Regulation of Nociceptive Transmission at the Periphery *Via* TRPA1-TRPV1 Interactions

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Abstract: TRPV1 and TRPA1 have traditionally been considered to function independently from each other as homomers, but their extensive co-expression in sensory neurons and recent evidence suggest that these channels can functionally interact and may form a complex as part of their normal function. Although TRPA1 and TRPV1 do not absolutely require interaction to maintain function in expression systems or even sensory neurons, their heteromerization may still result in dramatic effects on channel biophysical properties, pharmacology, signaling, regulation, and ultimately function. Understanding the regulation and functional significance of TRPA1-TRPV1 interaction is of tremendous clinical importance since first, both channels are the potential molecular targets for numerous therapeutic drugs; and second, TRPA1-TRPV1 co-expression is far more specific for nociceptive sensory neurons than expression patterns of TRPA1 or TRPV1 considered separately.

Keywords: TRPV1, TRPA1, nociceptors.

INTRODUCTION

Regulation of nociception at the peripheral level has an advantage in the possibility of developing analgesics devoid of central side effects. Nociceptive transmission at the periphery follows a relatively straightforward scheme. Tissue damage, inflammation or nerve injury triggers excitation of nociceptors (damage sensing sensory neurons). This in turn, recruits an array of voltage-gated channels which are responsible for the propagation of nociceptive signals to the central terminals of nociceptors. Such a simple scheme leaves two possibilities to regulate nociceptive transmission at the peripheral level. It can be controlled either on the level of nociceptor excitation or signal propagation. Both strategies have been extensively exploited during the last decade. Accumulated data has strongly demonstrated that the transient receptor potential V1 (TRPV1) and A1 (TRPA1) play critical roles in inflammation-induced or tissue damage-induced excitation of nociceptors [1-7]. Thus, TRPV1 is involved in thermal nociception as well as inflammatory thermal hyperalgesia. The TRPA1 channel at least partially contributes to inflammatory cold and mechanical hyperalgesia. TRPA1 participation in cold and mechanical acute nociception is still debated [2, 4, 6]. The contribution of TRPV1 and TRPA1 to peripheral nociception supports the idea that one of the approaches to control nociceptive transmission at the periphery could be to understand and manage cross-regulation between the TRPV1 and TRPA1 channels.

INTERACTION BETWEEN TRP CHANNELS

TRP channels have unique characteristics in terms of cell-type expression pattern [8], modes of activation [9], pharmacological profile and biophysical properties including voltage-dependency and ion permeability [10]. A large body of evidence demonstrated that a variety of TRP channels are capable to assembling into homo- or heterotetrameric channel complexes (see reviews [10, 11]. The subunit composition may influence the biophysical and regulatory properties of the resulting channel complex. Thus, the hetero-oligomer between TRP and TRP-like channels in *Drosophila melanogaster* has unique permeation properties and low constitutive activity [12]. Regulatory pathways of TRPL are altered by assembling with TRPγ [13]. In mammals, the formation of various TRP channel complexes of TRPC [14-16], TRPV [17-19], TRPM [20], or TRPP [11] channels results in novel phenotypes with characteristic biophysical features. Heteromerization can alter the stability of receptors on the plasma membrane by governing the trafficking properties of the receptors [21-23]. Desensitization of receptors can also be influenced by complex formation [24-27]. Finally, the sensitization modes of receptors by different cell stimulants (such as inflammatory mediators) could be regulated in a unique way within heteromeric complexes. Based upon these findings, it is possible that a functional interaction between TRPA1 and TRPV1 channels may occur via a variety of pathways within a heteromeric complex [2, 27-29].

