Targeting P2X3 receptor signaling to attenuate chronic pain

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Research areas of interest

• Ion channel function and expression
• Plasticity of ion channels under chronic pain states
• Potential targets for pain therapy
**ATP-gated receptor family**

- **P2Y receptors:** Metabotropic receptors  
P2Y1, P2Y2, P2Y3, P2Y4, P2Y5, P2Y6

- **P2X receptors:** Ionic Receptors
  - Homomeric: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, P2X7 (P2Z)
  - Heteromeric: P2X2/3, P2X4/6, 2X1/5
Structure of P2X receptors

- Two transmembrane domains
- Separated by an extracellular domain (approximately 280 amino acids).
- Channels form as multimers of several subunits

Ion Permeability

Small monovalent cation (Na\(^+\), K\(^+\))
Some to Ca\(^{2+}\) or anions
## Agonists and Antagonists

### Agonists
- ATP (for P2X and P2Y receptors)
- $\alpha,\beta$-m-ATP (specific for P2X3 and P2X2/3 receptors)

### Antagonists
- Suramin & PPADS (for P2X and P2Y receptors)
- TNP-ATP, A317491 (potent for P2X3 and P2X2/3 receptors at low dose)
Three Questions

1. Does inflammation/injury sensitze the P2X receptors?
2. What are the possible mechanisms underlying these changes?
3. Is it possible that targeting P2X receptor signaling suppresses pain?
Peripheral inflammation sensitizes P2X receptors

Xu & Huang, J Neurosci., 2002
Effect of nociceptive electrical stimulation

Xu & Huang, PNAS, 2004
Potentiation of ATP-induced currents in IBS-like rats with colonic hypersensitivity

Xu et al., GUT, 2008
Increase in expression of P2X3R in DRGs

A

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<thead>
<tr>
<th></th>
<th>TL DRG</th>
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<th>LS DRG</th>
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<tbody>
<tr>
<td></td>
<td>CON</td>
<td>AA</td>
<td>CON</td>
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<tr>
<td>P2X1</td>
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<td>P2X3</td>
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<tr>
<td>Actin</td>
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B

Relative densitometry

P2X1: CON 1.0, AA 1.6
P2X3: CON 1.2, AA 2.1

C

D

Relative densitometry

P2X1: CON 1.3, AA 2.0
P2X3: CON 1.5, AA 2.5

* Indicates significant difference between CON and AA
Upregulation of P2X3R mRNA Expression in DRG Neurons

Laser Capture Micro-dissection

RT-PCR
Reduction in Nocifensive Behaviors by P2X3R Antagonist in IBS rats

**AWR scores**

1. Normal behavior without response
2. Contraction of abdominal muscles
3. Lifting of abdominal wall
4. Body arching and lifting of pelvic structures

Xu et al. Gut 2008
Peripheral inflammation, nociceptive electric stimulation or Neonatal colonic inflammation sensitizes the P2X receptor function mediated by upregulation of P2X receptor expression in DRGs
DNA demethylation and transcription
p2x3r gene
(ATP受体)

MSP

BSP

Pain, 2015
p2x3r gene
(ATP受体)

Diabetes, 2015
Biochemical pathways for cytosine methylation & demethylation

S-adenosylhomocysteine → DNA Methyltransferase → 5-methyl Cytosine

Demethylase → 5-mC → DNA Hydrolytic D-amination

DNA METHYLATION

Cytosine → Thymidine

Repair by Uracl DNA Glycosylase

Uracil → DNA Replication

Singal R, Ginder G D Blood 1999;93:4059-4070
Downregulation of DNMT3b

A

B

Fold Increase

DNMT3a  DNMT3b

Gadd45a  MBD4  MBD2  TDG
Enhanced binding ability with p65 in diabetic pain
Upregulation of p65 and P2X3Rs

- A: Western blots showing upregulation of p65 and P2X3Rs in DM compared to CON.
- B: p65 mRNA levels in CON and DM.
- C: DM rats showing increased PWT (g).
- D: PWL (s) in DM compared to NS.
- E: CON rats showing increased PWT (g).
- F: PWL (s) in CON compared to NS.
- G: P2X3 mRNA levels in NS and PDTC.
- H: P2X3 mRNA levels in NS and PDTC.
Attenuation of pain by siRNA-mediated inhibition of P2XR expression
Working hypothesis

Physiological conditions

$p2x3r$ gene promoter

CpG island

Exon1

Methylated $\rightarrow$ Unmethylated

P2X3R expression

Pain
Working hypothesis

Diabetic Neuropathy → DNMT3a, DNMT3b → \(\text{M} \rightarrow \text{U} \rightarrow \text{CpG island} \rightarrow \text{p2x3r gene promoter} \rightarrow \text{Exon1} \rightarrow \text{P2X3R expression} \rightarrow \text{Pain} \)

\[\text{M} \text{ Methylated} \quad \text{U} \text{ Unmethylated}\]
Molecular Mechanisms Underlying Pain in Diabetic Neuropathy Uncovered

Pain associated with diabetes, and yet another major cellular target for the anxiolytic drug gabapentin and pregabalin, which are commonly used to relieve diabetes-induced pain in humans and other animals. However, more than 50% of patients using gabapentin or pregabalin experience side effects, such as excessive sedation, ataxia, dizziness, euphoria, and weight gain, all of which limit its clinical use (1).

Several studies conducted in recent years have reported on the plasticity of various ion channels expressed in DRG neurons in the peripheral nervous system. In this issue, a study by Zhang et al. (12) sheds more light on the issue of abnormal regulation of nociceptive ion channels in sensory neurons in animal models of type 1 diabetes. Here, they investigate the role of nuclear factor-κB (NF-κB) in the regulation of purinergic receptor P2X ligand-gated ion channel 3 (P2X3R) plasticity in DRG neurons in rats with painful PN. First, they show that the injection of nonspecific purinergic receptor antagonists suramin and dipyridamole into the spinal cord of diabetic rats decreases nociceptive behavior, as shown in the merge of P2X3+/Dil staining and Dil.
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Welcome To Visit Suzhou
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Residential houses