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REVIEW —Current Perspective—

Recent Advances in Neuropharmacology of Cutaneous Nociceptors

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ABSTRACT—Cutaneous nociceptors are peripheral receptive endings of primary sensory neurons activated by noxious stimuli. Nociceptors detect and signal the presence of tissue-damaging stimuli or the existence of tissue damage. In this short review, we will focus on the molecular mechanism of maintenance, activation, inhibition and sensitization in cutaneous nociceptors. Neurotrophic factors are essential to the development of nociceptors during embryogenesis. Recent evidences have indicated that nociceptors in the adult are maintained by either nerve growth factor (NGF) or glial cell line-derived neurotrophic factor (GDNF). A selective activator of nociceptors is capsaicin, natural product of capsicum peppers. Recently, the receptor for capsaicin (the vanilloid receptor 1: VR1) has been cloned, identified and characterized. VR1 seems to play an important role in the activation and sensitization of nociceptors. In contrast, peripheral endogenous cannabinoids such as anandamide are novel candidates for mediators that inhibit the excitation of nociceptors. Intracellular messengers and the mechanisms of signal transduction in nociceptors have also been studied. Our recent findings provide evidences demonstrate that an activation of both cAMP- and cGMP-second messenger systems is required to induce the sensitization of nociceptors. Such emerging evidences reviewed here would make a significant contribution to further understanding of the molecular mechanism of nociceptors.

Keywords: Pain, Capsaicin, Neurotrophic factor, Cannabinoid, Nitric oxide (NO)

The term nociceptor is defined as "a receptor preferentially sensitive to a noxious stimulus or to a stimulus which would become noxious if prolonged" by the committee on taxonomy of the International Association for the Study of Pain (1). The committee also defines the term noxious stimulus as "a stimulus that is damaging to normal tissues". Excitation of nociceptors by a noxious mechanical, thermal or chemical stimulus results in the production of nociceptive information. Several types of nociceptive primary afferent neurons carry nociceptive information to the spinal cord. Tissue damage or inflammation also causes hyperalgesia, an increased response to a stimulus that is normally painful. A sensitization of the nociceptor response to a stimulus is involved in the peripheral mechanism of hyperalgesia. Chemical mediators, which are locally released in damaged or inflamed tissue, play an important role in both producing pain and sensitizing nociceptors (see ref. 2). These chemicals have a variety of structures such as peptides, amines, amino acids, lipids, nucleotides and nucleosides. Recent studies have revealed the molecular mechanisms of actions of such chemical mediators on nociceptors. Some chemicals, such as ATP, glutamate and serotonin, act on membrane receptors that are directly linked to ion channels to cause membrane depolarization (3). Other chemicals act indirectly through membrane receptors that are linked to intracellular second messenger systems (see ref. 4). The cellular and molecular mechanisms of peripheral pain have recently been reviewed by Cesare and McNaughton (5). In this short review, we will focus on more recent observations that would contribute to the elucidation of molecular mechanisms of maintenance, activation, inhibition and sensitization in cutaneous nociceptors. We will not cover some mediators, which have recently been reviewed, such as excitatory amino acids, bradykinin (3, 5), nociceptin/orphanin FQ (6), purine nucleotides and nucleosides (7).

Maintenance of nociceptors by neurotrophic factors

Nerve growth factor (NGF) has an important role in the development of the peripheral nervous system such as primary afferent sensory neurons and postganglionic sympathetic neurons. Furthermore, NGF seems to play an important role in regulating the sensitivity of nociceptors in the adult (see ref. 8). Application of high pharmacological doses of NGF produces hyperalgesia in humans and adult animals (8). However, it has been difficult to study the physiological role of NGF in the adult because of the absence of a selective antagonist against the NGF receptors, TrkA and p75. A synthetic fusion protein, the extracellular domain of the TrkA receptor coupled to the Fc portion of human immunoglobulin (Ig) G, has been developed and used to neutralize endogenous NGF. Sequestration of cutaneous NGF with TrkA-IgG for 10–14 days results in the reduction of the chemical and thermal, but not mechanical, sensitivity of nociceptors in adult rats (9, 10). Bennett et al. (10) have also shown epidermal innervation density to decrease in 44% after chronic TrkA-IgG treatment. Nerve fibers that enter the epidermis are considered to be the unmyelinated terminal endings of nociceptors. These findings demonstrate that endogenous NGF in the adult specifically modulates the terminal arborization of unmyelinated nociceptive primary sensory neurons and the sensitivity of primary afferent nociceptors to thermal and chemical stimuli in normal states. In some inflammatory states, NGF produced in the inflamed tissue would also take part in the production of inflammatory hyperalgesia because acute administration of TrkA-IgG inhibits carrageenan-induced hyperalgesia (9).

