Functional expression of poly-histidine tagged voltage-gated sodium channels in insect cells

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The aim of this research was to develop biosensor technology using natural receptors for use in screening for new drugs and in detection of toxins. The role that voltage-gated sodium channels have in conducting electrical impulses in excitable tissues and as targets for drugs and toxins made them the receptor of choice for this research. Sodium channels initiate and propagate action potentials in both nerve and muscle cells. They are important pharmacological sites of action for local anaesthetics and antiepileptic drugs[1]. Many biological toxins, including shellfish toxins and insecticides, exert their toxic effects by modifying sodium channel function.

The gene encoding the human voltage-gated sodium ion channel skeletal muscle subtype (*hSkM1*) was obtained in a mammalian expression vector, pRC/CMV[2]. We introduced a 6x histidine tag at the C-terminus in order to facilitate purification of the protein by metal-affinity chromatography. The modified sodium channel gene (*hSkM1*-HT) was sub-cloned into the pFastbac vector, and expressed in *Sf*9 insect cells using the baculovirus expression system. Expression of sodium channel protein was determined by Western blotting and function was confirmed by patch-clamping. We also expressed *hSkM1*-HT in HEK 293 cells. Whole-cell sodium currents were recorded from both *Sf*9 insect cells and HEK 293 cells. Future work will involve purifying *hSkM1*-HT channels and reconstituting them into planar lipid bilayers where their activation properties will be assessed and effects of blockers examined.

(1) Denac, H., Naunyn-Schmiedeberg's Arch Pharmacol, 2000, 362: 453-479.
(2) George AL, Jr, et al., Annals of Neurology, 1992, 31 (2): 131-137.