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# A Preliminary Investigation of the Mechanism of *P2RX3*-mediated Central Sensitization in Migraine

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***Abstract:*** Migraine is a prevalent neurological disorder affecting approximately 15% of the global population, but its pathogenesis has not yet been elucidated. Migraine pathogenesis is importantly associated with central sensitization, and *P2RX3* plays a key role in central sensitization. In this study, we investigated the mechanism of *P2RX3* in central sensitization of migraine, with the aim of providing a new theoretical basis for migraine treatment.

***Keywords:*** Migraine; Central sensitization; *P2RX3*; CGRP; cAMP/MAPK/CREB

## 1. Introduction

*P2RX3* receptor has an important role in central sensitization of migraine, and the study of the molecules involved in its upstream and downstream signaling pathways may be the basis for investigating the pathogenesis of migraine and the development of new therapeutic agents. In this study, We explored the role of *P2RX3* in central sensitization of migraine using a mouse model of nitroglycerin migraine to further investigate the mechanism of *P2RX3*-mediated central sensitization in migraine.

## 2. Materials and methods

### 2.1 Animals

Twenty SPF-grade male C57BL/6J WT mice, weighing 25±5 g, aged 8-10 weeks, were purchased from Jiangsu Jicui Pharmachem Biotechnology Co.

### 2.2 Methods

(1) Construction of a mouse model of chronic migraine

On the day of the experiment, 20 mice were randomly divided into two groups of 10 mice each. The experimental group was given 10 mg/Kg nitroglycerin intraperitoneally, and the control group was given an equal volume of 0.9% saline, and nitroglycerin or saline was injected intraperitoneally for 9 consecutive days.

(2) Determination of pain threshold in the hind paw of mice

Changes in mechanical pain thresholds were recorded in each group of mice using the Von-Frey fiber wire test and hot plate.

(3) Real-time fluorescence quantitative PCR

Two hours after the end of the last injection of nitroglycerin or saline, 10 mice were randomly selected from 20 mice to be decapitated and processed, and stored in the refrigerator at -80°C after rapid stripping of TNC and TG tissues on ice. Total RNA was extracted using TRIzol® Reagent. The primer sequences were as follows:

| GENE | FORWARD(5'-3') | REVERSE(5'-3') |
|---|---|---|
| CGRP | TTTGAGGTCAATCTTGGAAAGCA | CTGAGCAGTGACACTAGAGCC |
| P2RX3 | AAAGCTGGACCATTGGGATCA | CGTGTCCCGCACTTGGTAG |
| TAC1 | AAGCGGGATGCTGATTCCTC | TCTTTCGTAGTTCTGCATTGCG |
| TACR1 | CTCCACCAACACTTCTGAGTC | TCACCACTGTATTGAATGCAGC |
| CRTC1 | CTATGGCACCGTGTACCTCTC | GGCTGGGTGTCATTGTGCT |
| MAPK8 | AGCAGAAGCAAACGTGACAAC | GCTGCACACACTATTCCTTGAG |
| CACNA1G | TGTCTCCGCACGGTCTGTAA | AGATACCCAAAGCGACCATCTT |
| GAPDH | CATGGCCTTCCGTGTTCCTA | CCTGCTTCACCACCTTCTTGAT |

# 3. Results

## 3.1 Repeated administration of nitroglycerin induces nociceptive sensitization in mice

As shown in Figure 1, repeated nitroglycerin administration resulted in a significant decrease in hindfoot pain threshold in mice, suggesting that we successfully constructed a nitroglycerin migraine mouse model.

