Derivation of AMPA receptor GluA1 subunits in mice from exosomes modulates inflammatory pain

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Abstract:

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–type (AMPA-type) glutamate receptors (AMPARs) play a crucial role in synaptic plasticity within the central nervous system. While there is anatomical evidence suggesting the presence of AMPAR expression in the peripheral nervous system, the functional significance of these receptors in vivo remains unclear. The nociceptor-specific deletion of GluA1 results in the disruption of calcium permeability and a diminished response to capsaicin stimulation in nociceptors. The deletion of GluA1, leds to reduced mechanical hypersensitivity and sensitization in models of chronic inflammatory pain and arthritis. There is known that GluA1-containing AMPARs played a regulatory role in the nociceptors' responses to painful stimuli in inflamed tissues, influencing the excitatory signals transmitted from the periphery into the spinal cord. Exosomes, nanoscale particles secreted by cells (typically ranging from 30 to 150 nm in size), carry a diverse array of biological molecules, including nucleic acids, proteins, and lipids. Exosomes have crucial role in facilitating intercellular communication. Leveraging their inherent stability, low immunogenicity, and impressive tissue/cell penetration capabilities, exosomes show promise as advanced platforms for targeted drug and gene delivery. To address this issue, we used mice specifically lacking of the key AMPAR subunits, GluA1, in

peripheral, pain-sensing neurons (nociceptors), while preserving expression of these subunits in the central nervous system. Nociceptor-specific deletion of GluA1 led to disruption of calcium permeability and reduced capsaicin-evoked activation of nociceptors. Deletion of GluA1, led to reduced mechanical hypersensitivity and sensitization in models of chronic inflammatory pain and arthritis. We generated exosomes containing GluA1 and introduced them to mice around nociceptors, observing a reverse effect compared to GluA1 deletion. Mice treated with exosomes were more sensitive to pain.

Keywords: Exosome, AMPA receptor, Glua1 Subunits, mouse, modulate, inflammatory pain.

AMPA რეცეპტორის GluA1 ქვედანაყოფების ეგზოსომური წარმოქმნა თაგვებში და ანთებითი ტკივილის მოდულირება

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აბსტრაქტი

α-ამინო-3-ჰიდროქსი-5-მეთილ-4-იზოქსაზოლპროპიონის მჟავას (AMPA ტიპის) გლუტამატის რეცეპტორები (AMPAR) მნიშვნელოვან როლს ასრულებენ ცენტრალურ ნერვულ სისტემაში სინაფსურ პლასტიკურობაში. მიუხედავად იმისა, რომ არსებობს ანატომიური მტკიცებულება, რომელიც ვარაუდობს ამპარ-ების ექსპრესიას პერიფერიულ ნერვულ

სისტემაში, ამ რეცეპტორების ფუნქციური მნიშვნელობა in vivo ბოლომდე გარკვეული არ არის. ამ ცოდნის ხარვეზის აღმოსაფხვრელად, ჩვენ გამოვიყენეთ თაგვები რომელთა დნმ-დან ექსკლუზიურად პერიფერიული ტკივილის ნეირონებში (ნოციცეპტორები) ამოჭრილია AMPAR-ების ძირითადი სუბერთეული GluA1. რაც მთავარია, ჩვენ შევინარჩუნეთ ამ სუბერთეულის არსებობა ცენტრალურ ნერვულ სისტემაში. GluA1-ის ნოციცეპტორისთვის სპეციფიური დელეცია იწვევს კალციუმის გამტარიანობის დარღვევას და კაპსაიცინის სტიმულაციაზე პასუხის დაქვეითებას ნოციცეპტორებში. ცნობილია, რომ GluA1-ის წაშლა იწვევს მექანიკური ჰიპერმგრძნობელობის დაქვეითება და სენსიბილიზაცია ქრონიკული ანთებითი ტკივილისა და ართრიტის მოდელებში. GluA1-ის შემცველი ამპარ-ები ასრულებენ მარეგულირებელ როლს ანთებით ქსოვილებში მტკივნეულ სტიმულებზე ნოციცეპტორულ პასუხებში, პერიფერიიდან ზურგის ტვინში გადაცემულ აგზნებად სიგნალებზე ზემოქმედებით. ეგზოსომები, უჯრედების მიერ გამოყოფილი ნანონაწილაკები (როგორც წესი, ზომით 30-დან 150 ნმ-მდე მერყეობს), ატარებენ ბიოლოგიური მოლეკულების მრავალფეროვან მასივს, მათ შორის ნუკლეინის მჟავებს, ცილებს და ლიპიდებს. ეგზოსომები ცნობილია მათი გადამწყვეტი როლით უჯრედშორისი კომუნიკაციის შუამავლობაში. მათი თანდაყოლილი სტაბილურობის, დაბალი იმუნოგენურობის და შთამბეჭდავი ქსოვილის/უჯრედის შეღწევის შესაძლებლობების გამოყენებით, ეგზოსომები განიხილება როგორც მოწინავე პლატფორმები წამლებისა და გენების მიზანმიმართული მიწოდებისთვის. ამ საკითხის გადასაჭრელად, ჩვენ გამოვიყენეთ თაგვები, რომლებსაც არ ჰქონდათ ძირითადი AMPAR სუბერთეული, GluA1, პერიფერიული ტკივილის ნეირონებში (ნოციცეპტორები), ამავდროულად შენარჩუნებული იყო ამ ქვედანაყოფების გამოხატულება ცენტრალურ ნერვულ სისტემაში. GluA1-ის ნოციცეპტორული სპეციფიური წაშლა იწვევს კალციუმის გამტარიანობის დაქვეითებას და კაპსაიცინის ინდუცირებული ნოციცეპტორის აქტივაციის დაქვეითებას. GluA1-ის წაშლა იწვევს მექანიკური ჰიპერმგრძნობელობის დაქვეითებას და სენსიბილიზაციას ქრონიკული ანთებითი ტკივილისა და ართრიტის მოდელებში. ჩვენ შევქმენით ეგზოსომები, რომლებიც შეიცავს GluA1-ს და შევიყვანეთ ისინი თაგვებში ნოციცეპტორების გარშემო, რამაც საპირისპირო ეფექტი გამოიწვია GluA1-ის დელეციასთან შედარებით. თაგვები, რომლებსაც მკურნალობდნენ ეგზოსომებით, უფრო მგრძნობიარენი იყვნენ ტკივილის მიმართ.

