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SENSORY AND SIGNALING MECHANISMS OF BRADYKININ, EICOSANOIDS, PLATELET-ACTIVATING FACTOR, AND NITRIC OXIDE IN PERIPHERAL NOCICEPTORS

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Pethő G, Reeh PW. Sensory and Signaling Mechanisms of Bradykinin, Eicosanoids, Platelet-Activating Factor, and Nitric Oxide in Peripheral Nociceptors. *Physiol Rev* 92: 1699–1775, 2012; doi:10.1152/physrev.00048.2010.—Peripheral mediators can contribute to the development and maintenance of inflammatory and neuropathic pain and its concomitants (hyperalgesia and allodynia) via two mechanisms. Activation or excitation by these substances of nociceptive nerve endings or fibers implicates generation of action potentials which then travel to the central nervous system and may induce pain sensation. Sensitization of nociceptors refers to their increased responsiveness to either thermal, mechanical, or chemical stimuli that may be translated to corresponding hyperalgesias. This review aims to give an account of the excitatory and sensitizing actions of inflammatory mediators including bradykinin, prostaglandins, thromboxanes, leukotrienes, platelet-activating factor, and nitric oxide on nociceptive primary afferent neurons. Manifestations, receptor molecules, and intracellular signaling mechanisms of the effects of these mediators are discussed in detail. With regard to signaling, most data reported have been obtained from transfected nonneuronal cells and somata of cultured sensory neurons as these structures are more accessible to direct study of sensory and signal transduction. The peripheral processes of sensory neurons, where painful stimuli actually affect the nociceptors in vivo, show marked differences with respect to biophysics, ultrastructure, and equipment with receptors and ion channels compared with cellular models. Therefore, an effort was made to highlight signaling mechanisms for which supporting data from molecular, cellular, and behavioral models are consistent with findings that reflect properties of peripheral nociceptive nerve endings. Identified molecular elements of these signaling pathways may serve as validated targets for development of novel types of analgesic drugs.

I. INTRODUCTION

Nociceptive primary afferent neurons represent the first neuron in the pain pathways. Major parts of these neurons are as follows: *1*) cell body (soma) located in the sensory dorsal root, trigeminal, nodose and jugular ganglia where most of the peptides and proteins required by the whole neuron are synthesized; *2*) peripheral terminal specialized for the detection of heat, cold, mechanical, and chemical stimuli that can induce pain; *3*) central terminal projecting to the dorsal horn of the spinal cord or to the brain stem; and *4*) axon interconnecting the above-mentioned elements. Membrane depolarizations induced in the peripheral terminal by an excitatory effect of thermal, mechanical, and/or chemical stimuli can evoke action potentials that propagate along the peripheral and central axon to the central nervous system. The peripheral ending-preterminal axon region contains three types of receptors/ion channels that mediate the above-mentioned process. First, various transducer proteins are responsible for conversion of the natural stimuli into locally spreading membrane depolarizations (receptor or generator potentials) mediated by influx of Na^+ and/or Ca^{2+}. They include several types of heat- or cold-activated ion channels, still largely putative mechanosensitive channels and a big family of G protein-coupled (metabotropic), ion channel-linked (ionotropic), or tyrosine kinase-linked receptors for various chemical mediators. Another set of ion channels in the peripheral terminal/preterminal axon region is involved in regulation of the electrical excitability, i.e., the efficiency of the conversion of receptor potentials into propagating action potentials. These include mainly various types of K^+ channels (e.g., voltage-gated, Ca^{2+}-activated, or ATP-sensitive channels) whose hyperpolarizing activity opposes the depolarizing effect induced by transducer protein activation. The preterminal axon is equipped with voltage-gated Na^+ channels that are acivated by membrane depolarization of sufficient magnitude and are responsible for the generation of action potentials. One subtype of these channels, $Na_v1.9$, however, appears not to be involved in spike generation; it rather contributes a depolarizing influence to resting potential and thereby regulates electrical excitability.

A huge array of endogenous chemicals contribute to sensory phenomena including pain and hyperalgesia that develop during inflammation and after tissue as well as nerve injury. Most of these mediators are not stored preformed but synthesized de novo at the site of injury. The agents contribute to pain via two principal mechanisms. Excitation of nociceptive nerve endings or fibers implicates generation of action potentials which then travel to the central nervous system and may directly induce pain sensations or, at least, "central sensitization" of spinal nociceptive transmission resulting in secondary hyperalgesia and allodynia, in particular to mechanical stimulation (625). Sensitization of nociceptors refers to their increased responsiveness to heat/cold, mechanical, or chemical stimulation that gives rise to primary hyperalgesia to these stimuli. If, for example, the threshold to noxious heat drops to 37°C or lower, a decrease by only 3–4°C, normal body temperature can become a driving force of nociceptor discharge and pain, and the discrimination between excitatory and sensitizing mechanisms becomes vain (431, 599). In fact, in most inflammatory and neuropathic disease states, both ongoing activity in sensory nerves and sensitization or hyperalgesia (to heating) are found in vivo. All three types of receptors/ion channels in the sensory nerve endings and preterminal axons (see above) are molecular targets for the intracellular signaling mechanisms underlying inflammatory mediator-induced spike generation and sensitization.

Bradykinin is one of the most potent pain-producing agents formed under inflammatory conditions, and a multitude of its excitatory and sensitizing effects on peripheral nociceptors have been described supporting its role as a prototype of peripheral pain mediators. A number of bradykinin effects have been shown to be mediated, at least in part, by products of the arachidonic acid-cyclooxygenase cascade, suggesting the existence of mutual interactions between the pain mediators (576). Acknowledging the significance of these secondary agents not only as bradykinin-related mediators but also as inflammatory mediators on their own right, this review aims to give an account of the excitatory and sensitizing actions of bradykinin, prostaglandins, leukotrienes, platelet-activating factor, and nitric oxide (NO) on nociceptive primary afferents. This group of selected mediators contains member(s) from both the peptide and lipid categories of endogenous locally acting regulatory substances (autacoids), and also NO that is a unique mediator both chemically and functionally. In accord with the recognition that inflammation (e.g., Wallerian degeneration) is a critical element of the pathological processes following nerve injury, contribution of these mediators to neuropathic pain is also discussed. The quasi-efferent tissue responses secondary to inflammatory mediator-induced release of neuropeptides such as substance P (SP) or calcitonin gene-related peptide (CGRP) from peptidergic nociceptors are not covered here, although this (measurable) neurosecretion can be used as an index of activation.

Although the major site where painful stimuli excite nociceptors in vivo is the peripheral terminal, this structure is inaccessible to direct investigation of membrane currents and intracellular signaling processes owing to its minute size. In contrast, somata of the primary afferent neurons are suitable for such examinations explaining why the bulk of data concerning inflammatory mediator effects on sensory neurons have been obtained studying the cultured cell bodies, assuming that they are reliable models of their peripheral terminals. This assumption has suffered a first major setback when mice with a targeted deletion of the heat-activated ion channel and capsaicin receptor TRPV1 (transient receptor potential vanniloid type 1) showed the expected loss of heat-activated current in capsaicin-sensitive dorsal root ganglion (DRG) neurons but almost normal nocifensive behavior and completely normal primary afferent discharge in response to moderate noxious heat (90, 777, 803). Another example is the functional expression of $GABA_A$ receptor channels in all DRG neurons as opposed to their lack in peripheral nerve axons and terminals (199). Thus selective trafficking, variable heteromerization, post-translational modifications of proteins may create relevant differences between the cellular model and the real nerve endings. In addition, biophysical parameters, e.g., surface-to-volume ratio, are entirely different, resulting in different mechanisms underlying (electrical) excitability (802). Even the ultrastructure is essentially different with cell bodies in the DRG being rich in endoplasmic reticulum and calcium store capacity, while in nerve endings, bare of endoplasmic reticulum, calcium-operated mechanisms such as neurosecretion depend entirely on influx from extracelluar space.

These differences nurture doubts whether signaling mechanisms revealed in the cellular models translate to nerve terminals, whole animal behavior and finally humans. On the other hand, behavioral studies are not considered the last resort in pain research providing unequivocal evidence. Just for example the ongoing debate should be mentioned whether or not the mustard oil receptor transient receptor potential ankyrin type 1 (TRPA1) is involved in noxious cold sensing and its deletion results in a behavioral deficit (333, 369). At least, the cognitional gap between cellular models and behavior can be filled with methodologies such as recording of action potentials from teased single-fibers or cell bodies in continuity with their peripheral receptive fields (777, 801). Another, more global, technique focusing on the periphery is the measurement of basal and stimulated release of neuropeptides as an index of nociceptor activation in a large variety of ex vivo preparations (33). These peripheral nerve axons of primary nociceptive neurons that can serve, to a certain extent, as a model of their terminals, sharing remarkable sensory and neurosecretory capabilities with the nerve endings (288). According to the above considerations, the major aim of the present review is to highlight those molecular mechanisms and membrane targets of the discussed inflammatory mediators that have been revealed both in cellular models and in paradigms reflecting the activity of peripheral nociceptors and which are therefore likely to play a role in peripheral nociception in vivo. To our knowledge, no other review employed systematically this approach. To facilitate this, in each chapter data referring to the cell body or the peripheral endings are dissected: in some parts of the review this distinction is made under different section headings but in other parts, for didactical reasons, data are separated in different paragraphs of the same section. Identification of these peripheral targets may help drug development during search for novel analgesics acting on peripheral nociceptors.