CO-EXPRESSION OF TRPA1 AND TRPV1

The expression pattern for TRPA1 and TRPV1 has been studied in detail. It was previously thought that the TRPV1 channel is mainly expressed in nociceptors [30]. However, subsequent research has demonstrated that TRPV1 is present in multiple regions of the brain, including cerebral cortex, hippocampus, thalamus and brain stem nucleus [31-33]; in certain blood cells [34]; fibroblasts [35]; and skin epidermis [36]. TRPA1 is also present in nociceptors of dorsal root ganglia (DRG) and trigeminal ganglia (TG) [37], as well as the nodose-petrosal ganglion complex [38, 39], inner ear [40] and skin [41]. Altogether, it appears that TRPA1 and

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TRPV1 are co-expressed in sensory ganglia and skin. Co-expression of the TRPV1 and TRPA1 channels in certain types of skin cells has not been reported. However, there is a wealth of information on co-expression of these channels in sensory neurons. Using in situ hybridization and immunohistochemistry, TRPA1 was localized to a subset of TRPV1-positive neurons [42, 43]. Quantification of TRPA1-positive sensory neurons demonstrated that under normal conditions, TRPA1 is expressed in ≈55% of TRPV1-positive neurons, while nerve growth factor (NGF) treatment of TG increases TRPA1 expression to ≈80% of TRPV1-positive cells [43]. Furthermore, recordings of capsaicin- (CAP; TRPV1-specific) and mustard oil-activated (MO; TRPA1-spesific) currents from cultured sensory neurons indicate that <3% of neurons express functional TRPA1, but not TRPV1 [44]. In summary, TRPA1 is almost exclusively present in a TRPV1-positive population of sensory neurons, and does not co-express with TRPV1 in other tissues. Such a specific pattern of TRPA1-TRPV1 co-expression could make drug(s) that target functional interaction between TRPA1 and TRPV1 devoid not only of central, but also peripheral side effects.

APPROACHES TO STUDY CROSS-REGULATION BETWEEN TRPV1 AND TRPV1

Approaches to study any interaction between channels and receptors are quite standard. These approaches are as follows: biochemical and cell biology; biophysical, pharmacological and physiological. Biochemical and cell biology approaches include co-immunoprecipitation (co-IP), FRET interaction, trafficking studies, and total internal reflection fluorescence (TIRF) microscopy [17, 45-52]. Thus, TRPA1 and TRPV1 can be co-IPed from expression systems and trigeminal sensory neurons [53]. Interaction between TRPV1 and TRPA1 on the plasma membrane has also been confirmed by TIRF based FRET [53]. Biophysical approaches are based on characterization of channel or receptor biophysical properties in the presence and absence of interacting protein (i.e. auxiliary protein, adaptor, receptor and/or channel) [54]. There are also well developed methods to study common pores for multi-subunit channels [55, 56]. For TRPA1-TRPV1 interaction, modulation of the whole-cell as well as intrinsic single-channel properties of TRPA1-mediated responses by the TRPV1 channel has been investigated in detail [44, 53]. Pharmacological approaches are one of the most effective ways to investigate these interactions. To investigate interactions between channels/receptors, availability of agonists and antagonists differentially interacting with separate and interacting subunits are necessary. Thus, the cannabinoid AM1241 can gate the TRPA1 channel, but not TRPV1. However, this agonist is more effective (≈5-fold) in activation of cells expressing both TRPA1 and TRPV1 channels [57]. TRPV1-specific antagonists such as capsazepine (CZP) and I-RTX do not alter mustard oil (MO) responses. Interestingly, in the presence of TRPV1, CZP and I-RTX potentiate MO responses (unpublished observation: Patil M. and Akopian A. N.). Heteromers could have specific antagonists that clearly distinguish complex formation between receptors [58]. Unfortunately, as of yet there are no antagonists that specifically recognize the TRPA1-TRPV1 complex. Another viable approach is to eliminate a subpopulation of sensory neurons by certain drugs. Thus, the highly potent TRPV1 agonist, RTX, can ablate TRPV1 positive neurons *in vivo* [59]. Some studies [60], but not others [2, 61] suggested that TRPA1 could be a mechano-sensor. Since TRPA1 is highly co-expressed with TRPV1, then ablation could reduce mechanical pain, if TRPA1 is indeed a mechano-gated channel. However, RTX-triggered elimination of TRPV1-positive neurons leads to attenuation in thermal hyperalgesia and potentiation of mechanical pain [59]. This study suggests that there are at least other mechano-gated channels in sensory neurons [62]. The physiological approach is based on disruptions of channel/receptor complexes *in vivo* and behavioral evaluation of those animals. The most straightforward method is to block complex function with complex-specific antagonists. Alternative strategies could be based on identification of interaction domains coupled with separation of complex subunits. Obviously, this strategy is viable if the interaction domains do not interfere with function of the separate subunits. Thus, in the case of a TRPA1-TRPV1 complex, interaction domains should not interfere with normal functioning of TRPA1 and TRPV1 channels.