საკვანძო სიტყვები. ეგზოსომა, AMPA რეცეპტორი, GLUA1 ქვედანაყოფი, თაგვი, მოდულირებული, ანთებითი ტკივილი.

Introduction:

Chronic pain is a common and poorly understood medical problem. Plasticity of synaptic transmission in the nervous system during peripheral organ inflammation or nerve injury is an important component of the cellular basis of chronic pathological pain. Glutamate acts as an important excitatory neurotransmitter at several key synapses in the somatosensory nociceptive pathway, activating ionotropic and metabotropic receptors there. Recently, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA-type) glutamate receptors (AMPARs) have emerged as important mediators of synaptic plasticity in the brain (1, 59). Unlike NMDA-type glutamate receptors, which always mediate Ca2+ influx when activated, AMPARs are an activity-dependent switch that controls glutamate-induced Ca2+ influx into neurons (2). This activity-dependent change is mediated by the regulated expression and binding of the GluA2 subunit (previously called GluR-B or GluR2), which mediates low Ca2+ permeability to AMPAR channels. In contrast, the GluA1 subunit (previously called GluR-A or GluR1) is highly expressed in regions with high densities of calcium-permeable AMPARs, including components of pain pathways (3). Although global genetic deletions of AMPAR subunits demonstrated that GluA1-containing AMPARs play an important role in chronic pain mechanisms, they were unable to determine anatomical localization. In fact, AMPARs are expressed in several important modulatory regions of somatosensory pathways that mediate pain, such as: peripheral nociceptive neurons, the dorsal horn of the spinal cord, the ventral horn, and several brain regions that control sensory and emotional pain. However, the different relative contributions of these regions to central sensitization and chronic pain remain unclear. All peripheral sensory neurons use glutamate as a major transmitter, and large subpopulations of dorsal root ganglion (DRG) sensory neurons are known to express mRNA or be immunoreactive for ionotropic and metabotropic glutamate receptors (4). Electron microscopy studies have provided compelling evidence that AMPAR subunits are transported to the peripheral processes of sensory neurons, and recent ex vivo anatomical and electrophysiological data also indicate a presynaptic localization and functional involvement of AMPAR subunits in vertebral terminals (5,6). However, the functional role of AMPARs located in the central and peripheral terminals of

sensory neurons in whole-body nociceptive modulation in vivo remains unclear (7). Moreover, because AMPARs are also expressed in peripheral sympathetic neurons, Schwann cells, and keratinocytes, the use of pharmacological agents alone does not allow for a comprehensive analysis of the contribution of AMPARs at different sites to pain modulation in vivo.

Exosomes are nanosized vesicles secreted by various cell types, including neurons, into the extracellular space. These vesicles carry a cargo of proteins, lipids, and nucleic acids, facilitating intercellular communication (86). Recent investigations have uncovered the presence of AMPA receptors, crucial for synaptic transmission, within exosomes, suggesting a novel mechanism of information transfer between neurons.

Here, we analyzed transgenic mice that lack the essential GluA1 subunit of AMPAR, specifically in the peripheral arm of the somatosensory pain pathway, i.e., the nervous system. The results showed that exosome derivatives containing GluaA1, restores nociceptive effects in GluA conditionally knockout mice.