Considerable evidence indicates that most agents considered as inflammatory mediators can also influence transmission of nociceptive signals at the level of the spinal cord or higher brain centers. It must be emphasized that only data showing their peripheral effects are covered in this review as the intricately entwined central mechanisms of primary afferent nociception are sufficient challenge for both writer and reader. Therefore, data obtained in experimental arrangements in which central actions of the mediators cannot be excluded are treated with caution. This refers to studies having used knockout animals or systemically applied receptor antagonists, channel blockers, and enzyme inhibitors possibly passing the blood-brain barrier. Upon description of these data, these experimental conditions and the consequent limitations are emphasized.

II. ROLE OF BRADYKININ IN PERIPHERAL MECHANISMS OF NOCICEPTION

A. Synthesis and Breakdown of Bradykinin

The nonapeptide bradykinin and the related decapeptide kallidin (lysyl-bradykinin) collectively termed kinins can be synthesized both intravascularly (in the plasma) and extravascularly in tissues. These peptides are cleaved from their protein precursors called kininogens by proteolytic enzymes, the kallikreins which are formed from their precursors, the prekallikreins. The plasma prekallikrein is converted to kallikrein when the clotting factor XII (Hageman) is activated by contact with negatively charged surfaces. Plasma kallikrein acts on high-molecular-weight kininogen producing bradykinin and kallidin which act preferentially on B_2 bradykinin receptors. The tissue prekallikrein is transformed to kallikrein in response to inflammation or tissue damage. The substrates for tissue kallikrein are high- and low-molecular-weight kininogens. The kinins are metabolized rapidly by kininase I and II and have a half-life of <1 min in plasma. Kininase I cleaves only the COOH-terminal arginine residue yielding des-Arg^9-bradykinin and des-Arg^{10}-kallidin which retain biological activity on B_1 bradykinin receptors, while kininase II, which is identical to angiotensin-converting enzyme, cleaves two amino acids from the COOH-terminal resulting in inactive fragments. Kininase activity is reduced in an acidic environment which can be a factor contributing to higher levels of bradykinin during inflammation (169, 268). Recently, an alternative pathway of degradation by membrane-bound metalloendopeptidases was revealed. In cultured rat trigeminal sensory neurons, the metalloendopeptidases 24.15 and 24.16 as well as B_2 bradykinin receptors were shown to associate with lipid rafts (317). Inhibition of these endopeptidases led to an increase in various bradykinin effects including inositol phosphate accumulation, TRPV1 sensitization to capsaicin, and heat hyperalgesia (317, 238). These data suggest that these ectoenzymes can breakdown bradykinin and thereby regulate the degree of B_2 receptor activation.

B. Expression and Role of Bradykinin Receptors

1. Types and general features of bradykinin receptors

Two types of receptors for bradykinin and related kinins, termed B_1 and B_2, have been characterized (for review, see Refs. 261, 467, 601, 603) and identified by molecular cloning (275, 480, 486). Bradykinin and kallidin preferentially act at the B_2 receptors while des-Arg^9-bradykinin and des-Arg^{10}-kallidin produced by kininase I from bradykinin and kallidin, respectively, are selective agonists of the B_1 receptors. The B_2 receptors are largely constitutive, being present at a relatively constant density on various cells including smooth muscle, endothelium, macrophages, postganglionic sympathetic fibers, and nociceptive primary afferent neurons (for review, see Refs. 261, 467). In contrast, the B_1 receptors are typically inducible, found only at a low level under resting conditions but largely upregulated upon tissue trauma, inflammation, or nerve injury as a result of increased gene transcription followed by protein synthesis (2, 468, 603). In uninflamed tissues, most effects of bradykinin are predominantly mediated by B_2 receptors as shown by a large number of studies employing selective peptide (e.g., HOE 140 also known as icatibant, NPC 18521) and non-peptide B_2 receptor antagonists (e.g., WIN 64338, FR 173657, NPC 18884, bradyzide) as well as B_2 receptor knockout mice (see sect. II*D*). Consistent with this, selective B_1 receptor agonists typically do not activate or sensitize nociceptors under normal conditions.

Of the extracellular inflammatory mediators, interleukin (IL)-1β and tumor necrosis factor (TNF)-α have been shown to induce B_1 receptors (83, 132, 135, 144, 227, 568, 570). B_1 receptor induction by bacterial lipopolysaccharide (LPS) was inhibited by glucocorticoid pretreatment, cyclooxygenase (COX) blockade, and agents inhibiting the synthesis or action of TNF-α (82, 84, 205, 608); moreover, endogenous glucocorticoid hormones were shown to exert a tonic inhibitory control on receptor expression through a NF-κB-mediated pathway (78). In a model of experimental colitis in mice, B_1 receptor upregulation depended on de novo protein synthesis, NF-κB activation, TNF-α production, and inducible NO synthase (iNOS) activity (267). Platelet-activating factor (PAF), a proinflammatory mediator, was shown to upregulate B_1 receptors in the rat skin through a pathway involving neutrophils and a NF-κB-TNF-α-IL-1β axis (181, 182). A low-dose phorbol ester treatment failed to alter B_1 receptor mRNA levels in the mouse paw; however, it increased the amount of B_1 receptor protein, suggesting that this upregulation occurred at the level of translation (187). In addition to inducibility, another striking difference between the two bradykinin receptor subtypes is that while B_2 receptors undergo a rapid agonist-induced desensitization (see sect. II*C4*), B_1 receptors do not exhibit any notable tachyphylaxis (27, 475).

Evidence has been provided that B_1 and B_2 receptors may contribute to development of pain and hyperalgesia not only in the periphery but also in the spinal cord and even higher centers (184, 206, 571, 592). These data make it difficult to interpret nociceptive deficits observed in studies employing kinin receptor knockout animals and systemically applied kinin receptor antagonists possibly passing the blood-brain barrier. However, the most widely used peptide bradykinin receptor antagonists, HOE 140 (icatibant) and des-Arg^9,Leu^8-bradykinin, blocking both B_2 and B_1 receptors, respectively, were reported not to have central effects on systemic administration (755, 757) meaning that data obtained with these agents are likely to reflect peripheral contribution of endogenous kinins (similarly to those gained with locally applied antagonists).

Experimental data concerning the upregulation and functional significance of bradykinin receptors in evoked nociception as well as in inflammatory nociception and hyperalgesia are summarized in **TABLE 1**.

2. *Role of bradykinin receptors in acute nocifensive behavior evoked by chemical agents in uninflamed tissues*

Compatible with the widely accepted constitutive expression of B_2 receptors, the early phase (0–5 min) of the formalin-induced nocifensive reaction reflecting acute and direct nociceptor activation was diminished by both peptide (104, 115) and non-peptide B_2 receptor antagonists (138, 139) in both rats and mice **(TABLE 1)**. The same holds true for the kaolin-, acetic acid-, or acetylcholine-induced acute abdominal writhing response as well as capsaicin-evoked paw licking in mice (138, 139, 273, 304). The hindpaw licking behavior in mice induced by intraplantar injection of trypsin, a nonselective agonist of proteinase-activated receptors (PAR), and recorded over 10 min was reduced in either B_2 or B_1 receptor gene deficient animals compared with wild-types (119). The latter result indicates that although B_1 receptors are weakly expressed (but inducible), they indeed can play a role in acute chemonociception perhaps as a conditioning factor. The peptide B_1 receptor antagonist des-Arg^9,Leu^8-bradykinin reduced the early phase of the nociceptive response to formalin in mice and rats (115, 652, 682). The early phase of the formalin-induced nociception (similarly to the acetic acid-induced writhing in mice) was also reduced by a non-peptide B_1 receptor antagonist in rats and in B_1 receptor knockout mice (571, 582). The algogenic effect of intraplantarly applied capsaicin was also diminished in B_1 receptor knockout mice (571). As the early phase of the formalin response similarly to capsaicin, acetic acid, or trypsin-evoked nocifension develops within 0.5–1 min and lasts for 5–10 min, these results suggest that the constitutive B_1 receptor expression in uninflamed tissue should not be underestimated (see sect. II*B5*) and/or that the induction process is rapid probably at the level of translation or translocation (187). In accord, selective B_1 receptor agonists such as des-Arg^9-bradykinin or des-Arg^{10}-kallidin were shown to *1*) excite and/or sensitize cutaneous nociceptors in naive monkeys or humans (172, 350), *2*) cause nocifensive behavior in the mouse paw (319, 582; see however Ref. 187), and *3*) induce mechanical hyperalgesia in the rat hindpaw (581).