NGF exerts its actions via either TrkA or p75 receptor. A recent study has demonstrated that NGF selectively increases the expression of bradykinin binding sites on cultured dorsal root ganglion neurons from adult mouse via the p75 receptor (11). This finding suggests that the hyperalgesic action of NGF might be mediated by the p75 receptor. Since the trophic action of NGF seems to be mediated by the TrkA receptor, NGF might exert the trophic and hyperalgesic actions via the TrkA and p75 receptor, respectively. If such dissociation is true, a selective TrkA agonist and p75 antagonist may be applied for the treatment of peripheral neuropathies and inflammatory pain, respectively.

In the adult rodent, primary afferent nociceptors are largely divided into two histochemically distinct classes (12). One constitutively synthesizes neuropeptides such as substance P and calcitonin gene-related peptide (CGRP), expresses the TrkA receptor, and is responsive to NGF. The other does not express neuropeptides and downregulates the TrkA receptor after birth. The latter class can be identified by the binding of the lectin IB4 and the expression of the P_2X_3 receptor (12). Since NGF does not have any trophic action on IB4-positive sensory neurons in the adult, investigators have searched for a candidate for the neurotrophic factor on those neurons. Recent studies have demonstrated that IB4-positive sensory neurons express the receptor components (RET, GFR-1 and GFR-2) for glial cell line-derived neurotrophic factor (GDNF) and respond to GDNF (13, 14). GDNF belongs to the superfamily of transforming growth factor-β. In vitro, GDNF supports the survival of IB4-positive small neurons that express Ret while failing to support the survival of neurons expressing TrkA and CGRP (13). In vivo, treatment of GDNF, but not NGF, prevents several axotomy-induced changes in IB4-positive neurons (14). These findings suggest that GDNF is a trophic factor for IB4-positive sensory neurons and may be useful in the treatment of peripheral neuropathies, which are resistant to NGF treatment. Further studies are required to elucidate whether GDNF, as well as NGF, regulates the sensitivity of nociceptors to a noxious stimulus in normal and inflammatory states.

Activation and sensitization of nociceptors via the capsaicin receptor

One of the recent hottest findings on nociceptors is the cloning and identification of the capsaicin receptor, vanilloid receptor subtype 1 (VR1) (15). Capsaicin (8-methyl-vanillyl-6-nonenamide) is the pungent ingredient in hot chilli peppers of the *Capsicum* family. Capsaicin and related compounds are collectively referred to as vanilloids. Vanilloids selectively excite nociceptive primary afferent sensory neurons by interacting at a specific membrane recognition site, the vanilloid receptor, and elicit a sensation of burning pain (see ref. 16). Julius and co-workers have succeeded in the isolation of complementary DNA encoding the capsaicin receptor by using an expression cloning strategy (15). The expressed capsaicin receptor protein is a non-selective cation channel with a notable preference for calcium ion. Under physiological conditions, an activation of the capsaicin receptor will cause depolarization of membrane potential and then an action potential in nociceptive primary sensory neurons through influx of calcium and sodium ions.

In newborn animals, application of capsaicin selectively causes cell death of primary sensory neurons with the capsaicin receptor (16). Caterina et al. (15) showed that capsaicin induces necrosis in non-neuronal cells, HEK293 cells, transfected with VR1. This evidence suggests that capsaicin-induced cell death is due to the continuous and excessive influx of calcium ions through the VR1 channel. A similar mechanism has been proposed in the glutamate-induced excitotoxicity through the NMDA receptor.

The types of sensory neurons that express the VR1 protein have been revealed by Tominaga et al. (17). Im-

munocytochemical analysis has indicated that approx. 85% of substance P- and 60–80% of IB4-positive primary afferent fibers express VR1 immunoreactivity. Therefore, VR1 could account for the capsaicin sensitivity of both nociceptor types. At the same time, this finding provides evidence that there is a substantial population of IB4-positive neurons that do not express VR1. There might be a subpopulation of IB4-positive neurons that is either resistant to capsaicin action or sensitive by virtue of another receptor subtype.

What kinds of functions would the VR1 channel have in vivo? A possible answer is suggested by the same authors (15, 17). The cloned capsaicin receptor, VR1, is activated by noxious, but not innocuous, heat. Analysis of heat-evoked single channel currents in patches excised from VR1-transfected HEK293 cells suggests that heat gates the VR1 channel directly (17). This finding can account for a burning pain sensation produced by topical application of capsaicin to skin. The VR1 channel would have a function as a transducer of noxious thermal stimuli in vivo.

