NEUROFIBROMIN, NERVE GROWTH FACTOR AND RAS: THEIR ROLES IN CONTROLLING THE EXCITABILITY OF MOUSE SENSORY NEURONS

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ABSTRACT

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Neurofibromin, nerve growth factor and Ras: their roles in controlling the excitability of mouse sensory neurons

Neurofibromin, the product of the *Nf1* gene, is a guanosine triphosphatase activating protein (GAP) for p21ras (Ras) that accelerates the conversion of active Ras-GTP to inactive Ras-GDP. It is likely that sensory neurons with reduced levels of neurofibromin have augmented Ras-GTP activity. In a mouse model with a heterozygous mutation of the *Nf1* gene (*Nf1+/-*), the patch-clamp recording technique is used to investigate the role of neurofibromin in controlling the state of neuronal excitability. Sensory neurons isolated from adult *Nf1+/-* mice generate more APs in response to a ramp of depolarizing current compared to *Nf1+/+* mice. In order to elucidate whether the activation of Ras underlies this augmented excitability, sensory neurons are exposed to nerve growth factor (NGF) that activates Ras. In *Nf1+/+* neurons, exposure to NGF increases the production of APs. To examine whether activation of Ras contributes to the NGF-induced sensitization in *Nf1+/+* neurons, an antibody that neutralizes Ras activity is internally perfused into neurons. The NGF-mediated augmentation of excitability is suppressed by the Ras-blocking antibody in *Nf1+/+* neurons, suggesting the NGF-induced sensitization in *Nf1+/+* neurons depends on the activation of Ras. Surprisingly, the excitability of *Nf1+/-* neurons is not altered by the blocking antibody, suggesting that this enhanced excitability may depend on

previous activation of downstream effectors of Ras. To determine the mechanism giving rise to augmented excitability of *Nf1+/-* neurons, isolated membrane currents are examined. Consistent with the enhanced excitability of *Nf1+/-* neurons, the peak current density of tetrodotoxin-resistant (TTX-R) and TTX-sensitive (TTX-S) sodium currents (INa) are significantly larger than in *Nf1+/+* neurons. Although the voltage for half-maximal activation ($V_{0.5}$) is not different, there is a significant depolarizing shift in the $V_{0.5}$ for steady-state inactivation of INa in *Nf1+/-* neurons. In summary, these results demonstrate that the enhanced production of APs in *Nf1+/-* neurons results from a larger current amplitude and a depolarized voltage dependence of steady-state inactivation of INa that leads to more sodium channels being available for the subsequent firing of APs. My investigation supports the idea that regulation of channels by the Ras cascade is an important determinant of neuronal excitability.

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