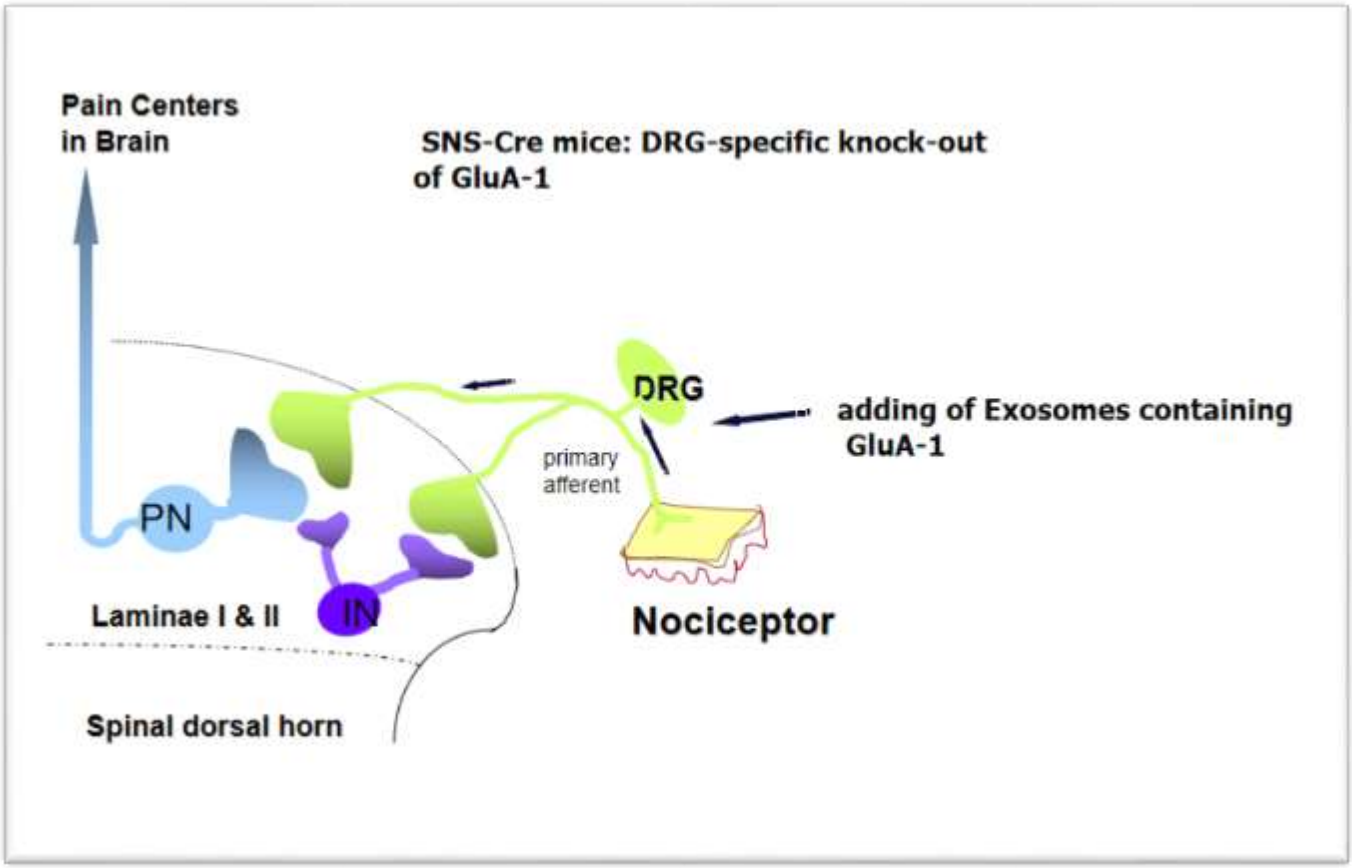


Fig.1 Central and somatic signals received by nociceptors in paraplegia, and the consequences of bringing nociceptors into a stable hyper functional state. Nociceptors receive injury-related signals in the

spinal cord (highly activated postsynaptic dorsal horn (DH) neurons, activated glial cells, and infiltrating immune cells) and in the dorsal root ganglion (DRG) (from other DRG neurons, satellite glial cells, blood, etc.)(9). Nociceptors have strong excitatory effects on pain pathways (referred to as DG neurons) and on circuits supporting somatic and visceral functions. LTP at DG synapses can be generated by somatic and peripheral AS, as well as after-discharge, which is facilitated by the hyper functional state of the nociceptor. Nociceptor activity causes central sensitization, promotes spontaneous and evoked pain, and enhances somatic and visceral reflexes (10). Nociceptor activity also results in positive feedback interactions with postsynaptic neurons, other somatic DRGs, inflammatory cells (microglia, infiltrating macrophages, and T cells), astrocytes, and satellite glial cells. PN - Proprioceptive Neuron: Proprioceptive neurons are specialized sensory neurons responsible for conveying information about the position and movement of body parts to the central nervous system (CNS)(11). They play a crucial role in proprioception, which is the sense of the relative position of neighboring parts of the body. IN - Interneuron: Interneurons are neurons that transmit signals between other neurons, acting as connectors or relays within the nervous system (60). In the context of the DRG, interneurons could be involved in processing and modulating sensory information before it reaches the spinal cord or higher brain centers. They contribute to the integration and coordination of signals within neural circuits (12).

