

## **Sustained firing of TRPA1 elicited by reactive agonists**

Marja Saarnilehto, Hugh Chapman, Katja Kuokkanen, Ari Koivisto

It has been recently shown that TRPA1 is activated through a direct mechanism of action that involves covalent binding of reactive agonists to three cytoplasmic cysteine thiols and a lysine in the channel protein. Further, intracellular calcium elevation was shown to activate TRPA1 in a manner that is synergistic to thiol reactivity-based activation. Previous studies show that TRPA1 responses inactivate within a few minutes during the maintained presence of agonists, which is caused by intracellular calcium-dependent desensitization.

We have recently shown that diabetes-induced mechanical hypersensitivity can be attenuated by TRPA1 antagonist treatment, which suggest that TRPA1 is chronically activated in vivo through increased production of endogenous agonists (Wei et al. 2009). We are therefore facing an apparent paradox: How TRPA1 activity can be sustained in vivo, if it is quickly inactivated under in vitro conditions? These findings prompted us to study the temporal kinetics of human TRPA1 activation in vitro in more detail. We found that TRPA1 is activated in 1 h FLIPR experiments in two phases by reactive agonists such as 4-HNE and formalin, whereas non-reactive agonists icilin and menthol induced only the first phase. Both phases could be blocked by TRPA1 selective antagonists. Surprisingly, reactive agonists were able to induce biphasic activation even in -3C neutralised mutant. Perforated patch clamp experiments revealed that maintained presence of reactive agonists elicit sustained firing of TRPA1, whereas non-reactive agonists induced only single depolarization. Our results suggest that reactive agonists elicit in vivo sustained firing of TRPA1 that may underlie chronic pain.

Department of In Vitro Pharmacology  
Orion Pharma  
Nonclinical R&D  
Tengströminkatu 8  
FI-20101 Turku  
Finland

Email: [ari-pekka.koivisto@orionpharma.com](mailto:ari-pekka.koivisto@orionpharma.com)

Reference: Wei H, Hämäläinen MM, Saarnilehto M, Koivisto A, Pertovaara A. *Anesthesiology* 111:147-154, 2009