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The Role of Pannexin-1 Channel- Review

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Abstract:- Three relations compose the Pannexin family of channel forming glycoproteins such as Pannexin-1 (Panx1, Gen-Bank AAQ89382.1, 426 amino-acids), Pannexin-2 (Panx2, Gen-Bank AAI01024.1, 74.4 kDa) and Pannexin-3 (Panx3, Gen-Bank AAK95655.1, 392 aminoacids, 44.7 kDa). Their primary performance is outlined by their capability to make single membrane channels that area unit regulated by post-translational modifications, channel intermixing and sub-cellular expression profiles. Pannexins (Panx) are tetra-spanning membrane proteins that mediate paracrine intercellular communication via release of purines such as ATP or UTP. Pannexins can form non-junctional transmembrane channels for the transport of molecules with a molecular weight of less than 1000 Da. These hemi-channels have been found in the plasma, ER, and Golgi membranes. They can form hemi-channels more easily than connexin subunits and transport Ca2+, ATP, inositol triphosphate, and other small molecules. Pannexins 1 is expressed in: brain, skeletal and heart muscle, testis, ovary. Pannexin 2 are found predominantly in the central nervous system, and for the Pannexin 3, these are involved in several embryonic tissues as well as in adult bone, cartilage and skin.

- ***Aim:***

The purpose of this study is to identify the role of pannexin channels and the importance of it to the organisms.

- ***Method:***

The information about the role of Pannexin channels is estimated by an electronic search in PubMed interface, Scopus and Science Direct, using the keywords "Pannexin Channels", and "Role of Pannexin channels". The inclusion criteria were: a) mechanism of Pannexin channels; b) Pannexin channels crucial for COVID-19 pathogenesis; c) pannexins as mediators of neuro-inflammation

- ***Results:***

Panx1 has received the greatest attention, although properties of this pannexin must be extrapolated with caution to the other pannexins. Although no germ-line mutations in genes producing pannexins have been connected to any disorders, elevated pannexin expression has been linked to disease onset and/or progression in numerous situations. However, disease can arise when pannexins are under expressed, underscoring the need of pannexin expression regulation.

- ***Conclusions:***

The role of the selective chloride conductance seen with voltage or caspase activation of the channel will be elucidated in the future. In addition, the signalling cascade in pyroptotic cell death including Panx1 requires clarification. Panx1 has been extensively documented as an ATP/UTP release channel even at physiological calcium levels. Post-translational changes appear to control their expression, intracellular location, intermixing, and maybe final function in many tissues.

Keywords:- *Pannexin Channels, Hemi-Channel, Neuro-Inflammation, COVID-19 Pathogenesis*

I. INTRODUCTION

Many Pannexin publications refer to pannexin oligomers as "hemi-channels", while others refer to them as "channels." This has caused confusion in the literature about the function of pannexins, which promotes the idea that pannexons serve as gap junction hemichannels and, as a result, have the same assembly and functional state as gap junctional intercellular channels. Based on sequence homology with invertebrate gap junctions and predicted topology similar to gap junction proteins, connexins (vertebrate form), and innexins, these integral membrane proteins were initially proposed to form gap junction-like structures (invertebrate form) (*Panuela. S et.al, 2007*). Four transmembrane segments, two extracellular loops, and cytoplasmic localization of the amino terminus, cytoplasmic loop, and carboxy terminus comprise the "connexin-like topology". Connexins have six conserved cysteine residues in their -extracellular loops, which allow for three-disulfide intra-connexin bridges5 (*Maeda. Sh et.al, 2009*), whereas pannexins have four conserved extracellular loop cysteines, which allows for two intra-pannexin disulfide bonds. It is also worth noting that the pannexin sequence is similar to innexins but not to connexins. The epithelial and endothelial barriers, neutrophils, macrophages and monocytes, dendritic cells, granulocytes, natural killer cells, and mast cells comprise the innate immune response, which is a critical step in the defence against infectious agents. Normally, this type of immune response is transient and does not target a specific pathogen. The adaptive (or acquired) immune response, on the other hand, is highly specific to a specific pathogen and generates immunological memory (*Kameritsch P, 2020).* Panx1 interaction with P2X7 receptors induces an immune response by releasing the pro-inflammatory cytokine interleukin (IL)-1 in response to ATP receptor stimulation, followed by caspase-1 activation. Panx1 has also been shown to activate the Toll-like receptor-independent inflammasome (which includes cryopyrin) in response to bacterial molecules passing from endosomes to cytosol. Furthermore, high extracellular K+ levels were found to potentiate P2X7-mediated activation of Panx1 channels in neuronal/astrocytic inflammasomes. However, researchers discovered that Panx1 is not required for the assembly of caspase-1-activating