3. *Accumulation of bradykinin under inflammatory conditions*

In various clinical (oral surgery, rheumatoid arthritis) and animal (carrageenan) models of inflammation, levels of bradykinin in plasma were found elevated (268). Of course, the concentration of bradykinin is elevated in inflammatory exudates. For example, following impacted third molar extraction in humans, immunoreactive bradykinin levels rose in the microdialysate of the surgical site and flurbiprofen (a nonselective COX inhibitor) pretreatment reduced this response, suggesting a role for prostanoids in bradykinin upregulation (688). In the same model, 1–3 h after surgery both B_2 (bradykinin and kallidin) and B_1 receptor ligands (des-Arg^9-bradykinin and des-Arg^{10}-kallidin) were upregulated along with increased B_2 and B_1 receptor mRNA levels in biopsy specimens (263). Similarly, elevated extracellular levels of bradykinin were detected by dental pulp microdialysis in patients with pulpitis (420). In trapezius muscle of patients with work-related trapezius myalgia, interstitial

Table 1. *Upregulation and functional role of bradykinin receptors in states associated with evoked nociception, inflammatory or neuropathic hyperalgesia*

Experimental Model	B_1 Receptor		B_2 Receptor	
	Upregulation	**Functional role**	**Upregulation**	**Functional role**
Evoked nocifensive reaction in uninflamed or inflamed tissue				
Formalin-induced nociception, 1st phase		115 (m), 652 (m), 682 (r), 582 (r), 571 (m)		115 (m), 104 (r) 139 (m), 138 (m)
Capsaicin-induced nocifensive reaction		571 (m)		138 (m)
Trypsin-induced nocifensive reaction		119 (m)		119 (m)
Kaolin-induced writhing				273 (m), 139 (m)
Acetic acid-induced writhing		582 (m)		273 (m), 139 (m) 138 (m), 304 (r)
Acetylcholine-induced writhing				139 (m)
Formalin-induced nociception, 2nd phase		115 (m), 104 (r), 652 (m), 682 (r), 619 (m), 571 (m), 241 (r), 582 (r), 582 (m)		115 (m), 104 (r) 259 (r), 139 (m) 138 (m)
PMA-induced nocifensive reaction	Protein: 187 (m)	187 (m)		188 (r)
Scorpion venom-induced nocifensive reaction		572 (r)		572 (r)
Sarcoma cell inoculation-induced nocifensive reaction		651 (m)		
Melanoma cell inoculation-induced nocifensive reaction	mRNA: 216 (m, DRG)	216 (m)		216 (m)
Inflammatory states associated with hyperalgesia				
Molar extraction-induced inflammation	mRNA: 263 (h)		mRNA: 263 (h)	
CFA-induced inflammation	Protein: 206 (r, DRG)	567 (r, M), 133 (r, M) 682 (r, M), 206 (r, M) 205 (m, M), 185 (m, H) 582 (r, H)		567 (r, M) 133 (r, M) 77 (r, M) 185 (m, H) Against: 619 (m, H) Against: 185 (m, H)
Enhanced bradykinin responsiveness of cutaneous nociceptors in CFA inflammation		Against: 39 (r)		39 (r)
Carrageenan-induced inflammation		619 (r, M) 581 (r, M) 157 (rabbit, M) 582 (r, H)		192 (r, M), 189 (r, M) 581 (r, M), 138 (r, M) 126 (m, M), 157 (rabbit, M), 619 (m, H), 303 (r, H)
Carrageenan- or bradykinin-induced mechanical hyperalgesia in LPS-primed mice		126 (m)		
Zymosan-induced mechanical hyperalgesia	mRNA: 48 (r)	48 (r)		48 (r)
LPS-induced mechanical hyperalgesia		581 (r)		192 (r), 189 (r) 581 (r)

Table 1—*Continued*

Experimental Model	B_1 Receptor		B_2 Receptor	
	Upregulation	Functional role	Upregulation	Functional role
Plantar incision	Against mRNA: 520 (m)	219 (r, H) Against: 419 (r, M) Against: 419 (r, H)	Against mRNA:520 (m)	219 (r, H) 520 (m, M) Against: 419 (r, M) Against: 419 (r, H)
Ultraviolet irradiation-induced heat hyperalgesia		567 (r) 241 (r)		
Bradykinin-induced heat hyperalgesia after ultraviolet irradiation		567 (r)		
Heat injury-induced heat hyperalgesia				219 (r)
Antigen-induced knee joint inflammation	Against protein: 650 (r, DRG)		Protein: 650 (r, DRG)	
NGF-induced heat hyperalgesia		615 (r)		
Capsaicin-induced mechanical hyperalgesia		134 (r)		134 (r)
IL-1β-, IL-2-, or IL-8-induced mechanical hyperalgesia		135 (r)		135 (r)
PAR-2 receptor activation-induced colonic hyperalgesia to capsaicin				340 (m)
PAR-4 receptor activation-induced mechanical sensitization of joint afferents				621 (r)
Exercise-induced delayed-onset muscle soreness		Against: 518 (r)		518 (r)
Scorpion venom-induced mechanical hyperalgesia		572 (r)		572 (r)
Melanoma cell inoculation-induced mechanical allodynia		Against: 216 (m)		216 (m)
Experimental pancreatitis-induced abdominal cutaneous hyperalgesia	mRNA: 99 (r, DRG) mRNA: 705 (m, DRG)	705 (m)	mRNA: 99 (r, DRG) mRNA: 705 (m, DRG)	99 (r) 705 (m)
Turpentine cystitis-induced mechanical hyperreflexia		310 (r)		310 (r)
Neuropathic states associated with hyperalgesia				
Ligation of the sciatic nerve	Protein: 574 (r) 168 (r)		Protein: 574 (r) 168 (r)	
Partial ligation of the sciatic nerve	Protein: 595 (m) 573 (r) mRNA: 183 (m)	183 (m, M) 573 (r, H) 183 (m, H) Against: 205 (m, M)	Protein: 573 (r)	573 (r, H)
Chronic constriction injury to the sciatic nerve	mRNA: 428 (r), 784 (r)	428 (r, H) 784 (r, H, M) 241 (r, H)	mRNA: 428 (r), 784 (r)	428 (r, H) 784 (r, H, M)
Infraorbital nerve constriction injury		444 (r, m, H)		444 (r, m, H)
Crush injury to the sciatic nerve			mRNA: 412 (m)	

Table I—*Continued*

Experimental Model	B_1 Receptor		B_2 Receptor	
	Upregulation	Functional role	Upregulation	Functional role
Spinal nerve ligation	Protein: 770 (r)	770 (r, H, M, C) 582 (r, H)	Protein: 770 (r)	770 (r, H, M, C)
Brachial plexus avulsion		592 (m, M)		
Streptozotocin-induced diabetes		226 (m, H), 225 (m, H) 224 (m, H), 222 (r, H) 223 (m, H), 73 (r, M)		73 (r, M)
Vincristine-induced neuropathy		73 (r, M)		73 (r, M)

Upregulation refers to increased mRNA or protein level. Functional role was revealed by using bradykinin receptor antagonists or bradykinin receptor knockout animals. r, Rat; m, mouse; c, cat; h, human; H, heat hyperalgesia; M, mechanical hyperalgesia; C, cold allodynia; against, the study provided evidence against the the upregulation or functional role of the bradykinin receptor. For other abbreviations, see text.

concentrations of kallidin and bradykinin were higher at rest and following exercise, respectively, compared with control, as assessed by microdialysis (233). During sustained isometric trapezius muscle contraction in humans, a correlation was found between tissue levels of kinins (bradykinin plus kallidin) and pain ratings (58). In mice in which melanoma cells were inoculated intraplantarly, tissue levels of bradykinin and related peptides were increased compared with healthy skin (216). Kininase activity is reduced in an acidic environment that can be a factor contributing to higher levels of bradykinin during inflammation (169).

A) ROLE OF BRADYKININ RECEPTORS IN NOCIFENSIVE BEHAVIOR INDUCED BY INFLAMMATION. In various models of inflammation, bradykinin receptors were shown to be involved in nocifensive behavior **(TABLE 1)**. The late phase (15–30 min) of the formalin-induced nocifensive reaction is considered to be aggravated by sensitizing actions of various inflammatory mediators released/produced in consequence of the assault. In this response, an involvement of B_1 receptor activation in both mice and rats was revealed using either peptide (104, 115, 619, 652, 682) or nonpeptide antagonists (241, 582) as well as gene-deficient mice (571). In addition, a contribution of B_2 receptor activation to the response became also evident using predominantly peptide antagonists (104, 115, 138, 139, 259). B_2 receptor antagonism also reduced formalin-induced edema (115, 139). Phorbol ester (PMA) injection into the mouse hindpaw evoked nocifensive behavior observed in a 15- to 45-min period that was slightly diminished by local B_2 receptor antagonism and abolished by the B_1 receptor antagonist des-Arg^9,Leu^8-bradykinin or in B_1 receptor knockout animals (188, 187). The nocifensive behavior and edema induced by scorpion venom injection into rat hindpaw was reduced by either B_2 or B_1 receptor antagonist given 30 min prior to toxin into the paw (572). In a murine model of bone cancer pain, selective antagonism of the B_1 receptor resulted in a diminishment of nociceptive behavior (651). Spontaneous licking observed from day 18 after inoculation of melanoma cells into hindpaws of mice was reduced by local injection of either a B_2 or B_1 receptor antagonist (216). In this model, B_1, but not B_2, receptor mRNA was upregulated in the L4/L5 dorsal root ganglia (DRGs).

