## Abstract

TRPA1 is a thermosensitive ion channel from the ankyrin subfamily of Transient Receptor Potential (TRP) receptors. These proteins play essential roles in the transduction of wide variety of environmental and endogenous signals. TRPA1, which is abundantly expressed in primary nociceptive neurons, is an important transducer of various noxious and irritant stimuli and is also involved in the detection of temperature changes. Similarly to other TRP channels, TRPA1 is comprised of four subunits, each with six transmembrane segments (S1–S6), flanked by the cytoplasmic N- and C-terminal ends. In native tissues, TRPA1 is supposed to be regulated by multiple phosphorylation sites that underlie TRPA1 activity under physiological and various pathophysiological conditions.

Using mutational approach, we predicted and explored the role of potential phosphorylation sites for protein kinase C in TRPA1 functioning. Our results identify candidate residues, at which phosho-mimicking mutations affected the channel's ability to respond to voltage and chemical stimuli, whereas the phospho-null mutations to alanine or glycine did not affect the channel activation. Particularly, we identify the serine 602 within the N-terminal ankyrin repeat domain 16, the substitution of which to aspartate completely abolished the TRPA1 activity but did not abrogate its cell surface expression. Moreover, the physicochemical properties of the residue at this position were found to be of critical importance and we used molecular modelling to explore the essential structural requirements for channel activation. We found that not only charge but also the size of this residue and its specific environment are important for the proper channel functioning.

We hypothesize that Ser602 might be a potential TRPA1 phosphorylation site vital for channel gating. Identification of the relevant kinase responsible for the abrogation of TRPA1 activity requires further studies. Alternatively, a proper conformation of the N-terminal ankyrin repeat 16 centred around the serine at position 602, is indispensable for proper channel gating.

**Key words**: Ankyrin transient receptor potential subtype 1; phosphorylation; TRP channel; serine/threonine kinase; gating; ankyrin repeat; PKC