Capsaicin is not an endogenous substance, and the natural ligand for VR1 is still unknown. Protons have been shown to modulate VR1 function (see ref. 16). Protons enhance the capsaicin-evoked response by increasing its potency without altering efficacy (15, 17). The heat-evoked response is also potentiated by protons in either VR1-expressing HEK293 cells or *Xenopus* oocytes. Furthermore, protons decrease the temperature threshold for VR1 activation so that even moderately acidic conditions ($pH \leq 5.9$) activate VR1 at room temperature (22℃) (17). These findings account for the fact that a reduction of tissue pH resulting from infection, inflammation and ischemia produces the increased response to noxious stimuli (hyperalgesia). The excess protons produced in inflamed or ischemic tissues would produce hyperalgesia by an enhancement of VR1 function.

Further future studies on the capsaicin receptor, VR1, must contribute to our understanding of nociceptor functions and the development of new analgesics.

Inhibition of nociceptors by endogenous cannabinoids

Inhibitory mechanisms of pain are present not only in the central nervous system but also in the peripheral sensory nervous system. For example, endogenous opioid peptides secreted from immune cells produce peripheral analgesia by inhibiting either the excitability of nociceptors or the release of proinflammatory neuropeptides via opioid receptors on peripheral sensory nerve endings (see ref. 18). During inflammation, endogenous opioid peptides released from activated immune cells seem to modulate the sensitivity of nociceptors.

Endogenous cannabinoids, as well as opioid peptides, have recently been indicated to participate in the peripheral regulation of pain. Endogenous cannabinoids isolated and characterized are amides and esters of fatty acids, such as anandamide (*N*-arachidonoylethanolamine) (19). Two subtypes of cannabinoid receptors, termed CB1 and CB2, have been characterized and cloned to date (see ref. 20). Cannabinoids produce antinociceptive actions in rodents at both spinal and supraspinal sites (20). Recently, Calignano et al. (21) have shown that local administration of anandamide and palmitylethanolamide (PEA) into the paw with formalin attenuates the formalin-induced licking behaviour in mice through the CB1- and CB2-like receptor, respectively. The same report has provided evidence that sufficient amounts of anandamide and PEA to activate cannabinoid receptors are present in the skin. These findings suggest peripheral endogenous cannabinoids to have an inhibitory role in pain initiation.

Similar inhibitory actions of endogenous cannabinoids on nociceptors has reported one after another (22, 23). Peripheral, but not systemic, administration of a low dose of anandamide reduces carrageenan-induced thermal hyperalgesia, carrageenan-induced edema and capsaicin-induced plasma extravasation via interaction with the peripheral CB1 receptor (23). Anandamide possesses an inhibitory effect on capsaicin-evoked release of CGRP from isolated hind-paw skin (23). Therefore, cannabinoids may produce such inhibitory actions on inflammation by inhibiting neurosecretion from capsaicin-sensitive primary afferent fibers. Endogenous cannabinoids produced in the inflammatory tissues may serve as an antiinflammatory mediator because they also possess an immunosuppressive action (19).

Anandamide and PEA are likely to produce the antinociceptive effects via interaction with the peripheral CB1 and CB2 receptor, respectively (21–23). Both CB1 and CB2 receptors inhibit adenylate cyclase via a pertussis toxin-sensitive G protein (19). Activation of the cAMP second messenger system has been indicated to be involved in the mechanism of sensitization of nociceptors (2). Therefore, the inhibition of cAMP formation in nociceptive sensory nerve endings via the CB1 or CB2 receptors might be involved in the mechanism of peripheral antihyperalgesic action of cannabinoids (Fig. 1).

There are numerous anecdotal reports that suggest the benefits of cannabinoid in certain therapeutic conditions (see ref. 20). However, the psychotropic action and restricted availability of cannabinoids have made its therapeutic use for humans very difficult. The peripheral endogenous cannabinoid system may become a new target for the development of novel analgesic agents without untoward psychotropic effects.

