

Meeting abstract

Open Access

## Double mutant gating perturbation analysis predicts a high conformational stability of the domain IV S6 segment of the voltage-gated Na<sup>+</sup> channel

René Cervenka, Touran Zarrabi, Péter Lukács, Xaver König, Karlheinz Hilber and Hannes Todt\*

Address: Institute of Pharmacology, Center of Biomolecular Medicine and Pharmacology, Medical University of Vienna, 1090 Vienna, Austria

Email: Hannes Todt\* - hannes.todt@meduniwien.ac.at

\* Corresponding author

from 15th Scientific Symposium of the Austrian Pharmacological Society (APHAR) Joint meeting with the Hungarian Society of Experimental and Clinical Pharmacology (MFT) and the Slovenian Pharmacological Society (SDF)  
Graz, Austria. 19-21 November 2009

Published: 12 November 2009

BMC Pharmacology 2009, 9(Suppl 2):A25 doi:10.1186/1471-2210-9-S2-A25

This abstract is available from: <http://www.biomedcentral.com/1471-2210/9/S2/A25>

© 2009 Cervenka et al; licensee BioMed Central Ltd.

### Background

The S6 segment of domain IV (DIV-S6) of voltage-gated Na<sup>+</sup> channels is considered to be a key player in gating and local anesthetic drug block. Thus, mutations at several sites of DIV-S6 are known to substantially alter the channel's inactivation properties.

### Methods

For a comprehensive analysis of the kinetic role of DIV-S6 in fast inactivation we performed a cysteine scanning analysis of sites 1575-1591 in the DIV-S6 of the rNa<sub>v</sub>1.4 channel. These mutations were engineered into the wild-type channel and into rNa<sub>v</sub>1.4 carrying the mutation K1237E. K1237 is located in the P-loop of domain III and mutations at this site have dramatic effects both on permeation and gating properties. Hence, K1237E most likely causes a complex conformational change of the channel. We sought to explore whether K1237E changes the pattern of gating perturbations produced by the serial cysteine replacements in DIV-S6. The constructs were expressed in *Xenopus laevis* oocytes and studied by means of two electrode voltage-clamp.

### Results

The half-point of availability following a 50 ms conditioning prepulse (V05) was  $-44 \pm 1$  mV and  $-51 \pm 1$  mV in wild-type and K1237E, respectively ( $p < 0.001$ ). Most

serial amino acid replacements by cysteines in DIV-S6 produced shifts in V05, both in the background of wild-type and in the background of K1237E, ranging from  $+17 \pm 1$  mV to  $-9 \pm 2$  mV. A plot of the shifts in V05 by single DIV-S6 mutants relative to wild-type vs. the shifts in V05 by double mutants relative to K1237E showed a significant positive correlation ( $r = 0.92$ ,  $p = 0.002$ ).

### Conclusion

This indicates that the general pattern of gating perturbations in DIV-S6 is not affected by K1237E, suggesting a high conformational stability of the DIV-S6 segment during the fast inactivated state.

### Acknowledgements

Funding support: Austrian Science Fund P210006-B11.