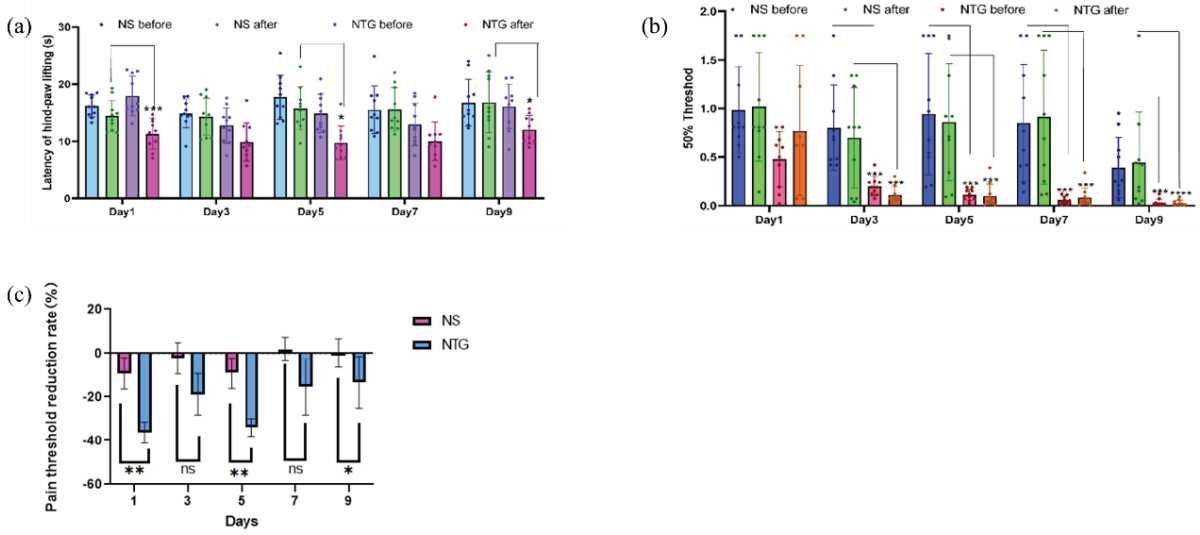



**Fig.1. Repeated NTG administration leads to hindfoot nociceptive sensitization in mice.**

**NS: 0.9% saline group; NTG: nitroglycerin group**

## 3.2 Repeated NTG administration leads to elevated levels of *P2RX3* expression in TNC and TG of mice

As shown in Figures 2, 3, repeated nitroglycerin administration resulted in significant upregulation of mRNA levels of genes such as *P2RX3* in the TNC/TG region of mice.

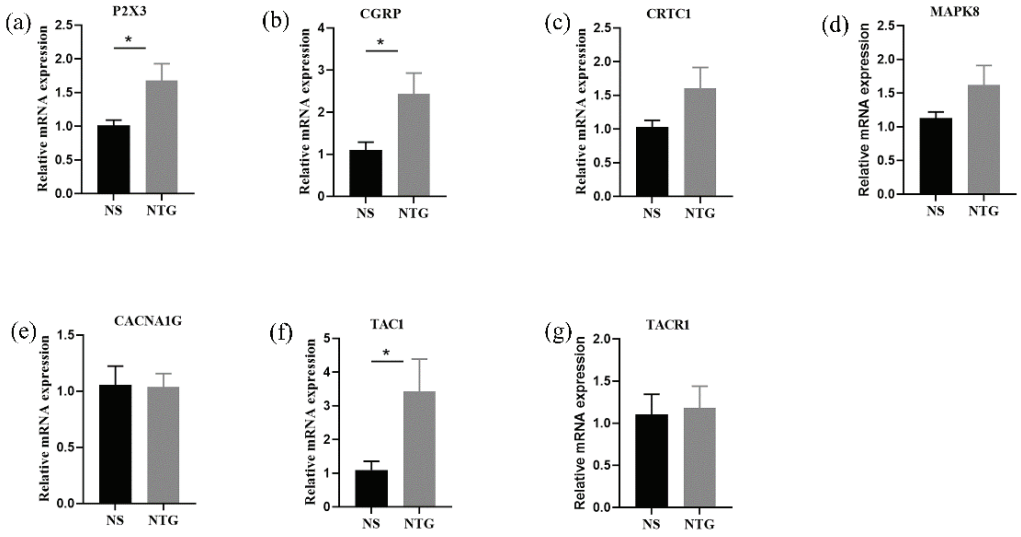
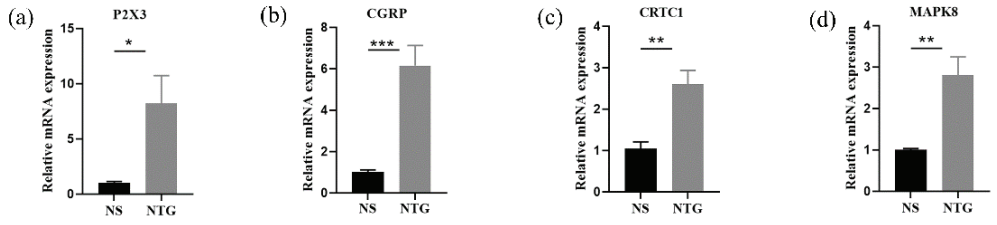