B) ROLE OF BRADYKININ RECEPTORS IN INFLAMMATORY HYPERALGESIA. In various models of persistent inflammation, activation of the induced, i.e., de novo synthesized B_1 receptors, has been shown to gain importance in the maintenance of hyperalgesia with or without concomitant activation of B_2 receptors depending on the model studied (see also Ref. 2 and **TABLE 1**). Some typical experimental paradigms demonstrating this are described below. It was also shown that during inflammation not only the B_1 receptors are upregulated but also their endogenous agonists des-Arg^9-bradykinin and des-Arg^{10}-kallidin (597). Moreover, des-Arg^{10}-kallidin was shown to activate NF-κB and to induce a homologous upregulation of B_1 receptors in cultured human lung fibroblasts (637). The induction of B_1 receptor-mediated hyperalgesia was shown to depend on prostanoid formation in some studies (126, 135, 581). Consistent with this, B_1 receptor agonists were shown to increase the release of PGI_2 from various cell types (80, 158, 724).

The carrageenan-induced mechanical hyperalgesia was diminished by either B_1 or B_2 receptor-selective antagonists in rats (126, 138, 189, 192, 581, 619). B_2 receptor antagonism, however, also reduced carrageenan-induced edema (138). In mice, the hyperalgesic effect of carrageenan 3–5 h after challenge was mediated by B_2 but not B_1 receptors, however, from 7 h after carrageenan injection hyperalgesia started to be mediated by B_1 receptors while the contribution of B_2 receptors diminished (126). Likewise, in a novel inflammatory pain model in the rabbit, carrageenan-induced mechanical hyperalgesia was prevented by icatibant, whereas an established hyperalgesia was reversed by des-Arg^9,Leu^8-bradykinin (157).

Mechanical hyperalgesia evoked by complete Freund's adjuvant (CFA) was inhibited by the selective B_1 receptor antagonist des-Arg^{10}-HOE 140 applied subcutaneously (206). One day after CFA treatment there was an increase in B_1 receptor protein expression in neurons of both ipsi- and contralateral DRGs (206). While in naive rats des-Arg^9-bradykinin failed to evoke mechanical hyperalgesia upon intra-articular or intradermal administration, following CFA pretreatment the B_1 agonist became able to evoke or aggravate hyperalgesia (133, 206, 354). In naive rats, the hyperalgesic effect of intra-articular or intradermal bradykinin was mediated by B_2 receptors, after CFA treatment both B_1 and B_2 receptors contributed to the response (133, 354). The heat hyperalgesia induced by CFA appeared intact in B_2 receptor knockout mice, but it was considerably reduced in B_1 receptor knockouts and by various B_1 receptor antagonist both in mice and rats (185, 582, 619). Genetic deletion of B_1 receptors caused no change in CFA-induced edema (185).

The heat hyperalgesia evoked by ultraviolet irradiation of the rat hindpaw was both prevented and reversed by peptide or non-peptide B_1 receptor antagonist but only slightly by icatibant (241, 567). This hyperalgesia was further increased by des-Arg^9-bradykinin or bradykinin, agents that failed to evoke heat hyperalgesia in naive animals (569). This aggravating effect of both agents was inhibited by des-Arg^9,Leu^8-bradykinin but not icatibant. Heat hyperalgesia induced by a mild heat injury to the rat hindpaw was reduced by icatibant applied intraplantarly 10 min after injury, whereas a B_1 receptor antagonist was largely ineffective (219). In contrast, incision-evoked heat hyperalgesia measured 18 h after injury in the rat was reduced by either icatibant or des-Arg^9,Leu^8-bradykinin applied locally (219; see, however, Ref. 419). A role for B_1 and/or B_2 receptors in mechanical or heat hyperalgesia was revealed in several other inflammatory paradigms **(TABLE 1)**.

All these data support the hypothesis that in the beginning of the inflammatory process kinin actions are predominantly mediated by the constitutive B_2 receptors which, however, soon tend to desensitize. Along with this, the kininase I products (des-Arg^9-bradykinin and des-Arg^{10}-kallidin) accumulate and start to act on the newly synthesized non-desensitizing B_1 receptors, thereby sustaining the actions of kinins. According to this mechanism, B_1 receptors may have an important role in persistent inflammatory pain which is reflected by the antinociceptive activity of novel non-peptide B_1 receptor antagonists (241, 582).

The above view is, however, not supported by some studies. Plantar incision-induced mechanical hyperalgesia was reduced by pretreatment with icatibant, but not des-Arg^{10}-HOE-140, from 2 h to 3 days post surgery in mice (520). The enhanced bradykinin responsiveness of cutaneous nociceptors in the inflamed isolated skin from CFA-pretreated rats was exclusively mediated by B_2 receptors (39). Although the thermal hyperalgesia and paw swelling induced by carrageenan was much less in mutant kininogen-deficient rats, a non-peptide B_2 receptor antagonist attenuated hyperalgesia and swelling in normal rats to a degree seen in the mutant animals (303). Unexpectedly, in DRG neurons from rats with antigen-induced knee joint inflammation, B_2, but not B_1, receptors were upregulated both in the acute and chronic phase of arthritis (650). In this study, only B_2 receptor expression was revealed both in control and inflamed rats.

4. *Role of bradykinin receptors in neuropathic hyperalgesia*

Similarly to inflammation, nerve injury, which may involve an inflammatory response, at least in some models, can also upregulate bradykinin receptors **(TABLE 1)**. Two and 10–14 days after unilateral sciatic nerve ligation in the rat, an increase in the number of both bradykinin receptor subtypes was observed in ipsilateral L4/L5 DRG neurons (168, 573, 574). This increase included a de novo expression of B_1 receptors and an enhancement of the density of preexisting B_2 receptors, and it was accompanied by an increase in the number of bradykinin binding sites on individual neurons. Chronic constriction injury (CCI) to the rat sciatic nerve led to similar changes: an increased B_2 receptor mRNA expression on day 2 and a marked increase in preexisting B_1 receptor mRNA expression 14 day after surgery (428, 784). Following crush injury of the sciatic nerve in mice, B_2 receptor mRNA level was elevated in DRG neurons peaking on day 7, and this upregulation was due to an increase in the mRNA content of the neurons (412). Partial ligation of the mouse sciatic nerve induced an upregulation of preexisting B_1 receptor mRNA expression in ipsilateral paw, sciatic nerve, and spinal cord (183). These latter data imply a role for B_1 receptor activation in central sensitization and generation of ectopic action potentials as nerve axons express various G protein-coupled receptors that sensitize their membranes to heat and, likely, other stimuli which could contribute to ectopic discharges (200). Somewhat discordant data have been obtained in another study employing the same model: preexisting B_2 receptor expression in small-diameter DRG neurons was strongly downregulated while B_1 receptors were induced in the large-diameter neurons and satellite cells of DRGs (595). In the above-mentioned models of neuropathic pain based on direct lesion of the sciatic nerve and inducing some kind of inflammation and sprouting, heat hyperalgesia was consistently shown to be mediated by both B_2 and B_1 receptors (183, 241, 428, 444, 573, 784). Regarding mechanical allodynia, a role for B_1 receptors was revealed in some (183, 784) but not other studies (205, 573).

Also relevant for neuropathic pain, 5–24 h after sural nerve transection in anesthetized rats, bradykinin applied to the nerve stump excited 7% of C-fibers and 1% of A-fibers tested, showing development of some ectopic chemosensitivity of axotomized sensory neurons (493).

In rats with L5/L6 spinal nerve ligation, an enhanced expression of B_1 and B_2 receptor protein in ipsilateral L4-L6 spinal nerves and hindpaw skin was revealed on day 12 after injury (770). B_1 and B_2 receptor levels were increased in the dorsal horn as well. In this model, an intraplantarly applied B_1 receptor agonist that was ineffective in naive rats evoked a nocifensive reaction, and an enhancement of B_2 the agonist-induced response was also noted. The nerve injury-induced cold and heat hyperalgesia as well as mechanical allodynia all were inhibited by either des-Arg^9-(Leu^8)-bradykinin or HOE 140. A novel non-peptide B_1 receptor antagonist reduced heat, but not mechanical, hyperalgesia 7 days after injury, and similar results were obtained in B_1 receptor knockout mice (582).

Brachial plexus avulsion-evoked mechanical and heat hyperalgesia in mice were abolished in B_1 receptor knockout mice for 80 days after injury, whereas in B_2 knockouts only a slight and transient (4–6 days) reduction of mechanical hyperalgesia was observed (592). Selective peptide and nonpeptide B_1 receptor antagonists applied either locally or systemically at the time of injury prevented development of mechanical hyperalgesia for 7–10 days thereafter, whereas upon intrathecal or intracerebroventricular application they were ineffective. In contrast, when applied 4 days after injury, both systemic and intrathecal applications reduced mechanical hyperalgesia. Finally, 30 days after injury, intracerebroventricular administration of these antagonists led to a pronounced antihyperalgesic effect. These results suggest a role for B_1 receptors in early stage of this type of nerve injury in the periphery followed by a later involvement of these receptors at the level of the spinal cord and subsequently at higher brain areas.

