

## Editorial



## One Door Opens, One Door Closes —Yielding Path to Bradykinin-Induced Pain

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It has been appreciated for some time that bradykinin (BK) is a significant endogenous mediator of inflammation (1) as well as a potent nociceptive mediator which may contribute to pain (2,3). Bradykinin is a 9-amino acid peptide which is clipped by the serine protease kallikrein from a 110-kDa precursor plasma  $\alpha$ -globulin known as kininogen. Tissue insult leads to the conversion of an inactive precursor, prekallikrein, to kallikrein, a process which is accelerated by clotting factor XII (Hageman factor) in the presence of negative charges (present on endothelial cell surfaces) (4). BK tends to act at sites where it is locally formed, as it is rapidly inactivated in the plasma and lungs by peptidases.

The hypersensitivity induced by BK is believed to occur via  $B_2R$  stimulation with activation of the G protein  $G_q$ , the  $\alpha$ -subunit of which activates phospholipase C in the cell membrane.  $PLC_\beta$  cleaves phosphatidylinositol 4,5 bisphosphate ( $PIP_2$ ) into inositol triphosphate ( $IP_3$ ), which contributes to BK-induced acute pain, and diacylglycerol (DAG) which contributes to BK-induced hypersensitivity. DAG subsequently activates calcium-independent protein kinase  $C_\epsilon$  ( $PKC_\epsilon$ ) with resultant phosphorylation and enhanced thermal sensitivity of TRPV1 channels.

$IP_3$ , on the other hand, is involved with acute BK-induced pain provocation, but the precise mechanisms by which this occurs have been uncertain. Recently Liu et al (5) have shed light on the molecular mechanisms which appear to underlie BK-induced pain. Liu et al (5) appear to have uncovered 2 events which largely explain the major mechanisms of acute nociceptive signaling induced by bradykinin.  $IP_3$  releases calcium ions from the endoplasmic reticulum resulting in a rise in intracellular calcium concentration. This rise in calcium results in 2 simultaneous events in sensory neurons (5). First, the increased intracellular calcium inhibits a potassium current carried by  $Kv\ 7.2/Kv\ 7.3$  channels known as “the M current,” leading to cell depolarization. Secondly, Liu and colleagues (5) report that the rise in calcium also activates a calcium-dependent chloride current (CaCC).

When applied exogenously to a human skin blister or injected into arteries supplying viscera in laboratory animals (2), BK induces 2 major effects: (a) severe spontaneous pain and (b) increased sensitivity to painful (hyperalgesia) and nonpainful (allodynia) thermal and mechanical stimuli (3,6). The findings of Liu and colleagues in a series of experiments indicate that the major effects of BK on the excitability of nociceptors are mediated by PLC- and  $Ca^{2+}$ -dependent inhibition of M-type  $K^+$  channels and by simultaneous opening of  $Ca^{2+}$ -activated  $Cl^-$  channels (CaCCs) encoded by transmembrane protein 16A (*Tmem16a*). Both these effects summate to contribute to membrane depolarization in primary sensory afferents, resulting in the generation of ascending nociceptive signals (5). In small nociceptive neurons from rat dorsal root ganglia, bradykinin binds to bradykinin 2 receptors ( $B_2$ ) which couple to  $G_{q11}$  and activate PLC (6). PLC hydrolyzes the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate ( $PIP_2$ ) and releases inositol 1,4,5-trisphosphate ( $IP_3$ ) into the

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cytosol; the latter can then trigger exit of  $\text{Ca}^{2+}$  from  $\text{IP}_3$ -sensitive intracellular stores. Both  $\text{PIP}_2$  depletion and  $\text{Ca}^{2+}$  release are powerful signaling events capable of regulating neuronal ion channels.

### M-Current

In a series of patch-clamp experiments, BK was demonstrated to lead to inhibition of M current which was always accompanied by the simultaneous activation of an inward current at  $-60$  mV (5). In efforts to investigate whether "M channel" inhibition or CaCC activation contributes to BK-induced animal nociceptive behaviors, Liu and colleagues preinjected the "M channel" opener retigabine or different CaCC blockers into the same plantar region of the hind paw where 5 minutes later BK was injected (5). They tested whether the "M channel" opener retigabine can reduce BK-induced nociceptive behavior, thinking that "M channels" inhibited by BK might be "rescued" with retigabine. Retigabine (5 nmol/site) was able to significantly attenuate BK-induced nociceptive responses (5). In patch-clamp experiments, application of retigabine effectively reversed the inhibitory effect of BK on M current at  $-60$  mV (5).

### Calcium-Dependent Chloride Current

Another important mechanism that contributes to BK-induced acute nociceptive signaling involves the activation of calcium-dependent chloride channels encoded by transmembrane protein 16A. The transmembrane protein 16 (*TMEM16* or anoctamin) protein family has been identified as a CaCC subunit (7-9). The gene encoding an essential CaCC subunit (*Tmem16A*- also known as *Ano 1*) has recently been identified (7-9).

