



Emerging Role of Pannexins in Seizures and Status Epilepticus

Targeting Pannexin1 Improves Seizure Outcome.

Santiago MF, Veliskova J, Patel NK, Lutz SE, Caille D, Charollais A, Meda P, Scemes E. *PLoS One* 2011;6(9):e25178. URL <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0025178>. Access 03/052012.

Imbalance of the excitatory neurotransmitter glutamate and of the inhibitory neurotransmitter GABA is one of several causes of seizures. ATP has also been implicated in epilepsy. However, little is known about the mechanisms involved in the release of ATP from cells and the consequences of the altered ATP signaling during seizures. Pannexin1 (Panx1) is found in astrocytes and in neurons at high levels in the embryonic and young postnatal brain, declining in adulthood. Panx1 forms large-conductance voltage sensitive plasma membrane channels permeable to ATP that are also activated by elevated extracellular K(+) and following P2 receptor stimulation. Based on these properties, we hypothesized that Panx1 channels may contribute to seizures by increasing the levels of extracellular ATP. Using pharmacological tools and two transgenic mice deficient for Panx1 we show here that interference with Panx1 ameliorates the outcome and shortens the duration of kainic acid-induced status epilepticus. These data thus indicate that the activation of Panx1 in juvenile mouse hippocampi contributes to neuronal hyperactivity in seizures.

Commentary

Electrical transmission in the central nervous system of invertebrates is maintained by a group of proteins called innexins. By contrast, electrical transmission in vertebrates is carried out by pore-making proteins called connexins. One would assume that the proteins mediating electrical transmission in vertebrates and invertebrates would share sequence homology, but that is not the case. Even though there are structural similarities between connexins and innexins, they show no sequence homology. Interestingly, in 2003, vertebrate homologs of innexins were cloned; these proteins are referred to as pannexins. There is no evidence that pannexins form gap junctions present between cortical interneurons and certain pyramidal neurons or mediate electrical transmission between these neurons (1).

Three types of pannexins are expressed in mammals: PX1, PX2, and PX3. PX2 is confined to the central nervous system, and PX3 is confined to the skin; PX1 is widely expressed. When expressed in expression systems, PX1 can form channels called pannexons. It is unclear whether PX2 can form channels by itself. However, coexistence of PX2 and PX1 suggest that PX1 and PX2 heterometric channels may form in cells expressing both of these proteins (2). In native cells, pannexins appear to form large pore channels in the plasma membrane, which can permeate molecules up to the size of 1 kDa, thus allowing passage of

molecules as large as ATP. The electrical properties of PX1 channels remain debated. In some cases, PX1-mediated currents are rectifying, whereas currents mediated by this channel are clearly linear in other studies. The activation of these channels may have some impact on their properties. When these channels are activated by depolarization, they appear to be rectifying, whereas activation by ligands, such as ATP and NMDA, produces a linear current voltage relationship (1).

The activators of PX1 channels are being studied. It is suggested that the PX1 channel is activated by purinergic receptors (i.e., receptors of ATP, UTP, and metabolites). Ionotropic ATP receptors belonging to the P2X family, which consists of P2X₁₋₇ receptors, can activate PX1 channels. Among these receptors, there is growing evidence that P2X₇ receptors are particularly active in activating pannexin receptors. Other receptor subtypes, such as P2X_{2,4} and ₅, also may be able to activate PX1 channels. A large body of evidence in the past has shown that prolonged oxygen glucose deprivation and ischemia can lead to opening of a large pore channel. It was presumed in the past that this large pore channel was a purinergic receptor, which underwent dilatation. However, with discovery of pannexins, it is now believed that purinergic receptors open a large conductance pore formed by pannexins. The physiological role of pannexins has been difficult to determine, especially because opening such a large pore is likely to kill neurons. Current understanding of these proteins is that they participate in brain pathophysiology, such as ischemia, excitotoxicity, epilepsy, and perhaps neuroinflammation

Prolonged activation of NMDA receptors causes two currents, referred to as primary and secondary currents. The



identity and ionic basis of the secondary current was poorly understood, even though it had been studied extensively (3). As the potential role of pannexins in epilepsy became apparent, it was found that this secondary NMDA receptor current was mediated by pannexin channels (4). PX1 is expressed on CA1 pyramidal neurons in the hippocampus at the postsynaptic density. The postsynaptic density also houses glutamatergic receptors, such as AMPA and NMDA receptors, besides a series of other proteins. This led to the hypothesis that NMDA receptor activation could cause opening of pannexin channels. Investigators used a combination of pharmacologic techniques and knockdown of PX1 channels by RNA interference technique to diminish this current. Furthermore, they demonstrated that prolonged epileptiform bursting triggered by lowered magnesium was attenuated by a peptide that blocked PX1 channels.

Challenges in understanding the role of PX1 channels in seizures and epilepsy have been a lack of specific drugs and a lack of ways to inhibit the channel. Many can drugs activate and inhibit these channels, but they have off-target effects.

Development of PX1 knockout mice circumvents this problem and allows additional exploration of the role of these channels in seizures and epilepsy. Santiago et al. focused on the role of ATP released from these channels during status epilepticus in wild-type and connexin1 knock-out mice. By using high potassium external medium to induce prolonged epileptiform bursting in hippocampal slices, the authors demonstrated diminished release of ATP from knock-out mice compared with controls. Similar effects were observed when a drug was used to inhibit pannexin. Furthermore, dye uptake to hippocampal neurons and astrocytes caused by high potassium was diminished by a PX1 inhibitor and in knockout mice. The authors also demonstrated that the PX1 channel was activated during status epilepticus induced by kainic acid in juvenile (P13-14) mice. Increases in extracellular ATP levels observed in control mice were diminished in knockout mice. Both behavioral and electrographic seizures were attenuated in these animals. The authors suggested that PX1 channels participate in generation and persistence of seizures or status epilepticus in vivo.

Overall, these studies contribute to the growing knowledge of the role of PX1 channels in induction of seizures. A status epilepticus model was used to understand the role of these channels. Status epilepticus is distinct from chronic recurrent

seizures observed in temporal lobe epilepsy or other forms of epilepsy. Only juvenile mice were used in this study, which is convenient, because the expression of PX1 declines with age. Thus, PX1 may have a more important role in acquisition of status epilepticus and seizures in younger animals compared with adults.

Furthermore, the mode of activation of PX1 during status epilepticus remains uncertain. Obvious candidates, such as NMDA receptor activation and prolonged depolarization, exist. How these two stimuli lead to PX1 insertion in the membrane is not known. It is proposed that activation of P2X₇ receptors open these channels, but this raises questions. Activation of purinergic receptors requires ATP in the extracellular space, but how does the ATP required for activation of purinergic receptors get into the extracellular space? In addition to opening pannexin channels, ATP diminishes the release of neurotransmitters, especially glutamate, and suppresses seizures (5). Thus, we are faced with a situation in which adenosine through purinergic receptors could both worsen and control seizures. Finally, current studies have focused largely on the PX1 channel. As noted in this commentary, the PX2 channel is also widely distributed in the central nervous system and may form heteromers with PX1. The role of PX2 channels in seizures and epilepsy remains unexplored.

by Jaideep Kapur, MD, PhD

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