Regarding metabolic neuropathy, a B_1 receptor agonist aggravated, whereas different peptide B_1 receptor antagonists diminished heat hyperalgesia induced by experimental diabetes in both rats and mice; moreover, hyperalgesia was totally absent in B_1 receptor knockout mice (222–226). The vincristine-induced mechanical hyperalgesia of rats was inhibited by either the B_1 receptor antagonist des-Arg^{10}-HOE 140 or the B_2 receptor antagonist HOE 140 (73).

The above data suggest that in several animal models of neuropathic pain, an upregulation/induction of B_1 receptors occurs along with a functional role in heat, and to a lesser degree, mechanical hyperalgesia. In most models, preexisting B_2 receptor density is also increased and B_2 receptor activation contributes to hyperalgesia too.

5. Localization of B_1 receptors

While there is unequivocal evidence that the B_2 bradykinin receptor protein and mRNA are localized in sensory neurons (412, 428, 573, 574, 595, 647, 648, 746, 770), conflicting data have been obtained concerning the localization of B_1 receptors. Some studies failed to detect either a constitutive expression or an induction of B_1 receptor mRNA or protein in cultured rat or mouse sensory neurons (65, 136, 574, 595, 648). Therefore, an indirect mechanism was proposed for the B_1 receptor-mediated hyperalgesia according to which activation of the induced B_1 receptors on nonneuronal cells leads to release of mediators, e.g., cytokines and prostanoids, which in turn sensitize the adjacent nociceptors. In other studies, B_1 receptor mRNA was detected in DRG neurons of the mouse, rat, and monkey as well as in plantar skin, sciatic nerve, and spinal cord of mice (183, 428, 647, 656). Constitutive B_1 receptor protein expression in both peptidergic (expressing SP and CGRP; Ref. 449) and nonpeptidergic rat sensory neurons (449, 779) giving rise to C and Aδ fibers has been shown with immunocytochemistry which would allow for a direct effect of B_1 receptor agonists on nociceptors (206, 448). In subsequent studies, Western blot analysis and quantitative autoradiography confirmed the presence of B_1 receptor protein in rat spinal nerves and DRG neurons (573, 770). Furthermore, a very low level of B_1 receptor mRNA expression and B_1 receptor agonist-induced translocation of PKCε (see sect. II*C1*) were revealed in freshly isolated rat and mouse sensory neurons of the nonpeptidergic IB_4-positive type (746). Both B_1 receptor expression and B_1 receptor-mediated PKCε translocation were strongly increased by glial cell-derived neurotrophic factor (GDNF) but not nerve growth factor (NGF), and after GDNF treatment, a B_1 receptor agonist induced a much more sustained facilitation of the heat-activated membrane current than that produced by B_2 receptor activation. In an earlier study, however, NGF, unlike GDNF, was able to increase the number of bradykinin binding sites on adult mouse sensory neurons via the neurotrophin receptor p75 (575).

C. Signal Transduction Mechanisms of Bradykinin Receptors

This section describes those aspects of bradykinin receptor signaling that were predominantly revealed in transfected host cells or somata of cultured sensory neurons when the readout was a response at the (sub)cellular level (e.g., alteration of intracellular second messenger or Ca^{2+} concentrations, induction of membrane current, or neuropeptide release, etc.). Signaling mechanisms revealed during analysis of the nocifensive/spike-generating as well as the various sensitizing actions of bradykinin (when the readout was an increase in pain behavior/spike discharge and heat, mechanical or chemical responsiveness of nociceptive neurons) are discussed in the respective sections (see sect. II*D*, *1–4*) and are mentioned here only briefly.

Both B_1 and B_2 receptors belong to the family of G protein-coupled plasma membrane receptors consisting of seven transmembrane regions. Generally they appear to utilize similar signal transduction mechanisms. As the B_2 receptors predominantly mediate the acute effects of bradykinin and kallidin, most of our knowledge concerning the biochemical background of the actions of the kinins refers to signaling mechanisms of the B_2 receptors. The most important signaling mechanisms of bradykinin receptors are shown in **FIGURE 1**.

1. PLC, PKC, and intracellular Ca^{2+} in bradykinin receptor signaling

A depolarizing effect of bradykinin on somata of rabbit nodose, i.e., vagal, ganglion neurons was observed 30 years ago (277). The depolarizing effect of bradykinin on cul-

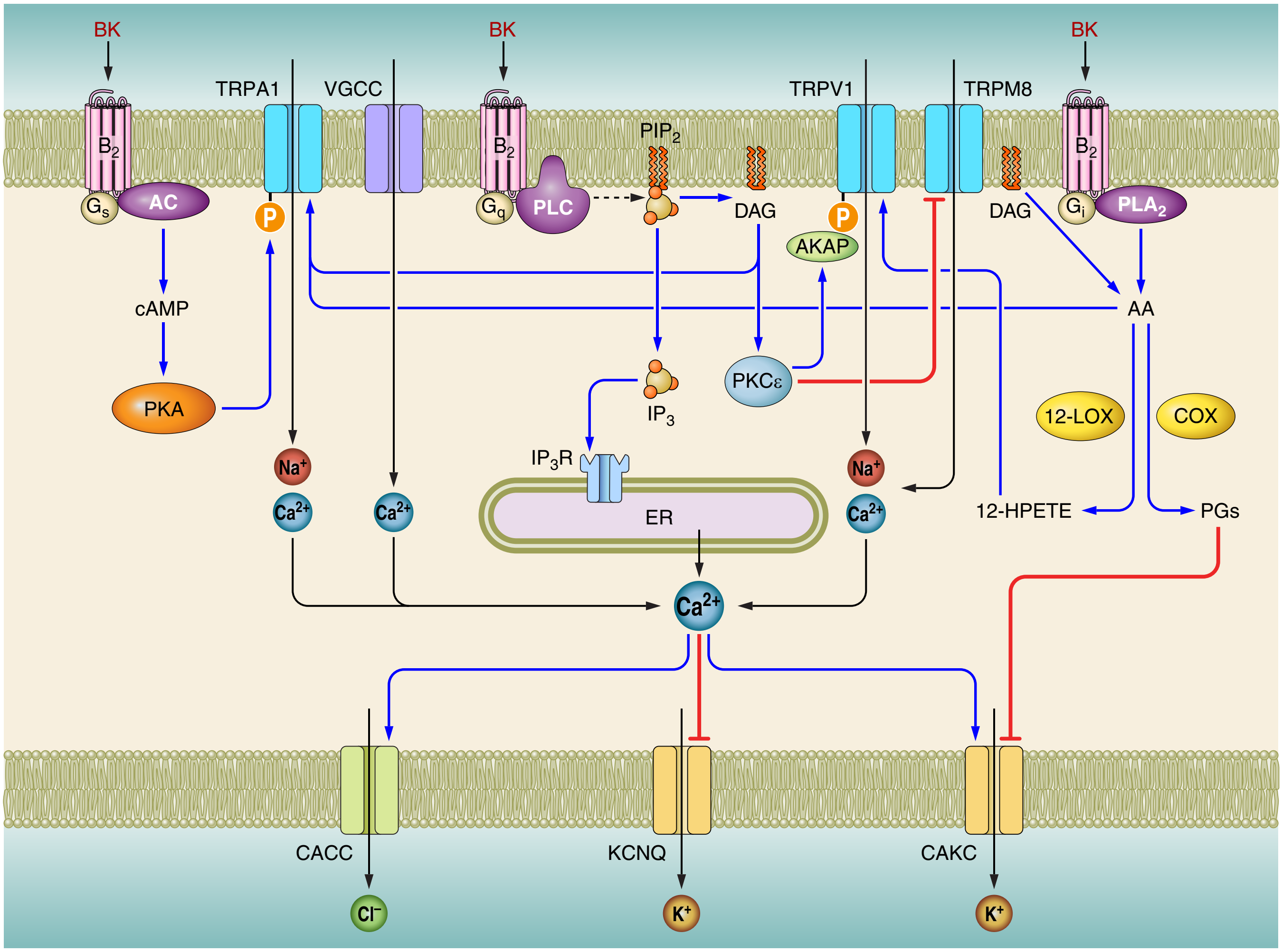


FIGURE 1. Schematic representation of bradykinin's (BK) most important signal transduction mechanisms in nociceptive sensory neurons. Blue arrow: activation of a target or stimulation of synthesis of a substance; red line: inhibition of a target; dashed black arrow: cleavage of a substance. Not shown are the minor outward K^+ currents in case of TRPV1, TRPM8, and TRPA1 channels. Also not shown are activation of MAPK enzymes and role of the GC-NO-cGMP-PKG pathway in tachyphylaxis. VGCC, voltage-gated Ca^{2+} channels; CACC, calcium-activated Cl^- channel; KCNQ, M-type K^+ channel (K_v7); CAKC, calcium-activated K^+ channel; ER, endoplasmic reticulum; IP_3R, IP_3 receptor. For other abbreviations, see text. AKAP is only shown when its involvement was directly revealed.