FUNCTIONAL CROSS-REGULATION BETWEEN TRPV1 AND TRPA1

Regulation of TRPA1 and TRPV1 homomers has been investigated in detail. However, recently published data suggest that cross-regulation between TRPA1 and TRPV1 may be involved in certain physiological processes. Firstly, we will briefly overview regulation of TRPV1 and TRPV1 as homomers. Modulation of TRPV1 and to a lesser extent TRPA1 by inflammatory mediators (i.e. factors released during inflammation and tissue damage) have been extensively investigated [63-65]. The common consensus is that TRPV1 activity (sensitization and desensitization) in sensory neurons is mainly regulated by a variety of kinases induced by inflammatory mediators or phosphotases activated by Ca^{2+} [51, 66-71]. Adapter proteins such as calmodulin and AKAP are also closely involved in regulation of TRPV1 channel activity [66, 72-74]. The molecular mechanism underlying regulation of TRPA1 activity does not have a consensus in opinions. One possible mechanism is that phosphatidylinositol 4,5-biphosphate (PIP_2) permanently inhibits TRPA1, and depletion of PIP_2 by inflammatory mediators coupled to phospholipase C (PLC) leads to sensitization of the TRPA1 channel [65, 75]. However, some evidence demonstrates that PIP_2 enhances TRPA1 activity and PIP_2 depletion contributes to desensitization of TRPA1 in sensory neurons [26, 76]. Another possible mechanism is that inflammation can increase intracellular Ca^{2+} resulting in fast trafficking of TRPA1 to the plasma membrane, thereby producing sensitization [64]. There are also conflicting reports regarding this finding. Thus, the vast majority of *in vitro* [26, 77], *in vivo* [78, 79] and clinical [80, 81] data suggest that TRPA1 is pharmacologically desensitized by repeated application of mustard oil, which increases intracellular Ca^{2+}. Finally, it was noted that phosphorylation does not alter TRPA1 activity [82].

There are several possible mechanisms underlying functional interaction between TRPA1 and TRPV1 channels. First, capsaicin and mustard oil pretreatments result in pharmacological [78, 79] and functional [81, 83] cross-

desensitization between TRPA1 and TRPV1. Mustard oil desensitizes TRPV1 via a Ca^{2+}-dependent mechanism involving the Ca^{2+}-dependent phosphotase, calcineurin [26, 78]. Mustard oil triggers Ca^{2+}-influx into sensory neurons and activates calcineurin, which then dephosphorylates and desensitizes the TRPV1 channel. Inhibition of sensory neurons via certain cannabinoids (including WIN55, 212-2, AM1241) -induced pharmacological desensitization of the TRPV1 channel, which recruits the same cellular signaling cascade [27, 57, 84, 85]. Desensitization of TRPA1 by capsaicin is also Ca^{2+}-dependent [78], but this process employs Ca^{2+}-evoked depletion of PIP_2 [26]. Capsaicin-gated massive Ca^{2+} influx activates Ca^{2+}-dependent PLCδ [26], which in turn leads to desensitization of TRPA1 [76]. Interestingly, mustard oil-triggered Ca^{2+}-influx can activate PLCδ only in expression system, but not sensory neurons [26]. Nevertheless, mustard oil is able to pharmacologically desensitize TRPA1 [78], and this desensitization is more pronounced in the absence of TRPV1 in sensory neurons [26]. Mustard oil responses could undergo pharmacological self-desensitization via two possible mechanisms: (1) mustard oil-generated modifications of the TRPA1 channels that needs time to recover [77], or/and (2) mustard oil triggers internalization of TRPA1 that could lead to reduction of TRPA1 activity [26, 64]. In TRPA1-TRPV1 containing sensory neurons, it appears that TRPV1 inhibits the internalization process for TRPA1 [26].

