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Role of mitoflash in synaptic plasticity during chronic low back pain

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Chronic non-specific low back pain (LBP) is one of the most burdensome health problems worldwide, the mechanism of its development still is not fully understood. Previous studies have revealed that there are neuroplastic changes at the level of the spinal cord in an animal model of nonspecific LBP that leads to a latent sensitization of dorsal horn neurons that may become manifest upon a second challenge, in which intramuscular injection with NGF appears to be a primary driver. This latent sensitization seems to be a first step in the transition from acute to chronic LBP. This transition comes along with activation of protein kinases, calcium influx and long-term structural and functional changes of ion channels in neuronal cells.

Several members of the transient receptor potential (TRP) superfamily, which are cation channels with high permeability for calcium, have the pivotal function as primary molecular sensors in nociceptive neurons, directly participate in transforming external stimulation into pain. A member of the melastatin subtype of TRP channel (TRPM3), known as a heat sensor involved in noxious heat sensation and inflammatory thermo-hyperalgesia, can also be activated by hypotonic cell swelling and neurosteroids (PregS), suggesting TRPM3 channel as a polyfunctional sensor with remarkable capability to detect a spacious spectrum of stimulus modalities containing those with physical and chemical nature. In terms of central synaptic plasticity implicated in learning and memory, TRPM3 has been proposed to serve as necessary intermediate regulator mediating PregS-induced increased glutamatergic transmission and postsynaptic AMPARs insertion, implying a propensity for TRPM3 to be a potential modulatory candidate of pain sensitization and underlying the hypothesis that the TRPM3-dependent and calcium-engaged mechanisms of nongenomic action of PregS in the nervous system may be involved in NGF-induced latent sensitization of chronic non-specific LBP. Aside from acting as the potent agonist for TRPM3, PregS injection has been shown to result in nocifensive behavioral responses in mice, suggesting the nocifensive nature of PregS. The serum level of PregS has been shown to elevate under stress that has been proposed to predispose non-specific LBP. The pain stress could in turn promote the release of PregS and further aggravated PregS-induced nociceptive sensation. This vicious circle might be another potential mechanism of non-specific LBP. The present study focuses on the DRG neuron that is the primary sensory neuron with outstanding regulation where pain messages are initiated, becoming an important regulatory site for synaptic plasticity at the presynaptic level.

Despite the extensive knowledge regarding the participation of non-canonical mitochondrial functions including calcium handling, redox signaling, and apoptosis in pathogenesis of prominent neurological diseases such as stroke, amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and Huntington's diseases, the mitochondrial functions in synaptic plasticity implicated in sensory processing and pain sensitization has been still under limited investigation in the past decades. Findings have shown that presynaptic mitochondria maintain synaptic transmission by calcium sequestration. Mechanistically, the increased calcium influx into presynaptic bouton through the specific calcium channels triggers glutamate release and excitatory postsynaptic currents (EPSCs), where the elevated presynaptic calcium is fast sequestered by local mitochondria, which triggers the subsequent release of mitochondrial calcium via mitochondrial Na^+/Ca^{2+} exchange, prolonging the calcium elevation in presynaptic terminals and leading to further glutamate release and EPSCs. Studies focusing on post-synaptic plasticity have shown that mitochondrial calcium uptake serves as the first step for the N-methyl-D-aspartate receptor (NMDAR)-mediated spinal cord synaptic plasticity, in which high levels of calcium must first be sequestered by mitochondria rather than directly trigger the spinal LTP. The recent research has indicated that the dynamic activity of mitochondria, known as mitoflash that is a short event consisting of mitochondrial membrane potential depolarization, reactive oxygen species production, and alkalization in the matrix, showing the spatiotemporal specific correlation with structural changes of

dendritic spines in the hippocampus, participates in the stabilization of the structural LTP of spines. Despite above advances, whether mitoflashes can be a downstream effector of TRPM3 activation that might be one mechanism of NGF-induced latent sensitization during non-specific LBP is still unknown. The mitochondrial function implicated in presynaptic mechanisms involved in pain sensitization are also rarely explored.