**Fig.2. Gene expression in TG after repeated NTG administration.**

**Data represented as mean ± standard error, n=5. *p < 0.05 vs control group**



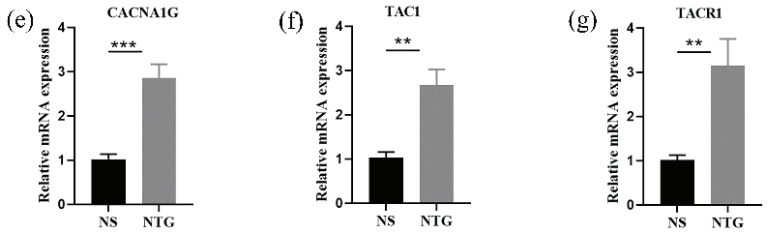



**Fig.3 Gene expression in TNCs after repeated NTG administration.**

**Data represented as mean ± standard error, n=5. *p < 0.05 vs control group**

## 4. Discussion

It has been confirmed that *P2RX3*, as a purinergic receptor mainly responsible for ATP signaling on sensory nerves, senses changes in extracellular ATP concentration and gates non-selective opening of its own cation channels, allowing Na+, K+, Ca2+ to pass through, with the most prominent effect being the increase in Ca2+ inward flow. When the organism is injured or nerve damage releases a large amount of ATP, activates the presynaptic membrane *P2RX3* receptor, causing a large amount of Ca2+influx, the increase in intracellular calcium concentration activates protein kinase A (protein kinase A, PKA), protein kinase C (protein kinase C, PKC), which leads to the phosphorylation of PKA, PKC, and at the same time, promotes the release of glutamate, which further activates NMDA receptor, and further activates NMDA receptor. release, which further activates NMDA receptors, leading to the generation of excitatory postsynaptic currents and causing central sensitization.

The cAMP signaling pathway is a cyclic nucleotide pathway, which is the main component of the cyclic nucleus. The cAMP signaling pathway is a kind of cyclic nucleotide signaling pathway, and the activation of cAMP signaling pathway is closely related to the signaling of extracellular hormones and neurotransmitters, and regulates the transmembrane ion transport in the cell membrane when activated.

Migraine mouse models are constructed with a variety of methods, and the nitroglycerin migraine mouse model is one of the more commonly used models. In this study, a nitroglycerin migraine mouse model was established, and the results showed that after 5 injections of nitroglycerin into the mice, the mechanical and thermal pain thresholds of the experimental group of mice were significantly reduced, which indicated that nitroglycerin caused nociceptive sensitization in the mice, suggesting that the success of in vitro constructed a migraine mouse model, which can be used for subsequent studies.

Calcitonin gene-related peptide, as a peptide neurotransmitter widely distributed in the central nervous system. Numerous studies have shown that exogenous injection of CGRP can trigger persistent migraine-like headache, suggesting that CGRP plays a key role in the pathomechanism of migraine and is a key neurotransmitter for pain signaling[1, 2]. TG neurons releasing of CGRP can increase the activity of purinergic gated channels in pain signaling through a paracrine manner. The specific pathway is summarized as CGRP increases ATP-gated purinergic P2Y receptor signaling in satellite glial cells and *P2RX3* receptors in other neurons[3]. CGRP can activate *P2RX3* in two ways, either directly by acting on the neuron and initiating the cAMP signaling pathway cascade to activate the *P2RX3* gene, or indirectly, by activating the *P2RX3* gene in the satellite glial cells first[4]. neurotrophic brain-derived neurotrophic factor (BDNF) genes in glia, and the release of BDNF genes stimulates *P2RX3* expression in neurons [5]. Signaling feedback from BDNF or *P2RX3* receptors may lead to increased CGRP synthesis, which activates pathways that increase CGRP transcription[6, 7]. The interaction between purinergic receptors and CGRP promotes depolarization of trigeminal afferents and transmission of injurious stimuli, which in turn ultimately leads to central sensitization of migraine.

Substance P (SP) encoded by the *TAC1* gene is widely distributed in the mammalian CNS and peripheral nervous system as well as the enteric nervous system, and its biological functions are mainly mediated through the high-affinity neurokinin 1 receptor (NK-1R)[8]. SP/NK-1R plays an important role in the pathophysiological processes of many diseases, such as pain, infectious and inflammatory diseases, and cancer. SP is considered to be the most potent neurogenic inflammatory trigger due to its association with increased vascular permeability as well as plasma protein extravasation [9]. It also enhances inflammation by stimulating the production of inflammatory mediators such as histamine, NO, cytokines, and kinins, as well as interacting with adhesion molecules leading to leukocyte migration[10]. *CRTC1* is a CREB-regulated transcriptional coactivator, which plays an important role in the fulfillment of CREB's biological functions.