tured rat DRG neurons was shown to be mediated by a pertussis toxin-insensitive G protein (76, 482–484, 719), and a similar result was obtained in the neonatal rat spinal cord-tail preparation in vitro in which the excitatory effect of bradykinin applied to the tail was recorded as a reflex depolarization of the ventral root (162). This G protein was subsequently identified as a $G_{q/11}$ activating PLC (254). The levels of the two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), produced by PLC are both increased in DRG neurons in response to bradykinin (76, 229, 309, 711, 719). The increasing effect of bradykinin on inositol phosphate levels was reduced by inhibition of COX-1 but not COX-2 (711). PLC activation was found to contribute to bradykinin's actions in several studies (37, 186, 436, 711, 762). It is DAG that mediates most of the excitatory effects of bradykinin by activating PKC which can phosphorylate various target proteins. In rat DRG neurons, the membrane depolarization underlying the sensory stimulant (spike-generating) effect of bradykinin appeared to result from an inward current due to opening by PKC-mediated phosphorylation of an ion channel permeable to both Na^+ and K^+ (76, 313, 482, 594). In accord, omission of extracellular Ca^{2+} had only a small reducing effect on the bradykinin-evoked inward current in sensory neurons (76). The identitiy of this ion channel is still not perfectly clear (see possible candidates in sect. II*C3*). Recent studies on nodose ganglion neurons of the guinea pig and DRG neurons of the rat, however, provided evidence for a chloride outward current as a major cause of the bradykinin-induced membrane depolarization (436, 546; see more details in sect. II*C4*).

The fundamental role of PKC in the sensory effects of bradykinin is indicated by a great amount of data. Early studies provided indirect evidence showing that staurosporine, an inhibitor of protein kinases, attenuated the depolarizing effect of bradykinin in the neonatal rat spinal cord-tail preparation in vitro (160, 162) and also in cultured DRG neurons (76, 483). Downregulation of PKC strongly reduced bradykinin-induced release of SP and CGRP from cultured rat sensory neurons (42). Phorbol esters, which activate PKC, mimicked most effects of bradykinin in the experimental models mentioned above (42, 76, 162). The involvement of PKC in the bradykinin-induced PGE_2 release from cultured rat trigeminal neurons was also established (315). Bradykinin shortened the afterhyperpolarization in rat sensory neurons by accelerating Ca^{2+} outward transport in a PKC-dependent way (737). Bradykinin was shown to cause translocation of PKCε in cultured DRG neurons (746). Considerable evidence has been provided for the involvement of PKC in the various excitatory (nocifensive or spike-generating) and sensitizing effects of bradykinin studying transfected cells, cultured sensory neurons (85, 94, 95, 120, 296, 411, 435, 585, 586, 685, 745, 764; see details in sect. II*C3*), nerve fiber discharge (252, 253, 497), nocifensive behavior (186), and neuropeptide release (200). It means that PKC is certainly involved in signal tranduction of bradykinin receptors not only in the soma but also in the peripheral terminal/axon of nociceptive sensory neurons.

In addition to PKC activation, bradykinin was shown to induce an elevation of the intracellular free Ca^{2+} concentration in somata of cultured sensory neurons (see below). This response is at least partly due to release of Ca^{2+} from intracellular stores via IP_3 formation as it was still observed in the absence of extracellular Ca^{2+} and could be inhibited by depletion of intracellular Ca^{2+} stores (45, 76, 309, 331, 385, 719). On the other hand, removal of extracellular Ca^{2+} did reduce the magnitude of the bradykinin-induced Ca^{2+} transients, suggesting that influx of Ca^{2+} occurs as well (45, 309). In a recent study on cultured rat DRG neurons, the bradykinin-induced elevation of intracellular Ca^{2+} concentration was biphasic, with a sharp transient increase followed by a slower and smaller secondary rise (436). Removal of extracellular Ca^{2+} abolished the secondary rise but not the initial one. Voltage-gated Ca^{2+} channel inhibitors reduced both phases of the bradykinin-induced Ca^{2+} transients (particularly the secondary rise). Blockade of voltage-gated Na^+ channels failed to influence the Ca^{2+} transients. These data suggest that the primary event is the release of Ca^{2+} from intracellular stores, which triggers a secondary Ca^{2+} influx, mainly through voltage-gated Ca^{2+} channels but independent of action potential firing (436). The bradykinin-induced influx of extracellular Ca^{2+} through voltage-sensitive Ca^{2+} channels seems to be important for the generation of cGMP and the resulting, B_2 receptor-mediated, tachyphylaxis to bradykinin (see sect. II*C5*), rather than for excitation of the affected neurons (75, 76, 434). However, it is unclear which mechanism applies to peripheral sensory nerve endings that essentially lack intracellular calcium sores, i.e., endoplasmic reticulum.

Exocytotic neuropeptide release from nociceptive primary sensory neurons occurs as a result of an increase in intracellular Ca^{2+} concentration. Bradykinin induced or facilitated release of CGRP from the isolated guinea pig heart and atria, rat and mouse trachea, and bovine dental pulp (209, 232, 240, 298, 356). Bradykinin evoked a moderate SP and CGRP release from the isolated rat skin (a response enhanced in experimental diabetes, Ref. 215) but not from isolated mouse sciatic nerve axons (29, 200, 348). Bradykinin caused a release of both SP and CGRP from cultured DRG neurons (42, 177, 523, 524, 663, 687, 710, 742), and this response was diminished by inhibition of PKC (42), block of N-, but not L- or P-, type voltage-gated Ca^{2+} channels (177). However, this synaptic N type of voltage-gated Ca^{2+} channels is not involved in depolarization-induced neuropeptide release from peripheral nerve fibers where low-threshold T and also L-type channels govern the exocytosis of CGRP that entirely depends on extracellular Ca^{2+} (669). In addition, long-term (24 h) bradykinin treatment increased the CGRP (Calca gene) mRNA content in cultured rat sensory neurons (687).

2. *Arachidonic acid derivatives in bradykinin receptor signaling*

Bradykinin can lead to a release of arachidonic acid in cultured sensory neurons, and this response was shown to depend on influx of extracellular Ca^{2+} (76, 229, 719). The mechanism of arachidonate formation can be metabolism of DAG by DAG lipase to arachidonic acid and monoacylglycerol, the latter being split by monoacylglycerol lipase to arachidonic acid and glycerol (17, 229). Alternatively or additionally, activation of phospholipase A_2 (PLA_2) through a G protein (probably G_i, Ref. 785) can result in arachidonic acid formation in sensory neurons (229) similarly to nonneuronal cells (74). The role of PLA_2 in some nociceptor-activating and sensitizing actions of bradykinin has been demonstrated (186, 653, 696). Arachidonic acid release may lead to production of prostanoids (prostaglandins and TXA_2) and leukotrienes by COX and lipoxygenase (LOX) enzymes, respectively.

Relevant for the role of secondary prostanoids in bradykinin signaling, bradykinin selectively enhanced PGI_2 release from guinea pig nodose neurons (767). Bradykinin, through activation of B_2 but not B_1 receptors, released PGE_2 from cultured rat trigeminal neurons which was reduced by PLA_2 inhibition and COX blockade (315). In cultured rat DRG neurons, a short-term (30 min) exposure to bradykinin led to a small PGE_2 release that was reduced predominantly by COX-1 inhibition (308). Conversely, a long-term (3 h) exposure to bradykinin induced a massive PGE_2 release that was abolished by a selective COX-2 inhibitor. COX-1 but not COX-2 immunoreactivity was revealed in somata of rat small-diameter DRG neurons (742, 108). However, a low-level COX-2 mRNA expression was also detected in DRG neurons (307, 509). Furthermore, COX-2 mRNA and protein levels were increased by either a short-term (2 min) or a prolonged (3 h) bradykinin exposure via B_2 but not B_1 receptor activation (308, 309, 552, 709). In the isolated rat skin, the bradykinin-evoked PGE_2 release was attenuated by either COX-1 or COX-2 inhibiton, and COX-1 immunoreactivity was present in nerve branches and endings as well as in nonneuronal elements (e.g., mast cells), while COX-2 immunoreactivity was weaker but showed similar neuronal and extraneuronal localization (478). Bradykinin-induced prostanoid formation is, of course, not restricted to sensory neurons as a source. In fact, the amount of PGE_2 released from isolated skin in response to bradykinin stimulation was not altered after (1 wk of) complete denervation (630). In several studies, the bradykinin-induced release of neuropeptides from sensory nerves or DRG neurons was reduced by COX inhibitors (232, 298, 379, 687, 710, 742) and restituted by supplementation of PGE_2, suggesting that secondary prostaglandin is required for bradykinin to release neuropeptides (28). The involvement of COX products in the nociceptor-activating (i.e., spike-generating) and sensitizing actions of bradykinin revealed in a great number of studies is described in section II*D*, *1*B and *2–4*.