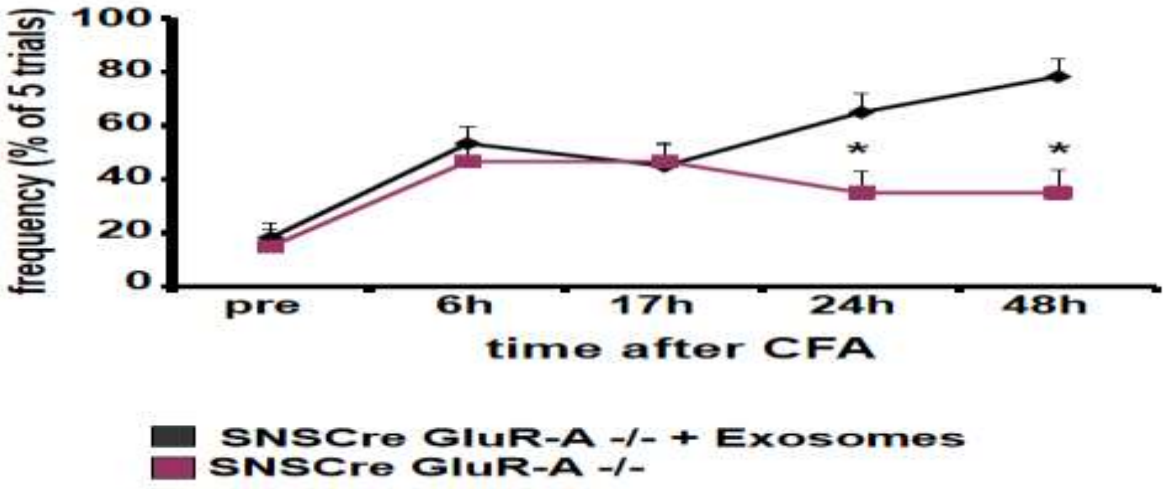
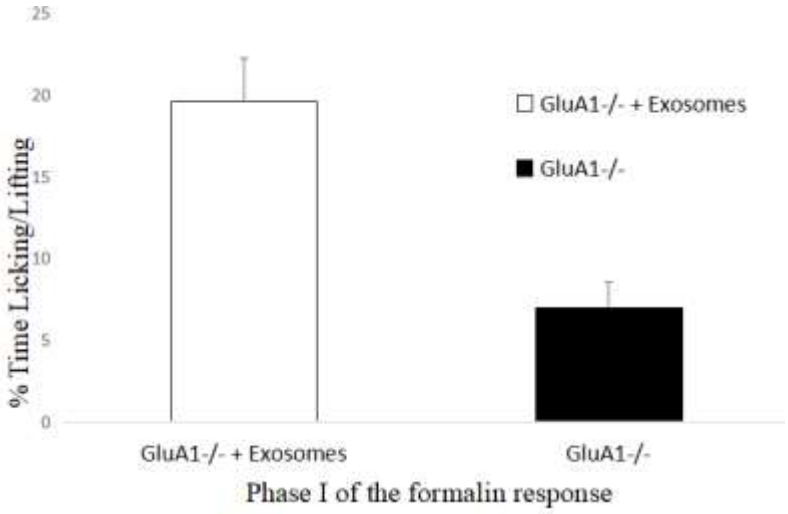


Fig. 2. CFA-induced mechanical hypersensitivity (13) was tested by applying gradual point pressure to von Frey hairs, and the minimum force producing a pull-off response in at least 2 out of 5 applications of von Frey hairs was (n = 20 per groups; $P < 0.01$, $^*P < 0.05$; Student's t test).



termed threshold.

Fig 3. Mice lacking GluA (SNS-GluA1-/-) exhibit markedly diminished pain responses to formalin stimulation. We manually evaluated the duration of licking and lifting behaviors in both exosome treated-SNS-GluA1-/-+ exosome (whch is close to wild-type (data not shown) and SNS-GluA1-/- littermates. SNS-GluA1-/- mice demonstrated significantly reduced licking and lifting throughout all phases of the formalin test compared to SNS-GluA1-/-+ exosome counterparts (n = 20 per groups; $P < 0.05$; Student's t test).

Materials and methods:

In contrast to mechanical hyperalgesia, mice developed CFA-induced thermal hyperalgesia, calculated as the percentage reduction in paw withdrawal latency in the inflamed paw compared to the contralateral non-inflamed paw. SNS GluA1-/- and GluA1-/-+. exosomes ($P < 0.05$) at similar levels ($P > 0.05$ between genotypes).

Exosome preparation:

Cell Culture and Exosome Isolation:

Human embryonic kidney (HEK) 293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were transfected with GluA1 protein expression plasmids using Lipofectamine 2000 according to the

manufacturer's instructions. After 48 hours, the culture media were collected, and exosomes were isolated. The collected media were subjected to a series of centrifugation steps to remove cell debris and large particles. The resulting supernatant was then ultracentrifuged at 100,000 g for 2 hours to pellet exosomes, which were subsequently resuspended in phosphate-buffered saline (PBS).