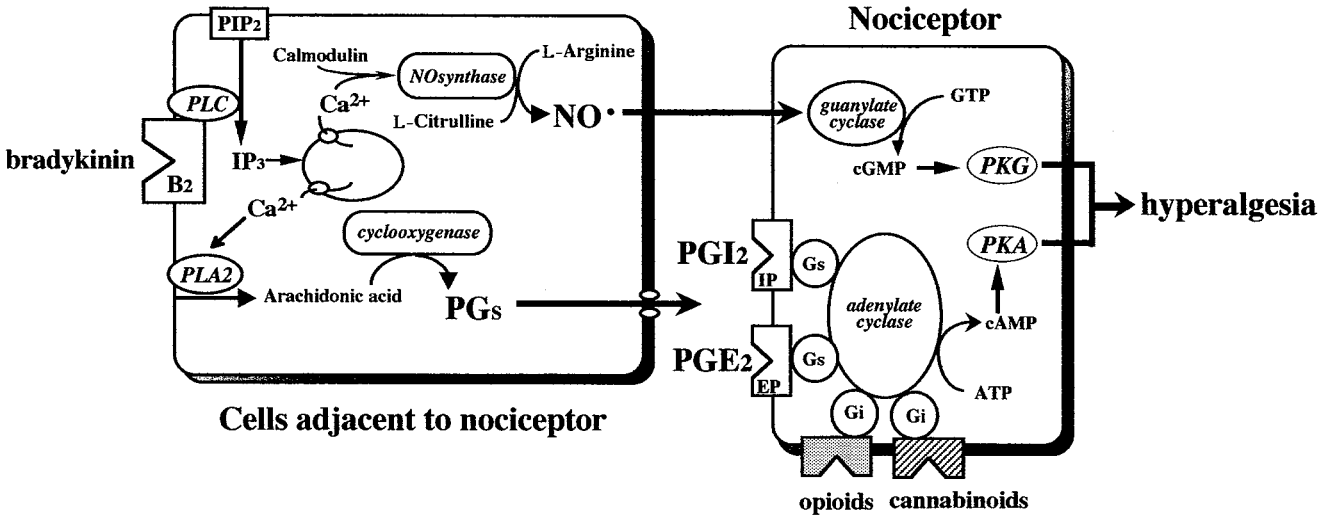

Fig. 1. Hypothetical schematic of transduction pathways involved in the production of peripheral hyperalgesia In the inflammatory state, chemical mediators such as bradykinin stimulate the production of PGs and NO in nociceptors or other cells adjacent to nociceptors. Released PGs such as PGE_2 and PGI_2 activate adenylate cyclase in nociceptors via the EP and IP receptor, respectively. Released NO penetrates into nociceptors and directly activates guanylate cyclase. Increased cAMP and cGMP then activates PKA and PKG, respectively. Activation of both PKA and PKG is essential to produce the peripheral mechanical hyperalgesia, while it remains unclear what kinds of protein are phosphorylated by PKA or PKG. Endogenous opioids and cannabinoids exert their antihyperalgesic action on nociceptors by inhibiting adenylate cyclase via their own receptors coupling to Gi protein.

Signal transduction involved in the sensitization of nociceptors

Numerous studies on the sensitization of nociceptors have focused on the molecular mechanism of action of prostaglandins (PGs) (see refs. 2 and 4). PGs such as PGE_2 and PGI_2 produce peripheral hyperalgesia by activating their own receptors on the peripheral sensory nerve endings (2, 24). In rat dorsal root ganglion cultures, activation of the EP or IP receptor results in the increase of intracellular cAMP levels (ref. 25 and our unpublished data). PGs-induced increase of intracellular cAMP seems to activate cAMP-dependent protein kinase (PKA) (2). Indeed, PGE_2-induced thermal hyperalgesia is reduced in mice that carry a null mutation in the gene that encodes the neuronal specific isoform of the type I regulatory subunit of PKA (26). These findings demonstrate that activation of the cAMP second messenger system is involved in the mechanism of PGs-induced sensitization of nociceptors (Fig. 1).

We have provided evidences that the nitric oxide (NO)-cGMP second messenger system also plays an important role in the sensitization of nociceptors (27). The peripheral mechanical hyperalgesia induced by bradykinin and carrageenan is abolished by intradermal administration of inhibitors of NO synthase, guanylate cyclase or cGMP-dependent protein kinase (PKG). Activation of the NO-cGMP pathway alone fails to produce peripheral hyperalgesia. Concomitant activation of the NO-cGMP pathway with the PGs-cAMP pathway synergistically induces peripheral hyperalgesia. Therefore, we hypothesize that activation of both cAMP- and cGMP-second messenger systems in nociceptors is required to produce peripheral hyperalgesia (Fig. 1).

Aley et al. (28), have recently reported that low levels of NO facilitate cAMP-dependent PGE_2-induced hyperalgesia, whereas higher levels of NO produces a cGMP-dependent hyperalgesia. Involvement of NO in the production of mechanical hyperalgesia is consistent with our findings. However, our data has demonstrated that NO is not involved in PGE_2-induced hyperalgesia (A. Nakamura et al., in preparation) and an activation of the NO-cGMP pathway alone fails to produce hyperalgesia (27). The cause of such a discrepancy is still unknown and further studies are necessary.

Non-steroidal antiinflammatory drugs such as aspirin and indomethacin exert their analgesic and antiinflammatory actions by inhibiting the PGs-cAMP pathway. Recently, NO has also been indicated to play an important role not only in the production of hyperalgesia, but also in other inflammatory processes (see ref. 29). Therefore, inhibitors of the NO-cGMP pathway may be useful as novel analgesics or antiinflammatory drugs.

Concluding remarks

It has become clearer that the nociceptor function is regulated by many endogenous chemicals and their own receptors. The chemicals and receptors reviewed here are involved in the maintenance, activation, inhibition and sensitization of nociceptors. Further studies on such chemicals and receptors would contribute not only to the understanding of the molecular mechanisms of nociceptors but also to the development of novel analgesics without central untoward effects.

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