Regarding the involvement of LOX products in bradykinin signaling, a nonselective LOX inhibitor reduced the depolarizing effect of bradykinin in rat sensory neurons (483). Several studies suggest a major involvement of 12-LOX products in bradykinin signaling (88, 653, 781; see more details in sect. II*C3*A). The involvement of LOX products in the nociceptor-activating and sensitizing actions of bradykinin is described in section II*D*, *1*B and *2–4*.

There is no doubt that both prostanoids and LOX products are important mediators of bradykinin actions also in peripheral terminals of nociceptive sensory neurons (see **TABLES 2** and **3**).

3. *TRP channels in bradykinin receptor signaling*

A) TRPV1 CHANNELS. In the last decade, a novel effector for bradykinin has been identified which is the capsaicin receptor, the first identified member of the thermosensitive ion channels. This receptor channel was initially named vanilloid receptor type 1 (VR1), reflecting the vanilloid structure of capsaicin (92), but it was subsequently renamed TRPV1, since it became the founding member of the vanilloid(-binding) subgroup of the genetically defined transient receptor potential (TRP) family of ion channels (for review, see Refs. 148, 780). TRPV1 is a nonselective cation channel located on polymodal nociceptive primary afferent neurons, and it is also activated by other chemical stimuli including low pH and anandamide in addition to noxious heat (>43°C; for review, see Refs. 89, 305). Activation of TRPV1 results in membrane depolarization due mainly to Na^+ influx and in release of neuropeptides (SP, CGRP) as a result of Ca^{2+} influx through the channel. Capsazepine and iodo-resiniferatoxin (I-RTX) are competitive antagonists of TRPV1 that bind to the intracellular capsaicin binding site, whereas ruthenium red is a functional antagonist blocking the pore of the ion channel. Three further TRPV channels sensitive to warmth or heat have subsequently been identified (TRPV2, TRPV3, and TRPV4), which have different thermal activation thresholds (91, 92, 257, 565, 661, 782).

Certain LOX products, namely the 12- and 15-(*S*)-hydroperoxy-eicosatetraenoic acids (HPETEs), 5- and 15-(*S*)-hydroxyeicosatetraenoic acids (HETEs) as well as leukotriene B_4 (LTB_4) directly activated TRPV1 channels from the cytoplasmic side in both sensory neurons and TRPV1-transfected host cells (301). Furthermore, arachidonic acid was also efficacious at TRPV1 receptors, albeit less, and its effect was reduced by inhibitors of the 5- and 12-LOX enzymes, suggesting that arachidonic acid is converted to HPETEs and/or HETEs within sensory neurons. Considering the ability of bradykinin to stimulate release of arachidonic acid from the membranes of sensory neurons (76, 229, 719), a hypothesis was put forward that algogenic and sensitizing mediators such as bradykinin may act on nociceptors by releasing arachidonic acid intracellularly, which is then converted to LOX products activating the TRPV1 receptor via an interaction with its cytosolic domains (301). Indirect support for this hypothesis was soon provided by a study pharmacologically showing that bradykinin effects on cultured rat DRG neurons and cutaneous nerve fibers could be antagonized by capsazepine, the competitive blocker of the ligand-binding site of TRPV1, and by LOX inhibitors, although in behavioral experiments the LOX antagonist baicalein by itself, in absence of bradykinin, prolonged the noxious heat withdrawal latency (653). Nonetheless, bradykinin stimulated via the 12-LOX pathway the production of 12-HETE in sensory neurons in vitro and in rat skin in vivo (653, 710, 761). Furthermore, 12-LOX is exclusively expressed in platelets, and activated platelets excite nociceptors and induce hyperalgesia (606, 641). Further support for the involvement TRPV1 and LOX products was provided by a study on guinea pig vagal afferents in which the action potential-generating effect of bradykinin was diminished by capsazepine or ruthenium red as well as by inhibition of 12-LOX or 5-LOX (88). The effect of bradykinin, however, was not altered by PLA_2 inhibition, similarly to a previous work (162), pointing to a PLA_2-independent liberation of arachidonic acid in vagal nociceptive nerve endings, perhaps involving DAG lipase. In acutely isolated cardiac DRG neurons, bradykinin depolarized and increased electrical excitability without evoking any currents, which effects were inhibited by the TRPV1 receptor antagonist I-RTX, by a selective 12-LOX inhibitor, an IP_3 antagonist and reduced by store depletion of buffering of intracellular Ca^{2+} (781). As the effect of bradykinin was not reduced by a Ca^{2+}-free extracellular solution, the likely localization of TRPV1 channels involved in the response is intracellular, most probably in the endoplasmic reticulum whose membrane also expresses TRPV1 receptors (228). After all, it is worth mentioning that the reported contributions of LOX products to bradykinin signaling are difficult to reconcile with studies concluding on a high degree of prostanoid involvement in bradykinin's actions (see sect. section II*D*, *1*B and *2–4*).

The fundamental role of the B_2 receptor-PLC-PKC signaling pathway also implicates TRPV1 as an effector. As the first clue, the noxious heat-activated, i.e., TRPV1-mediated, current in rat DRG neurons was sensitized by a short exposure to bradykinin or the PKC activating phorbol ester PMA, and these sensitizing effects were blocked by the nonselective protein kinase inhibitor staurosporine (95). In a subsequent study PKCε, a Ca^{2+}-independent isoform of PKC, was identified as the molecular entity responsible for this action (94). Phorbol ester was reported to induce channel activity in both TRPV1-transfected host cells and rat DRG neurons, and bradykinin itself was able to activate TRPV1 in a PKC-dependent manner providing the first direct evidence for the existence of B_2 receptor-PKC-TRPV1 pathway (585). Bradykinin was shown to lower the heat threshold of TRPV1 in host cells transfected with both TRPV1 and B_2 receptors through activation of PKCε (685). The heat threshold for activation in rat DRG neurons was also decreased by bradykinin in a PKC-dependent manner. Consistent with this, PKC activation by phorbol ester sensitized the TRPV1 receptor to capsaicin, heat, protons, and anandamide in TRPV1-transfected cells and rat or mouse DRG neurons (120, 745). Direct phosphorylation of the TRPV1 receptor by PKCε upon exposure to PMA was demonstrated, and two serine residues were identified as targets (52, 542). TRPV1 with point mutations introduced at these sites failed to be sensitized to either capsaicin or heat in response to PMA. With the use of an in vitro phosphorylation method on transfected cells and activators of either PKC or PKA, phosphorylation and sensitization of TRPV1 to capsaicin was shown, while bradykinin phosphorylated and activated TRPV1 essentially through PKC (411). In rat DRG neurons, the bradykinin-induced enhancement of the capsaicin-evoked, i.e., TRPV1-mediated, inward current was abolished by either a peptide inhibiting the interaction of the scaffolding protein AKAP79/150 with TRPV1 or by downregulating the expression of AKAP79 by the use of siRNA (794). In HEK293 cells coexpressing TRPV1 and the B_2 bradykinin receptor, bradykinin induced a sensitization to capsaicin which was abolished by *1*) expression of an AKAP79 mutant with the PKC site deleted, *2*) the use of siRNA to downregulate AKAP79, and *3*) mutation of the sites in TRPV1 that are targets for PKC-mediated phosphorylation, indicating the role of a B_2-PKC-AKAP-TRPV1 pathway in bradykinin signaling.

The data mentioned above suggest that two signal transduction pathways of bradykinin, namely, arachidonic acid mobilization and PKC activation, may finally converge on a common target, i.e., on TRPV1. In a conflicting study it has been postulated that the TRPV1 receptor is essential for the development of the various sensitizing effects of bradykinin (110). Bradykinin failed to induce heat hyperalgesia in TRPV1 receptor knockout mice and, using host cells expressing B_2 with or without TRPV1 receptors, bradykinin was shown to potentiate the effect of low pH or capsaicin.

Several further studies support the involvement of TRPV1 in various actions of bradykinin (45, 88, 186, 356, 410, 556, 611, 653; see also **TABLES 2** and **3**). Furthermore, in accord with the proposed role of TRPV1 in bradykinin signaling, sensitizing effects on capsaicin responses were reported from various reduced models (85, 186, 208, 552, 586, 616, 658, 679, 709, 711; see details in sect. II*D4* and **TABLE 3**). In contrast to other proinflammatory mediators including NGF and ATP, bradykinin failed to increase the expression of TRPV1 channels in the plasma membrane of cultured rat DRG neurons arguing against a role of bradykinin in transcriptional regulation of TRPV1 (85).

B) TRPM8 AND TRPA1 CHANNELS. Bradykinin was shown to inversely affect TRPV1 and TRPM8, the cool-sensing, menthol-activated member of the TRP family of ion channels (565, 586). In rat DRG neurons, bradykinin enhanced the capsaicin-evoked Ca^{2+} accumulation in parallel to a decrease of the menthol-induced, i.e., TRPM8-mediated, response with both bradykinin effects almost abolished by PKC inhibition. The inhibitory effect of bradykinin on TRPM8 function was suggested to be due to dephosphorylation of the channel mediated by a PKC-activated protein phosphatase. A downregulation of TRPM8 function may be of pathophysiological relevance because a combination with menthol was shown to reduce the nocifensive effect of intraplantar capsaicin (586). Therefore, the bradykinin-induced downregulation of TRPM8 function may enhance the pronociceptive action of the bradykinin-sensitized TRPV1 activity. In accord, bradykinin inhibited the effect of cooling on cultured sensory neurons by reducing the evoked Ca^{2+} influx and lowering the threshold temperature for activation via stimulation of PKC (435).