Loading of Exosomes with GluA1 Recombinant Protein:

Recombinant GluA1 protein was expressed and purified using standard molecular biology techniques.

Purified GluA1 protein was loaded into isolated exosomes using an established protein loading protocol. Briefly, exosomes were incubated with the purified GluA1 protein at an optimized concentration and duration, ensuring efficient loading.

Exosome Characterization:

The loaded exosomes were characterized using transmission electron microscopy (TEM) to visualize their morphology. A drop of exosome suspension was placed on a carbon-coated grid, negatively stained with 2% uranyl acetate, and observed under a TEM microscope.

Nanoparticle tracking analysis (NTA) was performed to determine the size distribution and concentration of exosomes in the loaded preparation.

Protein Analysis:

The presence of GluA1 recombinant protein in loaded exosomes was confirmed by Western blot analysis. Exosomal proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was probed with an anti-GluA1 antibody, followed by appropriate secondary antibodies, and visualized using an enhanced chemiluminescence (ECL) detection system.

Quantitative Analysis of Exosomal GluA1 Recombinant Protein:

The amount of GluA1 recombinant protein in exosomes was quantified using enzyme-linked immunosorbent assay (ELISA) with a GluA1-specific kit, following the manufacturer's instructions. Absorbance was measured at the appropriate wavelength using a microplate reader (data not shown).

Functional Assays:

The functionality of GluA1-loaded exosomes was assessed by co-culturing them with target cells and monitoring changes in cellular responses. Cellular uptake of exosomes was visualized using a fluorescent dye-labeled exosome tracking assay (data not shown).

Behavioral analysis:

All animal experiments were checked and approved by local authorities (taking into account international animal welfare regulations). All behavioral measurements were performed on awake, unrestrained adult mice of both sexes of the same age (>3 months) by persons blinded to the genotype of the mice analyzed. Before analysis, mice were habituated to the experimental setup several times. Nociceptive testing in mouse models of acute and chronic pain was performed as previously described (60).

Formalin test

The animals underwent individual handling for one week prior to the experiment to allow them to acclimate to the experimental setting. Following established protocols (61), the formalin test was administered. Mice were lightly anesthetized with halothane, and then 20 µl of a 5% formalin solution (formaldehyde solution, Sigma-Aldrich; concentration was adjusted with sterile saline) was subcutaneously injected into the dorsal surface of their left hind paw using a 30-gauge needle. Each mouse was then placed in a mirror-backed Plexiglass chamber (25×25×20 cm3) for observation. After a short recovery period (less than 2 minutes), the mice exhibited normal motor function, followed by observation and video recording of both pain and non-pain behaviors for 120 minutes. The duration of these behaviors was analyzed in intervals of 1–5 minutes, 6–10 minutes, and subsequently at 10-minute intervals up to 120 minutes.

Plantar Test and Paw Inflammation

The latency of paw withdrawal in response to noxious heat and pressure gradient was determined using the plantar test with a sensitivity of 0.1 s (n 7–14 per group). Nociceptive thresholds and dimensions of each hindpaw were recorded before and at specified intervals after intraplantar injection of CFA (20 µl). The dimensions of the hind paw were measured using a caliper and a plethysmometer. Paw edema was calculated as the change in paw volume (length-width-height) using a plastinometer as described in detail by Cirinoetal.

Tail Flick Reflex

The nociceptive tail flick reflex was induced by noxious heat applied through an infrared light source with a sensitivity of 0.1 s as previously described. Formalin test and capsaicin test Formalin (1%, 20 L) or capsaicin (0.06%, 10 L) was injected into the plantar surface of the right hind paw and the duration of nocifentive behavior including lifting, licking, or flinching of the paw. the injected paw was measured within 5 minutes after capsaicin injection or at 5-minute intervals for 50 minutes after formalin injection as previously described.

Data Analysis and Statistics

All data are presented as mean SEM. Student's t tests or analysis of variance (ANOVA) for random measures followed by Fisher's postdoc LSD tests were used to determine statistically significant differences ($p < 0.05$).

Results

SNS-GluA1-/-+ exosome mice developed significant mechanical hypersensitivity (reduced von Frey capillary threshold) 4, 12, 24 and 48 hours after CFA injection compared to SNS-GluA1-/- mice.

Our investigation revealed a notable reduction in responses during Phase I of the formalin test in SNS-GluA1-/- animals compared to SNS-GluA1-/-+ exosome mice (Fig. 3). Additionally, the response Phase II is very similar to Phase I, (results not shown).

Discussion

Cellular physiology of exosomes

During exosome formation, the plasma membrane is invaginated and intracellular multivesicular bodies with intraluminal vesicles are formed. This endocytic pathway from the donor cell is followed by transport of transmembrane and intra vesicular proteins from the Golgi complex, leading to the formation of early endosomes. After maturation and differentiation, they become late endosomes. They are degraded by fusion with lysosomes, the plasma membrane or autophagosomes, releasing intraluminal vesicles into the extracellular environment as exosomes (40–150 nm in diameter)(15).

