Peptide modulation of acid-sensing ion channels

Abstract

Acid-sensing ion channels (ASICs) are trimeric proton-gated cation channels that have been reported to be involved in numerous biological processes, including synaptic plasticity, initiation of pain, and neuronal death associated with ischemic stroke. A number of animal venom toxins and endogenous neuropeptides have been shown capable of modulating ASIC function and activity, both in vitro and in vivo. Thus, these peptides have great potential as therapeutics for the treatment of neurological disorders and provide molecular tools for the understanding and control of ASICs. Despite the recent advances in both structural and functional studies of peptide modulation of ASICs, the precise molecular mechanisms remain elusive, hampering the development of new and improved ASIC-modulating compounds.

This PhD project explored different aspects of peptide modulation of ASICs by investigating two different peptides, 1) a well-known toxin modulator of ASICs, Psalmotoxin-1 (PcTx1), and 2) the largely unexplored, highly potent, endogenous neuropeptide modulator of ASIC, big dynorphin (BigDyn).

In the first study presented here, we used two-electrode voltage-clamp electrophysiology (TEVC) to investigate the functional stoichiometry underlying PcTx1 inhibition. Through testing of a series of concatemeric ASIC1a constructs, containing a mutation known to drastically reduce the sensitivity of ASIC1a sensitivity towards PcTx1 in one, two, or all three ASIC1a subunits, we revealed that only two wild-type (wt) ASIC1a subunits are needed for wt-like inhibition at pH 7.4. This suggests that toxin inhibition of ASICs can be achieved through non-saturated ASIC1a toxin complexes.

In the second study, we employed a combination of molecular biology, electrophysiology, and synthetic peptide chemistry in order to decipher the molecular determinants of ASIC modulation by the neuropeptide BigDyn. Dynorphin peptides, as well as both truncated- and alanine-substituted dynorphin analogs, were produced by solid-phase peptide synthesis and their modulatory effects on ASICs were measured by TEVC. Our results showed that the modulation of ASIC1a by BigDyn is likely to be mediated through electrostatic interactions of positively charged amino acids in the N-terminal region of BigDyn that are crucial for the modulatory effects towards ASIC1a. In addition, by screening a series of ASIC1a mutants, we identified a double charge-neutralizing mutation of residues located in the acidic pocket that attenuated the modulatory effects of BigDyn.

Overall, the results presented here provide new insights into peptide modulation of ASICs that may contribute to the development of ASIC-targeting compounds in the future.