A migraine mouse model was established by nitroglycerin, and total RNA from TNC and TG tissues of mice in each group was extracted for qPCR experiments. The results showed that nitroglycerin injection resulted in up-regulation of CGRP expression in mice, while the mRNA levels of *P2RX3, CGRP, TAC1, TACR1, CACNA1G,* and *CRTC1* genes were significantly elevated. The combination of previous studies in the literature and our experimental results suggests that administration of nitroglycerin injections leads to the production of migraine, which

triggers an increase in the release of CGRP. The *P2RX3* receptor is sensitized to extracellular ATP in the presence of CGRP and has increased synthesis and translocation to the cell membrane. High expression of the *P2RX3* receptor leads to an increase in the opening of voltage-gated calcium channels controlled by voltage gates, which further enhances calcium Ion endocytosis. The increase in calcium inward flow activates adenylate cyclase to increase the intracellular cAMP concentration, which then activates the MAPK signaling pathway and phosphorylates and activates CREB; on the other hand, it triggers the activation of CaM kinase through the binding of calcium-binding proteins, which leads to the downstream phosphorylation of CREB. Activation of the MAPK/CREB signaling pathway may cause the expression of nociception-related genes such as *TAC1, TACR1, P2RX3,* and *CGRP*, which in turn enables the transcription of pain-mediated genes, further enhances *P2RX3*-mediated nociceptive transmission, and ultimately induces central sensitization of migraine.

## 5. Conclusion

*P2RX3* contributes to central sensitization of migraine by receiving CGRP signaling, increasing cell membrane calcium-gated channels to promote increased calcium inward flow, regulating activation of the cAMP/MAPK/CERB signaling pathway, and ultimately upregulating the expression of pain-related genes such as *TAC1* and *TACR1*.

## References


[1] ZUPAN M, ZALETEL M, VISOCNIK D, et al. Calcitonin gene-related peptide-induced hemodynamic changes in migraine with and without aura [J]. Acta Neurol Scand, 2021, 144(6): 616-22.

[2] GAETE P S, LILLO M A, PUEBLA M, et al. CGRP signalling inhibits NO production through pannexin-1 channel activation in endothelial cells [J]. Sci Rep, 2019, 9(1): 7932.

[3] CERUTI S, VILLA G, FUMAGALLI M, et al. Calcitonin gene-related peptide-mediated enhancement of purinergic neuron/glia communication by the algogenic factor bradykinin in mouse trigeminal ganglia from wild-type and R192Q Cav2.1 Knock-in mice: implications for basic mechanisms of migraine pain [J]. J Neurosci, 2011, 31(10): 3638-49.

[4] GINIATULLIN R, NISTRI A, FABBRETTI E. Molecular mechanisms of sensitization of pain-transducing P2X3 receptors by the migraine mediators CGRP and NGF [J]. Mol Neurobiol, 2008, 37(1): 83-90.

[5] RUSSO A F, HAY D L. CGRP physiology, pharmacology, and therapeutic targets: migraine and beyond [J]. Physiol Rev, 2023, 103(2): 1565-644.

[6] ZHANG Z, WINBORN C S, MARQUEZ DE PRADO B, et al. Sensitization of calcitonin gene-related peptide receptors by receptor activity-modifying protein-1 in the trigeminal ganglion [J]. J Neurosci, 2007, 27(10): 2693-703.

[7] HAANES K A, LABASTIDA-RAMIREZ A, BLIXT F W, et al. Exploration of purinergic receptors as potential anti-migraine targets using established pre-clinical migraine models [J]. Cephalalgia, 2019, 39(11): 1421-34.

[8] PENG L, AGOGO G O, GUO J, et al. Substance P and fibrotic diseases [J]. Neuropeptides, 2019, 76: 101941.

[9] HOLZER P. Neurogenic vasodilatation and plasma leakage in the skin [J]. Gen Pharmacol, 1998, 30(1): 5-11.

[10] CORRIGAN F, VINK R, TURNER R J. Inflammation in acute CNS injury: a focus on the role of substance P [J]. Br J Pharmacol, 2016, 173(4): 703-15.