inflammasome complexes in a recent study using Panx1 null mice (*Qu. Y et.al, 2011*). Panxs form large pore channels that open during membrane depolarization, changes in intracellular Ca2+ signalling, vasodilation, vasoconstriction, taste sensation, learning/memory, cellular differentiation, cell death, also during adaptive immune responses (*Chen. Y et.al, 2010).*

II. MATERIALS AND METHODS

❖ *Sources*

This study was carried through gathering information about the role of Pannexin-, as well as about the lastest researches about SARS-CoV-2-associated, and the inflammation that is caused by the opening of the Panx-1 channel. Databases published and related to the topic are provided from published materials and articles including: a) Medline (PubMed); b) National Center of Biotechnology Information (NCBI); c) Science Direct, researches about purinergic receptors and Pannexin-1 channels I the pathogenesis of SARS-CoV-19.

➢ *Pannexin-1 channels*

Panx1 is expressed at various levels in human tissues such as the brain (central nervous system in the cerebellum, cortex, neocortex and hippocampus interneurons), heart, skeletal muscle, skin, testis, ovary, placenta, prostate, lung, liver, small intestine, pancreas, colon, blood endothelium, erythrocytes (*Shestopalov. V.I 2008).* Panx1 has been shown to be a component of the P2X7 receptor complex required for ATP release. Additionally, ATP-induced ATP release was observed when Panx1 channels were activated via P2Y receptors and cytoplasmic Ca2 +, supporting Panx1's role in the initiation and propagation of regenerative Ca2 + signaling (*Locovei. S et.al, 2007).*

➢ *Panx1 muscle contraction*

After repeated twitches, the force created by muscle contraction rises. This reaction is known as potentiation, and it is based on an increase in intracellular free Ca2+ concentration caused by Ca2+ release from intracellular storage and Ca2+ input from the extracellular environment in fast and slow twitch muscles, respectively (*Sandonà et al, 2005*). Ca2+ uptake in the latter is dependent on the activation of purinergic ionotropic P2X4 receptors, which are abundant in this kind of muscle. Electrical stimulation at Panx1 channel activating frequencies (20 Hz) causes the development of a molecular marker that represents the change from a rapid to a slow myofiber (*Jorquera et al., 2013*). Panx1 channels in the T-tubule membrane are controlled for dihydropyridine receptors; at high frequencies (90 Hz), Panx1 channel activity is low and plasticity marker expression in myofibers does not alter. Electrical stimulation causes ATP release via Panx1 channels, resulting in an IP3-dependent intracellular Ca2+ signal that is directly related to gene expression alterations. Because apyrase, an ATP hydrolase, or suramin, a generic P2 receptors inhibitor, block both signals, both responses are dependent on the activation of P2 receptors by extracellular ATP. Panx1 channel inhibitors (10Panx1 and oleamide) decreased calcium transients and ATP release, preventing the metabotropic Ca2+ signal caused by extracellular ATP. As a result, it was postulated that a train of action potentials with a given frequency generates Ca2+ release events that differently activate Ca2+-dependent signalling pathways, determining the expression of genes responsible for the slow or fast muscle phenotype (*Tavi, 2011*). These signalling pathways include calcineurin-NFAT, Ca2+/calmodulin-dependent kinases II and IV, and protein kinase C These Ca2+ signals promote muscle plasticity by influencing the expression of many genes, including IL-6 and c-fos, as well as the transition of troponin isoforms from fast to slow fibre (*Jorquera et al., 2013*).

