Expression and function of proton-sensing G-protein-coupled receptors in inflammatory pain

Chia-Wei Huang, Ying-Ju Chen, Wen-Han Chang and Wei-Hsin Sun
Department of Life Sciences, National Central University, Chungli, Taiwan

Introduction

Inflammation often causes hypersensitivity to mechanical and thermal stimuli in the area around the wound. This phenomenon is partially due to the production and release of chemical mediators (e.g., prostaglandins, kinin, histamine, etc.) from the primary sensory terminal and from non-neural cells in the environment [14,15]. High local proton concentrations found in inflamed tissues (tissue acidosis) contribute directly to pain and hyperalgesia. Several lines of evidence have demonstrated that proton-sensing ion channels are related to acid-associated pain [1-7]. While the functions of proton-sensing G-protein-coupled receptors (GPCRs) in inflammation remains unclear. Proton-sensing GPCRs consisting of four members - ovarian cancer G-protein-coupled receptor 1 (OGR1), GPR4, G2A and T-cell death associated gene 8 (TDAG8), respond to proton stimulus with full activation at pH 6.4 – 6.8 [8-11]. These four receptors were found in DRG, and most (75%–82%) are present in small-diameter neurons that are responsible for nociception [12,13]. More than half of these genes are expressed in IB4-positive neurons that are involved in inflammatory or neuropathic pain. In this study, we try to investigate the role of proton-sensing GPCRs in inflammation.

Results

CFA induces mechanical and thermal hyperalgesia

Figure 1. Mechanical and thermal nociceptive responses were measured after CFA injection. Paw withdrawal threshold and latency declined significantly in ipsilateral paw of injected mice at 4 hours after injection and remained for 72 hours.

Up-regulation of TDAG8 gene after CFA injection

Figure 2. To investigate the change in gene expression levels of OGR1 family in DRG after CFA injection, quantitative polymerase chain reaction (Q-PCR) method was used. At 4 hours post injection, G2A gene was down regulated (2-3-fold), and the other three members remained the same expression levels. At 24 hours, only transcripts of TDAG8 increased significantly (2.5-4.3-fold), but the level was reduced at 72 hours (1.2-1.6-fold).

DRG neurons expressing TDAG8 increase in number with CFA-induced inflammation

Figure 3. The in situ hybridization method was used to investigate whether the expression pattern of TDAG8 changes in DRG neurons. After hybridization, the sections were co-stained with various antibodies against peripherin, N52 or Isletin B4 (IB4).

(A) After CFA injection, 27 ± 2% of total neurons expressed TDAG8 on the contralateral DRG, whereas the number was increased to 38 ± 2% on the ipsilateral DRG. The distribution of TDAG8-expressing neurons shifted slightly to a population of N52-positive neurons after CFA injection (10 ± 3% to 17 ± 2%). (B) TDAG8-expressing neurons were increased in number in both IB4-positive (21 ± 4% to 33 ± 4%) and IB4-negative (12 ± 3% to 21 ± 4%) neurons.

TDAG8 activation sensitizes TRPV1 response to capsaicin

Figure 4. (A) The role of TDAG8 in inflammatory pain is unclear, likely regulating other molecules (such as TRPV1) to influence mechanical or thermal hyperalgesia [1-7]. We further tested whether TDAG8 activation sensitizes the TRPV1 response to capsaicin. The addition of 5 nM capsaicin (0.001 ± 0.001 μmol/L) did not induce a significant response in TRPV1-expressing cells. (B) The enhanced to capsaicin by pre-treatment of pH 6.4 (3.0 ± 0.3-fold) slightly increased (Ca2+) in TRPV1-expressing cells (0.09 ± 0.01). The increase in the expression of both TDAG8 and TRPV1 induced a 5-fold enhanced (Ca2+) response (0.12 ± 0.03). The enhanced response also observed in the samples pre-treated by acid buffer (pH 6.4) at room temperature (0.074 ± 0.061; 3-fold increase). (B) The enhanced to capsaicin by pre-treatment of acid (pH 6.4) was detected in primary culture.

Conclusions

1. The number of TDAG8-expressing neurons increased in both IB4-positive and IB4-negative subpopulations.
2. Co-expression of TDAG8 and TRPV1 enhances [Ca2+]i response to low dose of capsaicin.
3. The [Ca2+]i responses to low dose of capsaicin were also increased in both IB4-positive and IB4-negative neurons after CFA injection, implying that initiation of TDAG8 might sensitize TRPV1 when inflammation.

References


Acknowledgements

We thank the staff of the biophysics and soft matter at National Central University to support the calcium imaging system.