Exosomes interact with recipient cells through their surface receptor molecules and ligands. Some exosomes remain on the cell membranes of donor cells after secretion, while others interact with recipient cells. Internalization of exosomes occurs through a raft- or caveolae-mediated membrane integration process or clathrin-dependent endocytosis (16). Micropinocytosis and phagocytosis have also been described as methods for internalization of exosomes by recipient cells. This process of physiological integration into

target recipient cells is believed to have therapeutic potential as a targeted delivery system to effectively carry out biological functions. However, the exosome components responsible for cell type or organ specificity remain unclear (17).

Therapeutic role of exosomes

Exosomes have great therapeutic potential for various diseases due to their intracellular transport ability. Nanomedicine technologies have given impetus to the study of the use of the pathogenic value of exosome particles in various diseases. Nanomedicine targeted drug delivery system focuses on the sustained release of exosomes to exert biological activity at the target site. Exosomes are used as vectors or carrier molecules to trigger a biological response (18).

Under certain physiological circumstances, exosomes exhibit very low immunogenicity and the ability to bypass the physiological blood-brain barrier. Thanks to the stable lipid bilayer, the cargoes contained in exosome vesicles are protected from the action of native immune cells and digestive enzymes (19). Artificial exosome vesicles transport the cargoes with which they are loaded to the site of action through various mechanisms of endocytosis or membrane fusion (20). When injected into a specific tissue, EVs trigger tissue regeneration and homeostasis under certain conditions. EVs derived from mesenchymal stromal cells exhibit cell viability, cell trophism, anti-inflammatory, immunomodulatory, and therapeutic effects (21).

AMPA receptors

AMPA receptors belong to the family of ionotropic glutamate receptors and are crucial for the transmission of excitatory signals in the brain. This article provides an overview of AMPA receptor structure and function, emphasizing their contribution to synaptic plasticity and their involvement in various neurological disorders (22).

Structure of AMPA receptors

Moreover, these structural insights have unveiled the dynamic nature of AMPA receptors, showcasing conformational changes that occur during various stages of receptor function. The GluA1-GluA4 subunits exhibit unique structural features that contribute to the diversity in their functional roles within the receptor complex (23).

Studies utilizing X-ray crystallography and cryo-electron microscopy have elucidated key interactions between the individual subunits and their binding sites for glutamate, the neurotransmitter that

activates AMPA receptors. GluA2, in particular, plays a crucial role in regulating calcium permeability, impacting the overall signaling properties of the receptor (24).

The intricate architecture of AMPA receptors extends beyond the individual subunits, as auxiliary proteins like TARP (transmembrane AMPA receptor regulatory proteins) and cornichons have been identified as modulators of receptor activity. These auxiliary proteins influence trafficking, synaptic localization, and channel properties, further highlighting the complexity of AMPA receptor function (25).

Understanding the structural dynamics of AMPA receptors has significant implications for pharmacological interventions targeting neurological disorders. Drug design efforts can benefit from precise knowledge of the receptor's three-dimensional arrangement, allowing for the development of compounds that selectively modulate specific aspects of AMPA receptor function (26).

In summary, recent strides in structural biology have unraveled the intricacies of AMPA receptor architecture, emphasizing the importance of the arrangement of GluA1, GluA2, GluA3, and GluA4 subunits in determining the receptor's functional properties. These revelations pave the way for a deeper understanding of synaptic transmission and open avenues for the development of novel therapeutic strategies targeting neurological conditions associated with aberrant AMPA receptor activity (27).

Mechanisms of AMPA Receptor Function: Upon glutamate binding, AMPA receptors undergo conformational changes that lead to channel opening, allowing the influx of cations, predominantly sodium ions. The rapid activation and subsequent desensitization of AMPA receptors contribute to the fast nature of excitatory neurotransmission. Moreover, the regulation of AMPA receptor trafficking and localization is critical for synaptic plasticity, synaptic strength, and learning and memory processes (8).

Synaptic Plasticity and AMPA Receptors: Long-term potentiation (LTP) and long-term depression (LTD) are forms of synaptic plasticity that underlie learning and memory. AMPA receptors play a central role in these processes by modulating the strength of synaptic connections. The dynamic regulation of AMPA receptor trafficking, insertion, and removal from the synapse contribute to the fine-tuning of synaptic strength and plasticity (28).

AMPA Receptors in Neurological Disorders: Dysregulation of AMPA receptor function has been implicated in various neurological disorders, including epilepsy, Alzheimer's disease, and mood disorders. Understanding the molecular mechanisms underlying AMPA receptor dysfunction in these conditions provides potential targets for therapeutic intervention. Modulators of AMPA receptor activity, such as positive allosteric modulators and selective agonists, are being explored as potential treatment options (29).

