

Short Communication

## Nerve growth factor, sphingomyelins, and sensitization in sensory neurons

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Because nerve growth factor (NGF) is elevated during inflammation, plays a causal role in the initiation of hyperalgesia, and is known to activate the sphingomyelin signalling pathway, we examined whether NGF and its putative second messenger, ceramide, could modulate the excitability of capsaicin-sensitive adult sensory neurons. Using the whole-cell patch-clamp recording technique, exposure of isolated sensory neurons to either 100 ng/mL NGF or 1 mmol/L N-acetyl sphingosine (C2-ceramide) produced a 3-4 fold increase in the number of action potentials (APs) evoked by a ramp of depolarizing current in a time-dependent manner. Intracellular perfusion with bacterial sphingomyelinase (SMase) also increased the number of APs suggesting that the release of native ceramide enhanced neuronal excitability. Glutathione, an inhibitor of neutral SMase, completely blocked the NGF-induced augmentation of AP firing, whereas dithiothreitol, an inhibitor of acidic SMase, was without effect. In the presence of glutathione and NGF, exogenous ceramide still enhanced the number of evoked APs, indicating that the sensitizing action of ceramide was downstream of NGF. To investigate the mechanisms of actions for NGF and ceramide, isolated membrane currents were examined. Both NGF and ceramide facilitated the peak amplitude of the TTX-resistant sodium current (TTX-R  $I_{Na}$ ) by approximately 1.5-fold and shifted the activation to more hyperpolarized voltages. In addition, NGF and ceramide suppressed an outward potassium current ( $I_K$ ) by ~35%. The inflammatory prostaglandin, PGE<sub>2</sub>, produced an additional suppression of  $I_K$  after exposure to ceramide (~35%), suggesting that these agents might act on different targets.

Based on the existing literature, it is not clear whether this NGF-induced sensitization is mediated by the high-

affinity TrkA receptor or the low-affinity p75 neurotrophin receptor. Pretreatment with the p75 blocking antibody completely prevents the NGF-induced increase in the number of APs evoked by the current ramp. Although the sensitization by NGF was blocked, the antibody had no effect on the capacity of ceramide, a putative downstream signalling molecule, to enhance the excitability.

Ceramide can be metabolized by ceramidase to sphingosine (Sph) and Sph to sphingosine 1-phosphate (S1P) by sphingosine kinase. It is well established that each of these products of sphingomyelin metabolism can act as intracellular signalling molecules. This raises the question as to whether the enhanced excitability produced by NGF was mediated directly by ceramide or required additional metabolism to Sph and/or S1P. Sph applied externally did not affect the neuronal excitability whereas internally perfused Sph augmented the number of APs evoked by the depolarizing ramp. Furthermore, internally perfused S1P enhanced the number of evoked APs. This sensitizing action of NGF, ceramide, and internally perfused Sph, were abolished by dimethylsphingosine (DMS), an inhibitor of sphingosine kinase. In contrast, internally perfused S1P enhanced the number of evoked APs in the presence of DMS. These observations support the idea that the metabolism of ceramide/Sph to S1P is critical for the sphingolipid-induced modulation of excitability. Thus, our findings indicate that the pro-inflammatory agent, NGF, can rapidly enhance the excitability of sensory neurons. This NGF-induced sensitization is mediated by activation of the sphingomyelin signalling pathway wherein intracellular S1P derived from ceramide, acts as an internal second messenger to regulate membrane excitability, however, the effector system whereby S1P modulates excitability remains undetermined.

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