The major aim in the current study is to address TRPM3-associated mechanisms of nerve growth factor (NGF)-induced latent sensitization of non-specific LBP, focusing on the presynaptic plasticity at the level of DRG neurons. The issues to be solved include (1) whether NGF can directly sensitize TRPM3-mediated response of DRGs to PregS challenge? (2) whether mitochondria take a role in the regulation of TRPM3-mediated calcium transients? (3) whether mitochondria can be a downstream target of TRPM3 activation and show bioenergetic and dynamic change upon PregS challenge? By NGF preincubation (50 ng/mL, 15-40 hrs) and acute NGF treatment (100 ng, 10 min) on DRGs, we established a surrogate model, aiming to simulate the effect of twice intramuscular NGF injection in animal models of non-specific LBP. We specifically target TRPM3-mediated calcium transients of DRGs upon PregS challenge, using calcium imaging and confocal imaging approaches, to investigate the sensitization of NGF on the response of DRGs to PregS. TRPM3-expressing HEK cells (HEK-TRPM3 cells) and mt-cp-YFP transient transfected HEK-TRPM3 cells were used to investigate spatiotemporal heterogeneity of mitochondrial calcium accumulation and bioenergetic and dynamic change of mitochondria following the TRPM3 activation.

In the present study, NGF preincubation (50 ng/mL, 15-40 hrs) enhanced initial response of DRGs to PregS (50 μM) from 0.38 to 0.46 (amplitude, $*p=0.17$), which was more pronounced for AUC from 4.93 to 6.00 ($*p=0.13$). While no significant upregulation in the fraction of TRPM3-expressing DRGs from 61.4% to 63.7%, nor the fraction of TRPV1-expressing DRGs from 91.4% to 88.9%, NGF preincubation accelerated the calcium influx through TRPM3 by 37% (rising velocity, from 0.19 to 0.26, $*p<0.05$) and slowed the intracellular calcium clearance by 31% (falling velocity, from -0.16 to -0.11, $*p<0.05$). Acute NGF treatment (100 ng, 10 min) attenuated the tachyphylaxis to repeated application of PregS (50 μM) from 47% to 29% (amplitude reduction, $*p=0.034$), which was more pronounced for AUC from 46% to 12% ($*p=0.001$). Acute NGF treatment also prolonged the rise time of PregS-induced calcium transients from 2.3 to 2.7 min ($*p=0.046$), slowed the intracellular calcium clearance by 50% (falling velocity, from -0.12 to -0.06, $*p<0.05$). In DRGs, PregS (50 μM)-induced calcium transients drove mitochondrial calcium sequestration followed by a steady-state mitochondrial calcium elevation (more than 10 mins). Compared to KCl (50 mM), PregS caused more mitochondrial calcium loading (41% vs. 20%, $*p=0.045$), which was comparable to Caffeine (20 mM) (buffer capacity, 41% vs. 42%, $p=0.977$). In TRPM3-expressing HEK cells, PregS (10 μM)-induced calcium transients caused a steady-state mitochondrial calcium elevation but failed to evoke the increased production of reactive oxygen species (ROS) that was effectively evoked by PregS (50 μM). PregS (50 μM) also significantly increased mitoflash events from 0.57 to 3.90 events/100 s/cell (frequency, $*p<0.001$), which was TRPM3-mediated and calcium-engaged.

The results indicate that NGF can acutely and directly sensitize the response of sensory neurons to PregS, which is TRPM3-mediated. The mechanisms may partly involve the functional upregulation of TRPM3 by NGF, which is mainly evidenced by accelerating the calcium influx by NGF preincubation and prolonging the rise time of PregS-induced calcium transients by acute NGF treatment. The post-translational modulation on calcium buffer by NGF preincubation and acute NGF treatment may participate in the sensitization of sensory neurons as well, evidenced by slowing the intracellular calcium clearance. For extracellular calcium influx-induced mitochondrial calcium sequestration, PregS is particularly effective for mitochondrial calcium loading, evidenced by higher buffer capacity than KCl. This mitochondrial calcium loading is a prerequisite for the signal transduction downstream TRPM3 activation, which involves increased ROS production and upregulation of mitoflash incidence by PregS.

To sum up, the present study suggests that TRPM3-mediated and mitochondrial-buffered calcium transients in DRGs are involved in the latent sensitization of non-specific LBP, where NGF-induced sensitization of the response to PregS, which is TRPM3-mediated, might be one of the earliest events initiating synaptic plasticity implicated in pain sensitization, especially at the presynaptic level. Beyond the traditional calcium modulation involving the mitochondrial function on delaying post-stimulus recovery and maintaining long-term cytosolic calcium signals, mitochondrial calcium regulation can take the form of frequency-encoded mitoflashes, as one of downstream mechanisms of TRPM3 activation, which might participate in pain sensitization and be a new sight toward the investigation of chronic non-specific LBP.