Lowering intracellular  $\text{Cl}^-$  ( $[\text{Cl}^-]_i$ ) by using a potassium acetate-based pipette solution almost completely abolished BK-induced inward current. Furthermore, the broad-spectrum chloride channel blocker 4,4'-diisothiocyanatostilbene- 2, 2'-disulphonic acid (DIDS, 100  $\mu\text{M}$ ) also abolished BK-induced inward current. Other  $\text{Cl}^-$  channel blockers, niflumic acid (NFA, 100  $\mu\text{M}$ ) and 5-nitro-2-(3 phenylpropylamino) benzoic acid (NPPB, 100  $\mu\text{M}$ ), also effectively blocked BK-induced inward currents. The above results suggest that the inward current evoked by BK was almost exclusively due to chlo-

ride channel opening (5). The B2R antagonist Hoe-140 and PLC blocker edelfosine both prevented the inward current responses, suggesting that this inward current is conducted by CaCCs activated via the PLC/ $\text{IP}_3$ / $\text{Ca}^{2+}$  pathway (5). Liu and colleagues used siRNA against *Tmem16a* to test whether it is a molecular correlate of the BK-induced  $\text{Cl}^-$  current in small DRG neurons. Transfection of siRNA significantly reduced levels of *Tmem16a* mRNA and protein. Liu et al (5) also tested whether pharmacological inhibition of CaCC would lead to anti-nociceptive effects. Preinjection of the  $\text{Cl}^-$  channel blockers DIDS (50 nmol/site) and NPPB (10 nmol/site) significantly attenuated the BK-induced nociceptive behaviors (5).

### SUMMARY

Thus, it appears that the acute pain which bradykinin can induce, is dependent on both the modulation of transmembrane protein 16 leading to opening of calcium dependent chloride channels with subsequent escape of negatively charged chloride ions resulting in a less negative intracellular environment (depolarization), as well as, the closing of M-type potassium channels which stops the escape of positively charged potassium ions resulting in a more positive intracellular environment (depolarization) from intracellular potassium accumulation (Fig. 1). These 2 processes both result in depolarization of sensory neurons that may facilitate nociception.

These two mechanisms each represent a potential for antinociceptive strategies aimed at: 1.) closing *TMEM16A* calcium-dependent chloride channels, and/or 2.) opening of M-type potassium channels. Retigabine and agents like it may provide analgesia by the second of these mechanisms. Retigabine's mechanism of action involves opening of neuronal Kv 7.2-7.5 voltage activated  $\text{K}^+$  channels responsible for generation of the M-current. Additionally, retigabine has also been shown to increase synthesis of gamma amino butyric acid (GABA) in rat hippocampal neurons and to allosterically potentiate GABA-induced chloride currents in cultured rat cortical neurons. Behavioral studies have shown retigabine can diminish pain behaviors involving animal models of neuropathic pain (10-13).

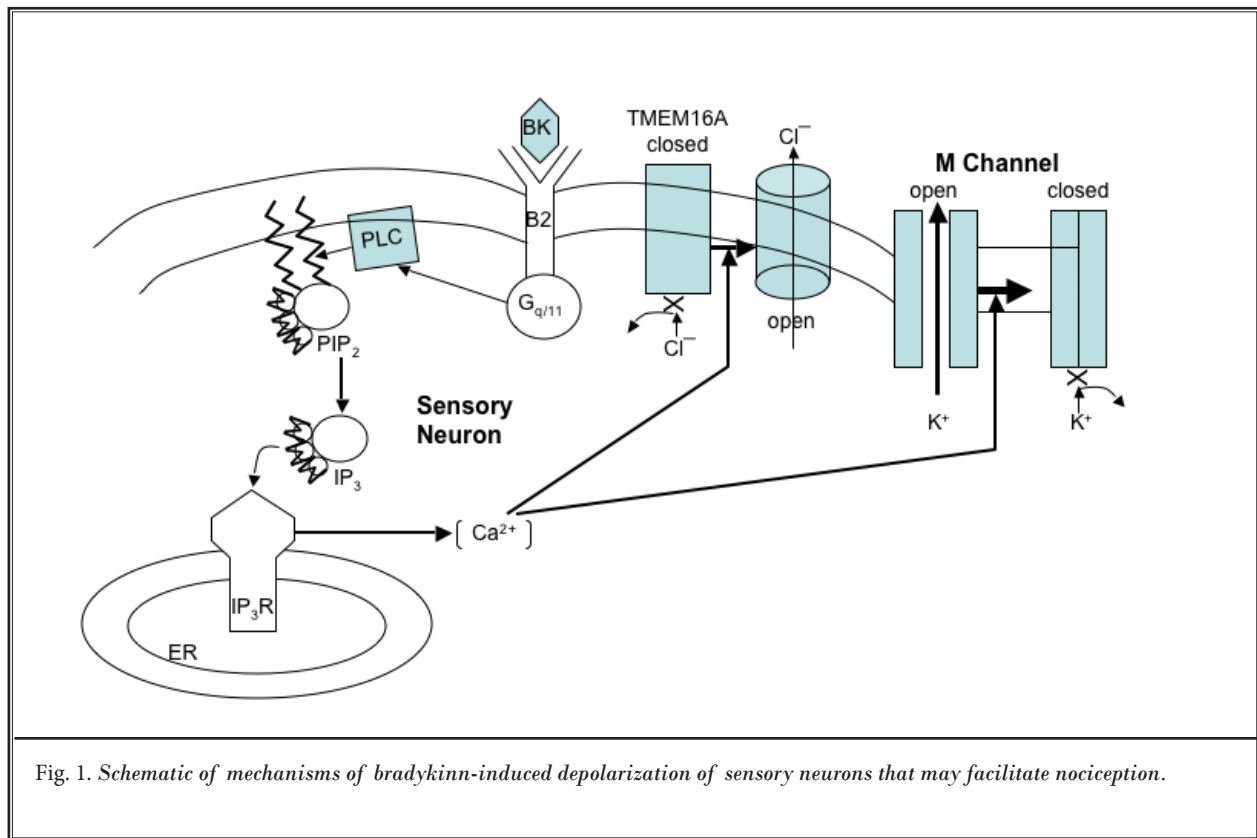


Fig. 1. Schematic of mechanisms of bradykinin-induced depolarization of sensory neurons that may facilitate nociception.

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