junction channels exist naturally, despite the fact that the related innexins do make gap

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junctions. In hindsight, the junctional coupling reported initially [1] was an artifact of the high levels of expression in the oocyte, where some pannexons were consequently without their usual glycosylation and were thus able to dock with pannexons overexpressed in an adjacent, contacting oocyte. Thus, pannexin was a protein in search of a function. Because of the similar pharmacology of innexins and connexins, it was intuited that pannexins would behave similarly. This assumption was proven to be correct; all gap junction inhibitors also inhibit pannexin channels, as summarized in a recent review [14].

Given the overlapping pharmacologies of connexins and pannexins, it was reasonable to consider that pannexins might be responsible for ATP release, as originally attributed to connexin "hemichannels". Systematic testing of this hypothesis in various cell types yielded evidence for a major role of Panx1 channels in ATP release under physiological and pathological conditions [15].

Invertebrates that are not chordates do not have connexins, but their cells can generate calcium waves. Because of the sequence similarity between pannexins and innexins, it was obvious to test the ability of innexins to form patent unpaired channels in addition to their well-documented formation of gap junction channels. Indeed, several innexins were found to be bifunctional, forming gap junctions and unpaired membrane channels, called "innexons", allowing the flux of small molecules including ATP across the plasma membrane [16,17].

3. Biophysical properties of Panx1 and innexin channels

Single channel records among the pannexins presently are only available for Panx1. This channel, called a "pannexon", is unusual in its high conductance and the presence of multiple subconductance states [18]. The maximal conductance of the Panx1 channel is ~500 pS. However, even with maximal stimulation, this high conductance typically is not sustained, but the channel dwells for variable durations in one of the multiple subconductance states (Fig. 1).

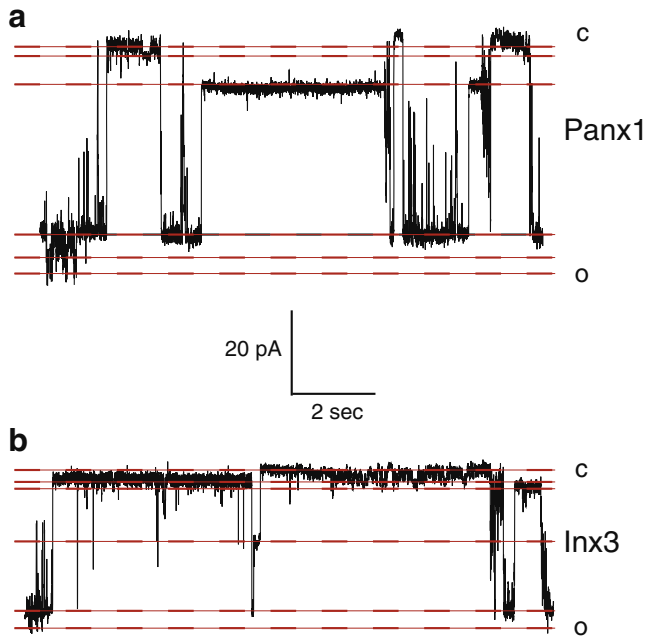

Fig. 1. Single channel activity in excised membrane patches containing Panx1 (a) or *Hm*inx3 (b). The membrane potential was clamped at −100 mV. Both the bath and the pipette solutions contained 150 mM potassium gluconate. Both channels have sub-conductance states, as previously reported for *Hm*inx2.

The biophysical properties of innexin membrane channels (innexons) are very similar to those of Panx channels, with single channel properties of innexons as complex as those observed for pannexons [17]. The maximal single channel conductance is ~500 pS and, like pannexons, innexons exhibit multiple subconductance states (Fig. 1). Furthermore, innexons open in response to mechanical stress and are activated by increased $[K^+]_o$ at the resting membrane potential like pannexons.

Concordant with the high single channel conductance, both innexin and Panx1 channels exhibit permeability to larger molecules than the small ions typically permeating ion channels [17–21]. Channels formed by both proteins allow the passage of both cationic and anionic dyes, which also are known to cross from cell to cell through gap junctions. Physiologically more relevant is the permeation of the signal molecule ATP from the cytoplasm to the extracellular space through innexin and Panx1 channels.

The similarity between pannexons and innexons also extends to the pharmacological properties of the channels. Both types of channels are inhibited by cytoplasmic acidification, carbenoxolone, arachidonic acid, and Brilliant Blue G [17,22,23]. Thus, the overall similarity between innexins and pannexins extends well beyond the limited similarity between the amino acid sequences.

4. Activation mechanisms for innexin and pannexin channels

Both innexin and pannexin channels can be activated by membrane potential changes [18,24]. While activation by voltage is a convenient method to study basic properties of pannexin channels, this activation method is far from physiological, since positive holding potentials in excess of +20 mV are required for Panx1 channel opening. Panx2 channels open at even more extreme positive membrane potentials [25]. Innexin channels are somewhat more sensitive to voltage changes and can be activated by depolarization to ~−20 mV. The positive potentials required to open pannexin channels are unlikely to occur in vivo. Indeed, membrane potential-induced ATP release from nerve terminals is mainly, if not exclusively, vesicular [26–29]. Positive potentials are typically only associated with action potentials, which are generally very brief and thus would not support efficient ATP release from cells. More significantly, the electrochemical gradient would not be favorable for such release either. While the concentration gradient represents a driving force for ATP efflux, the electrical gradient would oppose such flux. Indeed, a recent study demonstrated that ATP flux through connexin "hemichannels", which only open at extremely positive potentials, did not occur while the membrane potential was stepped to +80 mV [30]. Instead, a brief ATP release was associated with the tail currents after stepping the membrane potential back to −40 mV. While tail currents are very useful analysis tools, they are an experimental artifact. In vivo, membrane channels do not work under voltage clamp conditions. It would need a combination of slowly inactivating depolarizing channels combined with fast opening hyperpolarizing channels to approximate the situation encountered experimentally in tail currents. However, most ATP release in vivo occurs at or near the resting membrane potential, where connexin hemichannels are closed and Panx1 channels are activated by other means than membrane voltage changes.

We have pointed out early on that the activation of Panx1 channels by physiological stimuli can occur at the resting membrane potential [11]. A series of stimuli have been identified to open pannexin and innexin channels by voltage independent means. These physiological stimuli include low oxygen environment, mechanical stress, increased cytoplasmic calcium ion concentration, and more indirect activation of the channels by ligands to membrane receptors. Under pathological conditions, the channel