Neurofibromatosis: The role of guanosine triphosphatase activating proteins in sensory neuron function

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Abstract: Neurofibromatosis type 1 (NF1) is a common autosomal dominant disease characterized by formation of multiple benign and malignant tumors. People with this disorder also experience chronic pain, which can be disabling. Neurofibromin, the protein product of the \textit{Nf1} gene, is a guanosine triphosphatase activating protein (GAP) for p21Ras (Ras). Loss of \textit{Nf1} results in an increase in activity of the Ras transduction cascade. Because of the growing evidence suggesting involvement of downstream components of the Ras transduction cascade in the sensitization of nociceptive sensory neurons, we examined the stimulus-evoked release of the neuropeptides, substance P (SP) and calcitonin gene-related peptide (CGRP), from primary sensory neurons of mice with a mutation of the \textit{Nf1} gene (\textit{Nf1}+\textasciitilde). Measuring the levels of SP and CGRP by radioimmunoassay, we demonstrated that capsaicin-stimulated release of neuropeptides is 3-5 folds higher in spinal cord slices from \textit{Nf1}+\textasciitilde mice than that from wildtype mouse tissue. In addition, the potassium- and capsaicin-stimulated release of CGRP from the culture of sensory neurons isolated from \textit{Nf1}+\textasciitilde mice was more than double that from the culture of wildtype neurons. Using patch-clamp electrophysiological techniques, we also examined the excitability of capsaicin-sensitive sensory neurons. It was found that the number of action potentials generated by the neurons from \textit{Nf1}+\textasciitilde mice, responding to a ramp of depolarizing current, was more than three times of that generated by wildtype neurons. Consistent with that observation, neurons from \textit{Nf1}+\textasciitilde mice had lower firing thresholds, lower rheobase currents and shorter firing latencies compared with wildtype neurons. These data clearly demonstrate that GAPs, such as neurofibromin, can alter the excitability of nociceptive sensory neurons. The augmented response of sensory neurons with altered Ras signaling may explain the abnormal pain sensations experienced by people with NF1 and suggests an important role of GAPs in the mechanism of sensory neuron sensitization.

Key words: calcitonin gene-related peptide; dorsal root ganglia; neurofibromin; nerve growth factor; nociceptors; Ras

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disease characterized by formation of benign and malignant tumors[1]. Although, the hallmark of NF1 is the formation of various benign and malignant tumors, other common problems experienced by patients with NF1 are abnormal painful sensations, such as hyperalgesia, dysesthesias and allodynia, and painful itching or pruritus, particularly in response to injury, minor trauma or tumor growth[2-4]. Neurofibromin, the protein product of the \textit{Nf1} gene, is a guanosine triphosphate activating protein (GAP) for p21Ras (Ras)[5-7].

Ras is involved in cell signaling pathways that are important for cell survival, growth and proliferation in many cells types[8-10]. Activation of growth factor or other tyrosine kinase receptors initiates a series of protein interactions that lead to guanine nucleotide exchange factors (GEFs) exchanging GTP for GDP bound to the inactive form of Ras. Active Ras-GTP recruits the kinase, Raf, to the membrane and thereby activates a cascade of downstream effectors such as extracellular signal-regulated kinase (ERK), part of the mitogen activating protein kinase (MAPK) pathway. Recent evidence demonstrates that Ras-GTP also activates phosphoinositide-3-kinase (PI-3K), which leads to activation of both Akt (a critical survival signal in many cell types) and Jun-N-terminal kinase (JNK)[11]. Activation of PI-3K and small RhoGTPases in malignant peripheral nerve sheath tumor cell lines, rat fibroblasts and murine mast cells initiates a signaling cascade that leads to crosstalk between the Ras-Raf-Mek-ERK and the Ras-PI-3K-Rac-PAK pathways[12-14]. The Ras-PI-3K-Rac-PAK pathway is important in cytoskeletal function, such as chemotaxis and degranulation[15]. Ras-GTP is converted back to the inactive form, Ras-GDP, through hydrolysis. The hydrolysis reaction is accelerated thousands of folds by GAPs, thereby limiting the strength and duration of downstream signals. Decreased neurofibromin production in NF1 results in an

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increase in both basal and cytokine-stimulated Ras activity in human cells and animal models of this genetic disorder[16-19]. This serves as an example that the normal production and function of GAPs are the key to the regulation of the Ras transduction cascades.

There are growing evidences that cytokines and growth factors can activate downstream effectors of Ras transduction cascade to affect changes in adult sensory neurons. Stimulation of peripheral sensory neurons results in an increase in pERK in dorsal root ganglion (DRG) cells[20], as does peripheral inflammation[21]. In addition, spinal nerve ligation in the rat increases levels of phosphorylated p38 in DRG five hours post-procedure, particularly in small diameter neurons that are IB4-positive[22]. This increase was blocked by an antagonist of tumor necrosis factor and so this response may not be dependent on Ras activation. Activation of p38 by nerve growth factor (NGF) treatment is associated with increases in the expression of the TRPV1 receptor in the DRG neurons[23], as are NGF and GDNF-induced increases in pERK and pAkt[24]. The mitogen-activated protein kinase (MAPKK) inhibitor, PD98059, reduced the capsaicin sensitivity of neurons that were treated with NGF for a week[25], although PD98059 did not inhibit the increase in capsaicin sensitivity caused by acute NGF treatment[26]. As GAPs are a group of key regulators of Ras-GTP levels, it is important to elucidate the role of GAPs in these potential mechanisms of peripheral sensitzation.

Because of the growing evidences suggesting involvement of downstream components of the Ras transduction cascade in the sensitization of nociceptive sensory neurons, we examined the stimulus-evoked release of the neuropeptides, substance P (SP) and calcitonin gene-related peptide (CGRP), from primary sensory neurons of mice with a mutation of the Nf1 gene (Nf1−/−). Measuring immunoreactive SP (iSP) and CGRP (iCGRP) by radioimmunoassay, we demonstrated that capsaicin-stimulated release of neuropeptides is 3-5 folds higher in spinal cord slices from Nf1−/− mice than that from wildtype mouse tissue[27]. In addition, the potassium- and capsaicin-stimulated release of iCGRP from the cultures of sensory neurons isolated from Nf1−/− mice was more than double that from cultures of wildtype neurons. Using patch-clamp electrophysiological techniques, we also examined the excitability of capsaicin-sensitive sensory neurons isolated from DRG of adult Nf1−/− mice[28]. It was found that the number of action potentials generated by sensory neurons from adult Nf1−/− mice, responding to a ramp of depolarizing current was more than three times of that generated by wildtype neurons. Consistent with the greater number of action potentials, Nf1+/− neurons had lower firing thresholds, lower rheobase currents, and shorter firing latencies compared with wildtype neurons. Treatment of wildtype sensory neurons with NGF mimicked the enhanced neuropeptide release and excitability observed in Nf1−/− neurons[27,28].

These data clearly suggest that GAPs, such as neurofibromin, can play a key role in the excitability regulation of nociceptive sensory neurons. Thus, sensory neurons from mice with a heterozygous mutation of the Nf1 gene that is analogous to the human disease NF1, exhibit increased sensitivity to chemical and electrical stimulation. This augmented responsiveness may explain the abnormal pain sensations experienced by people with NF1 and suggests an important role of GAPs in the regulation of nociceptive sensory neuron sensitization.

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