Reactive oxygen species are second messengers of neurokinin signaling in peripheral sensory neurons

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AUTHOR SUMMARY

Mammals are equipped with several systems to sense the environment. A somatosensory system collects tactile information from the outside world, monitors the status of the body, and also informs the brain about the occurrence of injury, thus generating a sensation that we know as pain. Physiological pain is crucial for survival; however, disease or injury can result in pathological pain (e.g., arthritis pain, migraine, neuropathic pain) that has no benefit to the organism but brings suffering and distress. Such pain is difficult to treat with conventional analgescics and, despite much effort, the success of new treatment strategies is limited. The somatosensory system consists of the peripheral sensory nerves, which have highly sensitive endings in the skin and viscera from which they reach the spinal cord. However, peripheral nerves are not mere “receivers” of the primary external stimuli; they also have the ability to respond independently to external stimulation by releasing chemical factors such as neuropeptides into the surrounding tissues. Thus, pain-sensing (nociceptive) nerves produce several neuropeptides and can release them at their peripheral endings and into the spinal cord. For example, the peripheral release of one such neuropeptide, known as “substance P,” can produce local (“neurogenic”) inflammation. There is growing evidence that the peripheral sensory nerves themselves also are equipped with substance P receptors (e.g., specialized protein receptors, called “neurokinin receptors”), of which three (NK1, NK2, and NK3) have been identified to date. However, until now, the purposes of these receptors in sensory nerves and the sequence of intracellular events they trigger have not been elucidated.

Neurokinin receptors belong to a group of plasma membrane receptors called “G protein-coupled receptors”; they signal through the intracellular signaling cascade involving G protein alpha subunits q or 11 (Gq/11) and phospholipase C. Phospholipase C hydrolyzes plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) to produce the plasma membrane-bound second messenger diacylglycerol and a soluble second messenger, inositol trisphosphate (IP3). This signaling cascade is responsible for the action of many inflammatory mediators (chemical components of the tissue immune response that serve as signaling molecules), which are able to excite nociceptive nerves and so cause inflammatory pain. A classical example of such inflammatory mediators is bradykinin, a peptide that is released by damaged tissue; bradykinin excites nociceptive nerve endings causing acute “spontaneous” pain (that is, pain not induced by external stimulation). Bradykinin also increases the sensitivity of peripheral nerves to thermal and mechanical stimulation (hyperalgesia). Previous research suggests that the spontaneous pain induced by bradykinin is mediated by the IP3-induced release of Ca2+ from specific intracellular reservoirs of Ca2+ ions called the “endoplasmic reticulum” (Fig. P1). IP3 opens Ca2+-permeable pores within the endoplasmic reticulum, allowing the release of Ca2+ into the cytosol. This Ca2+ release mediates several excitatory activities in nociceptors, including inhibition of M-type K+ channels (which are needed to maintain nociceptive neurons in a low-excitability state) and activation of a Ca2+-activated Cl− channel (also called “TMEM16A” or “ANO1”), which makes nociceptive neurons more excitable (1) (Fig. P1). In contrast, bradykinin-induced hyperalgesia is linked to the sensitization of sensory receptors, such as the heat sensor TRPV1 (Fig. P1), an effect that makes nociceptive neurons “overreact” upon presentation with external thermal stimuli. Surprisingly, we found that although injection of bradykinin in the rat hind paw produces both spontaneous pain (i.e., the injection is painful) and thermal hyperalgesia, injection of substance P produces only hyperalgesia but is not painful. The present study explains the difference in painful effects of substance P and bradykinin and allows us to understand the cellular basis of the separate mechanisms responsible for spontaneous inflammatory pain and hyperalgesia.

Electrophysiological measurements and imaging of cultured dorsal root ganglia (DRG) sensory neurons established that...
(i) substance P can induce phospholipase C activity in sensory neurons; (ii) substance P increases neuronal responses to the TRPV1 agonist capsaicin, suggesting sensitization of TRPV1; (iii) in contrast to bradykinin, substance P usually does not induce release of Ca^{2+} from the endoplasmic reticulum; and (iv), intriguingly, substance P not only failed to inhibit M current (a current conducted by the M-type K^+ channels) in sensory neurons but instead caused marked augmentation of M current, an effect that was mirrored by decreased excitability. Thus, substance P signaling in sensory neurons displayed marked deviation from that expected by the G_{q/11}-mediated pathway.

The M current-augmenting effect of substance P was entirely unexpected. Therefore, we investigated it further. The effect of substance P resembled that of the augmentation of M current by reactive oxygen species that we reported earlier (2). Reactive oxygen species are highly reactive oxygen-containing small molecules such as hydrogen peroxide, which are produced by various enzymes of cellular metabolism and which increasingly are recognized as intracellular signaling molecules. The effect of substance P on M current was reversed by the application of a reducing agent, DTT, suggesting that, like reactive oxygen species, substance P induces oxidative modification of the M-channel protein. Therefore we hypothesized that substance P can induce the production and release of endogenous reactive oxygen species in DRG neurons. Indeed, imaging of cytosolic concentrations of reactive oxygen species with a specific dye indicated that substance P does induce elevated levels of cytosolic reactive oxygen species. Moreover, genetically introducing a molecular sensor of reactive oxygen species into cultured DRG neurons revealed “sparks” of mitochondrial release of reactive oxygen species in response to substance P. We then used a respirometry technique that allows measurement of the activity of the mitochondrial electron-transport chain, which is a main source of intracellular reactive oxygen species. Substance P induced marked inhibition of electron-transport chain activity, possibly at a complex III (one of the four major electron-transporting complexes of electron-transport chain), an observation that is consistent with the reported release of reactive oxygen species induced by the inhibition of the electron-transport chain complex III (3). Consistently, inhibition of complex III mimicked the substance P-induced augmentation of M current.

Because substance P signaling in sensory neurons deviated from the G_{q/11} pathway, we investigated if this signaling is mediated by another G protein alpha subunit. Indeed, the pharmacological evidence suggested that endogenous neurokinin receptors signal through the G_{i/o}-coupled pathway. Interestingly, overexpression of cloned NK1 in the immortalized cell line or in cultured DRG neurons resulted in reconstitution of the classical G_{q/11} cascade featuring robust release of Ca^{2+} from the endoplasmic reticulum and inhibition of M current. This observation strongly suggests that endogenous and overexpressed neurokinin receptors in neurons couple to different signaling pathways, probably because of the association of endogenous neurokinin receptors with another, yet to be identified, G_{i/o}-coupled receptor.

Our present study provides a reconstruction of the molecular events triggered in the peripheral nociceptive neuron by substance P. We report a signaling cascade in which endogenous reactive oxygen species are used as second messengers to augment M channel activity acutely, reducing the excitability of nociceptive neurons. Moreover, we provide clear evidence that spontaneous inflammatory pain and hyperalgesia can be induced by distinct underlying mechanisms within a single nociceptive neuron (Fig. P1). The latter finding is of particular importance for designing future strategies for analgesic drug discovery, because spontaneous pain and hyperalgesia are very poorly distinguished conceptually at present. In fact, most animal models currently used for testing analgesic drug efficacy are based on hyperalgesia measurements (4) even though most patients with chronic pain suffer from spontaneous pain rather than from hyperalgesia. Accordingly, despite the rapid progress in our understanding of the mechanisms of hyperalgesia, there has been rather limited achievement in treatment of pain in humans (5).

Our study provides a mechanistic concept for the difference between these two types of pain and highlights the need to change current approaches to animal pain models and drug testing.