Second, bradykinin (BK)-induced thermal and mechanical hyperalgesia is mutually transduced by the TRPA1 and TRPV1 channels [2, 4]. It was proposed that intracellular Ca^{2+} ($[Ca^{2+}]_i$) could be an intermediate for this type of functional interaction of TRPA1 and TRPV1 [2]. Thus, $[Ca^{2+}]_i$ could be accumulated via BK-induced intracellular store depletion, TRPV1 activation by BK-triggered PIP_2 depletion [86] and/or TRPV1 activation by BK-produced 12-lipoxygenase metabolites of arachidonic acid [87]. An increase of $[Ca^{2+}]_i$ to >1μM levels may activate TRPA1 [88, 89]. Through this pathway, activation of TRPV1 by BK could eventually result in TRPA1 functioning. Such TRPA1-mediated activation may result in neurogenic inflammation and mechanical or thermal BK-induced hyperalgesia [2, 4]. This hypothesis for TRPA1-TRPV1 functional interaction still needs to be developed, since a) it is not clear how TRPA1 is activated by $[Ca^{2+}]_i$ in sensory neurons [90], in which TRPA1 and TRPV1 co-expressed; b) capsaicin-evoked powerful Ca^{2+} influx would be required to activate TRPA1 and amplify capsaicin-gated responses, but capsaicin responses [2] as well as nociception [57] are not affected in TRPA1 null-mutant (KO) mice; and c) recently published data imply that BK may not activate the TRPV1 channel (Liu *et al.*, 2010).

Third, a functional interaction between TRPA1 and TRPV1 channels may take place within a heteromeric complex. Three possible pathways that underlie this interaction can be proposed. (1) BK can indirectly activate TRPA1, probably via generation of diacylglycerol (DAG) [91]. TRPV1 as a component of the complex may act as a modulator that is responsible for sensitization of TRPA1-mediated BK responses. (2) The pharmacological desensitization of TRPA1-mediated responses in sensory neurons lacking TRPV1 is more pronounced [26]. Therefore, it could be suggested that the absence of TRPV1 in sensory neurons may lead to a faster desensitization of BK responses in sensory neurons, and this, in turn, may suppress the development of inflammatory hyperalgesia. Interestingly, an increased rate of functional desensitization of BK-induced action potentials in TRPV1 KO mouse peripheral sensory fibers has previously been reported [92]. (3) Finally, TRPA1-mediated BK responses in sensory neurons could be modulated by a combination of TRPV1 and $[Ca^{2+}]_i$. Thus, $[Ca^{2+}]_i$ may influence ligand affinity and/or signaling or other properties of a TRPA1-TRPV1 complex. In summary, to understand mechanisms underlying functional interaction between the TRPA1 and TRPV1 channels, TRPA1 and TRPV1 *in vitro* and *in vivo* characteristics need to be studied in the presence and absence of TRPV1 and TRPA1 channels, respectively.

MODULATION OF TRPA1-MEDIATED RESPONSES BY TRPV1

Biophysical, pharmacological and regulatory properties of TRPA1 have been assessed mainly in TRPA1 expression systems. Recent studies have shown that TRPA1 and TRPV1 can be co-immunoprecipitated (co-IPed) from a TRPA1-TRPV1 expression system as well as from sensory neurons [53]. Moreover, FRET interaction between TRPA1 and TRPV1 occurs on the plasma membrane [53]. Therefore, modulation of TRPA1-mediated responses by the TRPV1 channel will be reviewed.

At physiological conditions (Vh=-60 mV; 2 mM extracellular Ca^{2+}), the mustard oil-gated current (I_{MO}) has a larger response and higher open probability (Po) in a TRPA1-containing expression system compared to TRPA1-TRPV1-co-expressing cells [44]; Staruschenko *et al.*, 2010). However, I_{MO} is larger in wild-type (WT) than in TRPV1 KO sensory neurons [44]. Further, I_{MO} Po still remained higher in TRPA1-expressing sensory neurons (i.e. TRPV1 KO) (Staruschenko *et al.*, 2010). These observations imply that TRPV1 controls the density of functional TRPA1 channels on the plasma membrane. It is still not clear whether TRPA1 density is controlled by the TRPV1 channel on transcriptional, translational and/or post-translational levels.