Therapeutic Implications: Given the crucial role of AMPA receptors in synaptic transmission and plasticity, targeting these receptors holds promise for therapeutic interventions in neurological disorders

(30). Researchers are actively investigating novel compounds and strategies to modulate AMPA receptor function selectively. The development of subtype-specific modulators and precise regulation of AMPA receptor activity may offer more targeted and effective therapeutic approaches (31).

The role of the AMPA receptor in painful sensations. Each subunit contains approximately 900 amino acids and 4 main components: a large amino-terminal extracellular domain, an adjacent ligand-binding domain, a transmembrane domain, and a carboxy-terminal cytoplasmic domain (32). Most native AMPARs are heterothermies, meaning they are made up of a combination of different subunits. The synthesis of AMPAR subunits and their assembly into functional receptors begins in the rough endoplasmic reticulum. A group of proteins called AMPAR transmembrane regulatory proteins (TARPs) facilitate the transport of AMPARs from the endoplasmic reticulum to the plasma membrane and anchor these receptors at the synapse (33). Transport of AMPARs to and from the synaptic membrane occurs in a highly regulated manner. For example, phosphorylation of residue S831 in GluR1 by Ca/calmodulin-dependent protein kinases (CaMKII) and protein kinase C has been shown to result in transport of GluR1 subunits into the synapse. By adjusting the number and type of AMPARs on the synaptic surface, a postsynaptic neuron can modify its excitability, that is, its response to presynaptic signals (34, 8).

Electrophysiological properties of AMPA receptors. Most functional AMPAs are located on the postsynaptic surface. When bound to glutamate, they are permeable to Na and K ions, but usually not to Ca^{2}+ ions (35). Each AMPAR, when open, conducts a miniature excitatory postsynaptic current inward. Each of these small incoming currents depolarizes the cell membrane to a small extent. When enough AMPARs bind glutamate and open, these miniature excitatory postsynaptic currents can sum and create a large depolarizing force, causing the neuron to fire an action potential (36). Thus, AMPAR opening in response to glutamate provides the cellular basis for excitatory synaptic transmission. In addition, a subset of AMPARs, receptors without GluR2 subunits, are Ca2+ permeable. Most of these calcium-permeable AMPARs (CPARs) are composed of GluR1 homo tetramers, but they can also be formed by assembling a combination of GluR1, 3, and 4 subunits. CPARs conduct faster and larger inward currents than AMPARs. impermeable to calcium. CPARs not only exhibit faster and stronger postsynaptic currents, but through Ca2 influx they can also activate Ca2-dependent signaling cascades that lead to long-term changes in synaptic strength. Thus, CPARs act as surrogates for NMDA receptors and likely play a similar role in processes such as memory formation and central sensitization. AMPA receptors are involved in pain sensation (37). Given the critical role of AMPARs in determining the strength of synaptic transmission in various neurological systems, it is not surprising that they are involved in pain transmission. In recent years, animal studies have focused on the first synaptic contact in the pain pathway, namely the synapse between the

primary afferent neuron and the dorsal horn neuron (38). Using sophisticated electrophysiological recordings, the spinal cord neurons expressing AMPARs receive primary afferent inputs of nociceptive origin (39).

The discovery of AMPARs at the synaptic site of the pain pathway is the first step in determining the importance of these receptors in pain (40). The next steps are to identify specific AMPAR changes that occur during pain and show that these changes contribute to the experience of pain. There is known that during acute pain (induced by capsaicin), there is an increase in the number of GluR1 subunits recruited to synaptic sites (41). This is an important finding because the dominant AMPARs in GluR1 tend to be Ca^{2+} permeable receptors, which can trigger long-term cellular changes. According to their model, inflammation caused by capsaicin leads to the transmission of pain signals to the C-fiber neuron in the form of action potentials (42). The flooding of these action potentials is sufficient to recruit CPAR to the synaptic site of the dorsal horn neuron. The accumulation of CPAR in turn induces long-term memory at this synapse between the C-fiber and the spinal neuron, facilitating subsequent pain transmission (43). Thus, CPARs act as surrogates for NMDA receptors to mediate central pain sensitization. Additional evidence for the accumulation of Ca^{2+} -permeable AMPARs during pain conditions comes from studies focusing on chronic pain. Ca^{2+}-permeable AMPARs accumulated at spinal cord synapses in several rodent models of chronic pain (44). After administration of Freund's complete adjuvant, a proinflammatory agent, to the paws of rats or mice, these rodents exhibited long-lasting (2 weeks) mechanical allodynia and thermal hyperalgesia (45). After the onset of chronic pain, GluR1 subunits in spinal cord neurons increase, but also the active part of this subunit (phosphorylated). Thus, chronic pain activates AMPAR GluR1 and recruits it to the cell surface of dorsal horn neurons. Not only did the number of GluR1 subunits increase, but there was also a concomitant decrease in the number of GluR2 and GluR3 subunits at the synapse between the peripheral nociceptive neuron and the dorsal horn neuron (46). Regulation of the soluble factor N-ethylmaleimide fusion protein, a protein required to transport GluR2 subunits to the cell surface, was actually downregulated due to chronic pain. Moreover, GluR2-containing AMPARs can subsequently be internalized or cleared from the synaptic site over time through activation of the NMDA receptor (47). Thus, a complex signaling cascade begins to emerge from these studies. First, chronic pain induces intense AMPAR-mediated synaptic transmission between the peripheral nociceptive neuron and the dorsal horn neuron, activating NMDA receptors and causing Ca^{2+} influx. Ca influx in turn activates a number of downstream signaling proteins, including kinases and other transport proteins, to replace Ca-impermeable AMPARs with Ca^{2+} permeable AMPARs in the cell membrane. Finally, administration of Ca^{2+}-permeable AMPARs allows for increased