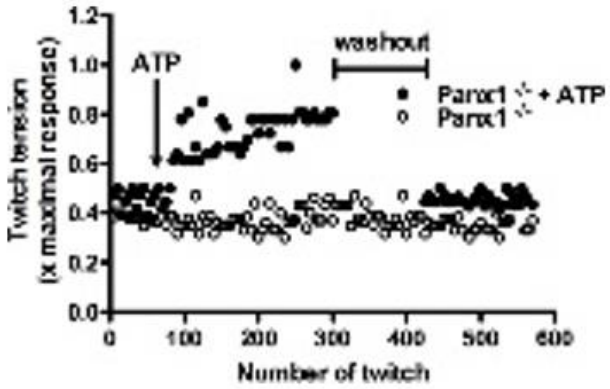

Fig 1:- Exogenous ATP reverses the absence of potentiation of muscular contraction

➢ *Neuro-inflammation*

Cancer, ischemia, platelet activation, seizure, immune cell migration, and HIV viral replication are all connected with Panx1 channel activation. (*Freeman T. J et.al 2019*). Cellular damage in the brain frequently triggers complex inflammatory cascades that begin with:
(1) the release of PAMPs and DAMPs from damaged neuron and glia,
(2) activation of brain resident microglia and astrocytes,
(3) the production of chemokines and cytokines, and
(4) the recruitment of peripheral cells, including leukocytes, to the site of injury. ATP is a crucial mediator produced from injured and dying cells into the extracellular environment during inflammation (*Kwon H. S et.al 2020*). Extracellular ATP has been demonstrated to operate as a danger signal, mediating inflammation through a variety of mechanisms, including inflammasome activation and immune cell infiltration. ATP operates as a major "find-me" signal in the

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CNS, attracting monocytes, macrophages, and microglia to the site of damage. Uncontrolled inflammasome activation can cause inflammation-driven cell death, known as pyroptosis, which can spread across tissues and may be one of the processes behind secondary damage.

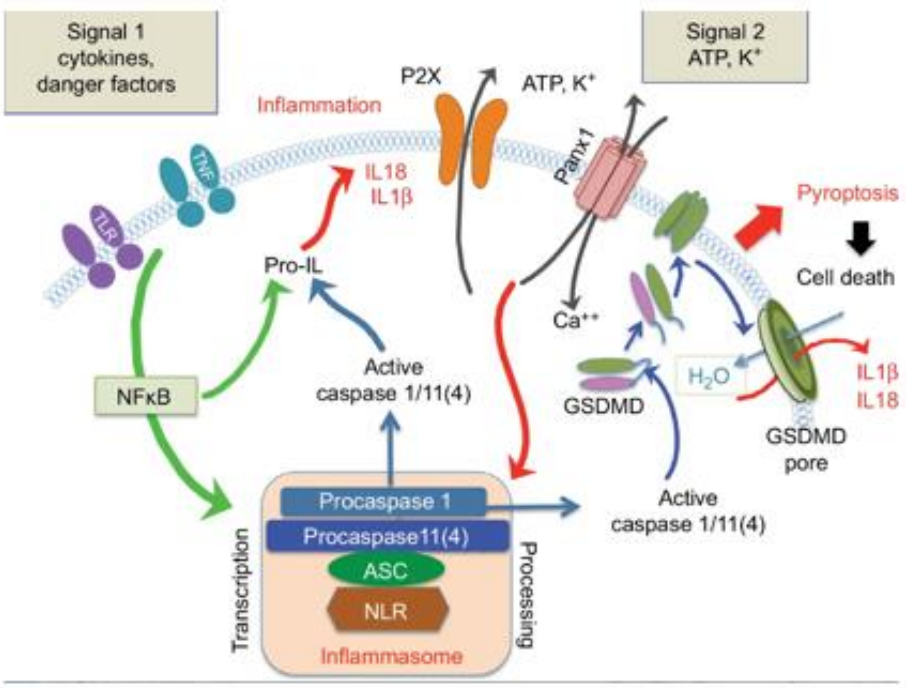

Fig 2:- Inflammasome- activation cascade

Inflammasomes have a role in negative outcomes in brain trauma; for example, important inflammasome proteins NLRP3, ASC, and Caspase-1 are increased in neurons, microglia, and astrocytes (*De Rivera Vaccari J.P et.al, 2014*). Because many CNS injuries and disorders include some level of neuro-inflammation, therapeutic regulation of the neuro-inflammatory response may be a promising method for treating brain injuries and neurological diseases. Indeed, several emerging treatment targets for neurodegenerative illnesses that were previously assumed to be neuronal in origin, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), alter astrocyte and microglia immunological responses (*Zhou Y.et.al 2020*).