Reports indicate that extracellular Ca^{2+} either does not affect [91, 93] or enhance [37, 94] TRPA1-mediated responses in TRPA1 expression systems. In TRPA1-TRPV1 expressing sensory neurons, I_{MO} and WIN 55,212-2 cannabinoid-gated currents (I_{WIN}), which are specifically mediated by TRPA1 in sensory neurons [57], are suppressed by physiological concentrations of extracellular Ca^{2+} [44]. One possible explanation for this effect is that extracellular Ca^{2+} promotes TRPA1-TRPV1 association, but co-IP of TRPA1 and TRPV1 was not significantly affected after culturing TRPA1-TRPV1 cells in Ca^{2+}-free media (Staruschenko *et al.*, 2010).

TRPA1-mediated responses have either an almost linear current-voltage (I-V) relationship [26, 37, 91] or inward rectification of I_{MO} I-V [94]. In contrast, I_{MO} and I_{WIN} I-V have pronounced outward rectification in sensory neurons and TRPA1-TRPV1 expressing cells [44]. This outward rectification is not detected in Ca^{2+}-free conditions. Such voltage-dependency of TRPA1-mediated currents in sensory neurons at physiological conditions (i.e. 2mM extracellular Ca^{2+})

implies that TRPV1 suppresses TRPA1 activity under physiological conditions. Indeed, single-channel recordings of I_{MO} at physiological conditions in the presence of TRPV1 showed that TRPV1 lowered I_{MO} open probability in sensory neurons (Staruschenko *et al.*, 2010). The modulation of TRPA1 single-channel activity by the TRPV1 channel happened only in the presence of extracellular Ca^{2+}, while the absence of $[Ca^{2+}]_i$ does not affect this modulation (Staruschenko *et al.*, 2010). Since Ca^{2+} does not interfere with TRPA1-TRPV1 association, TRPV1 may modify the TRPA1 channel's pore in a way that external Ca^{2+} could partially block the influx of Na^+ ions into cells. Such a phenomenon where divalent cations block the entrance of monovalent cations through pores has been reported for several Ca^{2+} permeable channels, including TRPV4 [95], TRPV5 [96] and TRPV6 [97].

TRPV1 can also modulate pharmacological properties of the TRPA1 channel. Thus, the TRPA1 agonist AM1241 (synthetic cannabinoid activating CB2 receptor) is more potent in the presence of TRPV1 [57]. A similar observation has been made for AM630 (synthetic antagonist for CB2), which is able to activate TRPA1 (unpublished observation). Another interesting finding was obtained with I-RTX, the allosteric TRPV1 channel blocker. I-RTX does not affect mustard oil responses in TRPA1 expressing sensory neurons (i.e. TRPV1 KO sensory neurons), but up-regulates TRPA1 responses in sensory neurons co-expressing TRPA1 and TRPV1 (unpublished observation Patil M. and Akopian A. N.).

CONCLUSION

What mechanisms could underlie functional interaction of TRPA1 and TRPV1 channels? Studies on modulation of TRPA1 by the TRPV1 channel suggest that a) density of the TRPA1 channel is regulated by TRPV1; and b) TRPV1 directly affects activity of TRPA1 under physiological conditions. If it is presumed that TRPV1 plays the role of regulatory subunit in a TRPA1-TRPV1 complex, then modification of TRPV1 by tissue damage, inflammation or even nerve injury could be translated to activity of the TRPA1 channel. This presumption could also be applied for certain ligands (be so agonists or antagonists), including endogenous agonists, interacting with TRPV1. Thus, these ligands could either inhibit or facilitate activity of TRPA1. These possibilities need to be investigated individually to draw further conclusions. Nevertheless, it is a promising direction for investigating the regulation of nociceptive transmission, since both channels demonstrate pivotal contributions to nociceptor excitation after tissue damage and inflammation, and in addition, TRPA1 and TRPV1 are predominantly co-expressed in nociceptors.

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