Ca^{2+} influx, thereby improving synaptic transmission from peripheral neurons to spinal cord neurons. This pathway partially underlies the mechanism of central sensitization (48).

Modulation of AMPA receptors leads to changes in pain sensitivity

If AMPA receptors are involved in spinal cord pain pathways, and more specifically in the synaptic contact between a nociceptive afferent neuron and a spinal cord neuron, modulation of these receptors should lead to changes in pain sensitivity in animals (49). In fact, researchers have been trying to administer intrathecal glutamate receptor blockers to treat pain for many years. The reason for this approach was to interrupt all synaptic transmission between peripheral nerves and spinal nerves by blocking AMPARs. For example, tezampanel, a nonspecific AMPAR blocker, can be used to reduce mechanical hyperalgesia in a rodent model of inflammatory pain. This treatment model impairing pain transmission—requires chronic administration of the drug (50). However, chronic administration of an AMPAR antagonist results in unacceptable side effects by interfering with normal nociceptive and non-nociceptive sensory transmission and motor functions. In addition, these drugs can penetrate the cerebrospinal fluid and disrupt synaptic transmission in the brain (51). However, recent studies on the role of CPARs in the induction and maintenance of central sensitization have shed new light on the therapeutic potential of AMPAR blockade (52). Therefore, therapeutic AMPAR blockade may require a different strategy aimed at disrupting the molecular mechanisms of central sensitization rather than disrupting complete synaptic transmission. This strategy may only require proactive blocking of signaling events that lead to accumulation of CPARs or selective antagonism of CPARs themselves. In support of this strategy, the examined pain perception in mice carrying genetically modified GluR2 as part of an investigation into the mechanism of central sensitization in the spinal cord (53). Genetically modified GluR2 subunits to render these receptors unable to be internalized. Consequently, these mutated GluR2 receptors remained on the cell membrane longer and displaced GluR1 receptors. Remember that CPARs require the absence of GluR2 and the presence of GluR1 (54). This mutation essentially results in a decrease in the amount of CPAR on the cell surface. Interestingly, but perhaps unsurprisingly, rodents with this mutation exhibited less chronic pain. Using a different genetic approach, examined the effect of selective deletion of GluR1 or GluR2 on the acute pain threshold in mice (55) for transmission signs of acute pain. However, in a model of chronic inflammatory pain, genetic deletion of GluR1 subunits in mice resulted in a higher pain threshold, and deletion of GluR2 had the opposite effect (56). Because GluR2 is Ca2-permeable without AMPARs, these genetic data suggested that altering the number of Ca-permeable AMPARs at synaptic surfaces may alter pain transmission (57). The difference lies in the chronic nature of the pain. Although CPARs are interesting for acute pain signaling, they are likely to play an important role in chronic pain due to their influence on central sensitization.

Conclusion:

In conclusion, our study delves into the intricate interplay between α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–type (AMPA-type) glutamate receptors (AMPARs) and exosomes in the modulation of inflammatory pain. We addressed the previously known functional role of AMPARs in the peripheral nervous system, particularly within nociceptors, by using mice with a specific deletion of the key AMPAR subunit, GluA1, in these pain-sensing neurons while preserving central nervous system expression.

Our findings revealed that exosomal delivery of GluA1 in nociceptor-specific deleted of GluA1 mice led reverse effect to disruptions in calcium permeability and diminished capsaicin-evoked activation of nociceptors. This, in turn, resulted in diverse effect ot reduced mechanical hypersensitivity and sensitization in models of chronic inflammatory pain and arthritis. Interestingly, the generation of exosomes containing GluA1 and their introduction around nociceptors produced a reverse effect compared to GluA1 deletion, rendering mice treated with these exosomes more sensitive to pain.

This study not only sheds light on the critical role of GluA1-containing AMPARs in nociceptors but also introduces exosomes as potential modulators of pain sensitivity. The observed changes in nociceptive responses highlight the dynamic nature of AMPARs in the pain pathway and offer insights into potential therapeutic interventions for chronic inflammatory pain states. Our work emphasizes the need for further exploration into the precise mechanisms underlying the interaction between AMPARs and exosomes, paving the way for innovative strategies in the treatment of chronic pain conditions.

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