➢ *Stroke*

Ischemic stroke triggers a complicated chain of molecular processes that result in necrosis and apoptosis, followed by neuro-inflammation. Reduced intracellular ATP causes membrane potential to depolarize, resulting in the activation of different ion channels and the breakdown of ionic homeostasis (*Weilinger N.L et.al 2016*). On the other side, neuronal Panx1 channels were found to activate early following oxygen and glucose deprivation. Neuronal necrosis and debris can activate microglia and astrocytes in response to ischemia insult, triggering subsequent neuro-inflammatory processes such as leukocyte infiltration. IL-1 is primarily generated by inflammasome complexes that include the proteins NLRP3, ASC, NEK7, and caspase-1. Extracellular ATP acting on P2X7 receptors is a crucial signalling route that might contribute to inflammasome activation (*Savio L.E.B 2018).*

➢ *Role in HIV Infection*

HIV infection produces biphasic opening of Panx-1 hemi-channels in peripheral blood mononuclear cells (PBMCs) and CD+4 T lymphocytes. When the virus binds to its receptor (CD4) and co-receptors (CXCR4 and/or CCR5), Panx-1 hemi-channels open. The virus caused ATP release and subsequent purinergic receptor activation by opening Panx-1 hemi-channels. (*Orellana et.al 2013*). The binding of gp120 to primary human macrophages causes the release of ATP, allowing for autocrine activation of purinergic receptors. Panx-1 hemi-channels, purinergic receptors, and extracellular ATP all play important roles in HIV infection and replication in immune cells, potentially by assisting with viral entrance and various stages of the viral life cycle (*Seror et.al*, 2011).

➢ *SARS-CoV-2*

Human coronavirus 229E (hCoV-229E) or its isolated S protein, one of the primary viruses causing the common cold, stimulate the temporary opening of Pannexin-1 (Panx-1) channels in human lung epithelial cells. SARS-CoV-2 induces a higher and longer duration of Panx-1 channel opening than hCoV-229E/S protein, leading in increased ATP, PGE2, and IL-1 release. When compared to hCoV-229E or its pure S protein, SARS-CoV-2 S protein opens Panx-1 channels aggressively and for a longer period of time, indicating a distinct opening mechanism (*Lu R. et.al, 2021*). Angiotensin-converting enzyme 2 (ACE-2), endocytosis, and furin are required for Panx-1 channel opening triggered by SARS-CoV-2 spike protein. ATP, PGE2, and IL-1 are released into the extracellular space following Panx-1 channel opening mediated by SARS-CoV-2 S protein. Lung

tissue and nasal swab studies show that Panx-1 mRNA and protein levels are elevated in immunological and lung cell populations, suggesting the Panx-1 channel's critical involvement in COVID-19 pathogenesis. Local and systemic inflammation, as well as HIV entrance into immune cells, have been linked to Panx-1 channel opening via a process involving the local release of intracellular components such as ATP, NAD+, prostaglandins, and other inflammatory lipids through the channel pore (*Gajardo G. et.al, 2020*).

III. CONCLUSION

Extracellular ATP operates as a primary danger signal in many CNS lesions, including spinal cord injury, TBI, and brain ischemia, inducing an organized inflammatory response that may lead to damage worsening. As a result, ATP release via Panx1 channels might be a promising therapeutic strategy for lowering inflammation in CNS injury. Indeed, several studies have demonstrated that Panx1 channel inhibitors are effective in animal models of cerebral ischemia, seizure, neuropathic pain, and TBI. More research is needed to create Panx1 inhibitors with low off-target effects, as many existing inhibitors also target other large-pore channels, such as connexin hemichannels and CALHM1 channels. Despite extensive research linking PANX1 mechanosensitivity to a variety of illnesses, the mechanism by which PANX1 channels are responsive to mechanical stimuli remains unexplained. Furthermore, while the structure of PANX1 was discovered using cryo-EM, the mechanism of its mechanosensitivity was not explained. PANX1 may have a domain that interacts with the lipid bilayer and perceives mechanical force, but further research is needed to confirm this. The field will continue to unravel the real nature of pannexin channels and their critical roles in health and illness with the emergence of new single and double Panx-KO mice models that will allow compensatory mechanisms to be investigated.

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