An involvement of TRPA1, the ion channel activated by noxious cold and multiple endogenous and exogenous chemicals including mustard oil (allyl isothiocyanate; Refs. 324, 677), in the excitatory action of bradykinin was also revealed (37). TRPA1-expressing Chinese hamster ovary cells transiently transfected with the B_2 bradykinin receptor showed current responses when exposed to bradykinin, and this current was similar to that evoked by noxious cold or cinnamaldehyde, an established agonist at TRPA1 receptors. The intracellular Ca^{2+} accumulation evoked by bradykinin was blocked by PLC inhibition, and a membrane permeable analog of DAG also directly activated TRPA1. These data show that PLC activation is required for TRPA1 activation possibly by generation of DAG. Alternatively, PLC may act by depleting the membrane of PIP_2 which was reported to inhibit TRPA1 in some (130, 359) but not other studies (6, 332). As arachidonic acid as well as a nonmetabolizable analog of it also activated TRPA1 (37), one might speculate that arachidonic acid, formed from DAG by DAG lipase and/or by PLA_2, induces TRPA1 activity without being converted to COX or LOX products.

A study on TRPA1 knockout mice provided further evidence for involvement of this channel in the actions of bradykinin (45). The bradykinin-induced Ca^{2+} uptake in trigeminal sensory neurons and heat hyperalgesia were attenuated and absent, respectively, in TRPA1-deficient mice compared with wild-type animals. The Ca^{2+} uptake response was diminished to the same degree in neurons from TRPV1-deficient mice or by ruthenium red, suggesting a functional coupling between TRPA1 and TRPV1. A model was proposed by the authors according to which TRPV1 acts upstream of TRPA1 in bradykinin signaling. B_2 receptor activation causes LOX activation and PLC stimulation leading to PIP_2 hydrolysis, PKC activation, and IP_3-mediated release of Ca^{2+} from intracellular stores. The consequent modest TRPV1 activation (for mechanisms, see sect. II*C3*A) results in further influx of Ca^{2+} which would then activate TRPA1 (324) contributing the bulk of the excitatory effect. The model is supported by coexpression of TRPV1 and TRPA1 in sensory neurons (677), but the role of intracellular Ca^{2+} in activation of TRPA1 is controversial (37). It is worth mentioning that CFA-induced inflammation produced a similar degree of heat hyperalgesia in TRPA1 knockout and wild-type mice showing that TRPA1, unlike TRPV1, is not a prerequisite for development of inflammatory thermal hyperalgesia (45). Bradykinin increased TRPA1-mediated currents in both transfected cells and rat DRG neurons via activation of both PLC (but not PKC) and PKA, providing the first evidence that the cAMP-PKA pathway contributes to the TRPA1-activating effect of bradykinin in addition to PLC activation (762). Indeed, bradykinin was shown to elevate cAMP levels in sensory neurons (see sect. II*C4*F). In vivo, a sub-nocifensive dose of bradykinin applied intraplantarly potentiated nociception evoked by the TRPA1 receptor agonist mustard oil. Bradykinin's acute nocifensive effect in the mouse and mechanical sensitizing actions on guinea pig esophageal and murine colonic afferents were shown to depend on TRPA1 activation (67, 400, 791).

The above data show that considerable evidence supports the involvement of both TRPV1 and TRPA1 channels in bradykinin-induced sensory transduction not only is somata but also in peripheral terminals of nociceptive sensory neurons in a variety of experimental models (**TABLES 2** and **3**). However, the complexity of the ion channel background of bradykinin's nociceptor-activating action is illustrated by a study on TRPV1-deficient mice, in which the nocifensive reaction evoked by a low dose of bradykinin applied intraplantarly was reduced compared with wild-type animals, but the effect of a higher dose was indistinguishable in the two genotypes of mice (337). It must be emphasized that in several studies the excitatory effects of bradykinin were not inhibited by either TRPV1 or TRPA1-selective antagonists, deletion of the TRPV1 or TRPA1 gene, or the broad-spectrum TRP channel inhibitor ruthenium red (20, 67, 151, 161, 232, 309, 337, 356, 374, 436, 546, 591, 611, 791, 792). These data indicate that non-TRP ion channels may also contribute to the excitatory/sensitizing effects of bradykinin (see sect. II*C4*).

4. Other targets of bradykinin receptor signaling

A) Ca^{2+}-ACTIVATED K^+ CHANNELS. In a subpopulation of nodose ganglion neurons of the rabbit in vitro, two temporally distinct components of spike afterhyperpolarization (AHP) were identified: a fast one lasting for <0.5 s and a slow one persisting for several seconds (203, 276). Evidence has been provided that the latter one is due to a Ca^{2+}-dependent outward K^+ current. Later it was shown that the slow AHP in vagal sensory neurons involved influx of Ca^{2+} through N-type voltage-gated Ca^{2+} channels followed by Ca^{2+}-induced Ca^{2+} release from endoplasmic reticulum via ryanodine channels and subsequent activation of Ca^{2+}-activated K^+ channels (113). In rabbit and guinea pig nodose ganglion neurons, bradykinin inhibited the slow AHP, and this effect was shown to be mediated by prostanoids, among them PGI_2 (736, 767, 769). The shortening of the AHP may be involved in the neuronal excitatory action of bradykinin, because AHP plays a role in controlling the response pattern of sensory neurons, and it is responsible for the slowing of the firing rate known as spike frequency adaptation (113, 768). In rat DRG neurons, bradykinin shortened the AHP by accelerating the Ca^{2+} efflux via PKC-dependent facilitation of the plasma membrane Ca^{2+} pump isoform 4 (737). In a recent study, a combination of bradykinin, PGE_2, and serotonin decreased a hyperpolarizing Ca^{2+}-dependent K^+ current in rat trigeminal sensory neurons (743). It must be emphasized that the contribution of Ca^{2+}-activated K^+ channels to bradykinin signaling in non-vagal sensory neurons is largely unknown, and no support for a role of these channels in peripheral endings of nociceptive sensory neurons is available. Therefore, further studies are required in this direction.

B) M-TYPE K^+ (K_V7 OR KCNQ) CHANNELS. The M current (mediated by K_v7 or KCNQ channels) was first described as an outward K^+ current induced by activation of M_2 muscarinic receptors. Recently, bradykinin has been shown to inhibit the M current in cultured rat DRG neurons through a B_2 receptor-PLC-IP_3-Ca^{2+} pathway (436). In addition, an involvement of M current inhibition in the bradykinin-induced membrane depolarization and action potential firing was revealed. The M channel opener retigabine reversed the inhibitory effect of bradykinin on the M current and diminished the nocifensive reaction induced by intraplantar bradykinin injection. However, the role of this type of channels in the function of the peripheral endings of nociceptors is completely obscure.

C) Ca^{2+}-ACTIVATED Cl^- CHANNELS. In nodose ganglion neurons of the guinea pig, the B_2 receptor activation-induced membrane depolarization was reduced by the TRPV1 antagonist I-RTX, but an early component of the response was due to a decrease in resting K^+ conductance followed by an increase in Ca^{2+}-activated Cl^- conductance (546). The latter mechanism, mediated by Ca^{2+}-activated Cl^- channels, can contribute to membrane depolarization, because primary afferent neurons have elevated intracellular Cl^- concentrations owing to constitutive activity of the Na^+-K^+-$2Cl^-$ cotransporter that allows for an outflow of Cl^- (580, 686). As mentioned in section IIC4A, bradykinin can inhibit, with a depolarizing effect, Ca^{2+}-dependent K^+ currents in nodose ganglion neurons of the guinea pig. Whether both bradykinin effects occur in the same neurons was not known (see below). In an ex vivo innervated trachea/bronchus preparation of the guinea pig, the bradykinin-induced action potential discharges in C-fibers were partially diminished by either I-RTX or the Ca^{2+}-activated Cl^- channel inhibitor niflumic acid (410). The combination of both inhibitors abolished the bradykinin effect showing the additive involvement of TRPV1 and Ca^{2+}-activated Cl^- channels in the excitatory action of bradykinin in vagal afferents. In this study, $GABA_A$ receptor activation evoked action potentials in bradykinin-sensitive jugular ganglion neurons, indicating that the reversal potential for Cl^- in these cells is in fact more positive than the action potential threshold owing to the elevated intracellular Cl^- concentration. The hypothesis that Cl^- efflux is a major component of bradykinin-induced discharge in vagal afferent C-fiber terminals is analogous to odorant receptor potentials in the dendrites of olfactory neurons (399). It has been revealed that the combination of bradykinin, PGE_2, ATP, and NGF further elevated the Cl^- concentration in DRG neurons within 2 h of exposure, and this alteration coincided with enhanced phosphorylation of the Na^+-K^+-$2Cl^-$ cotransporter, suggesting that an increased activity of the transporter caused the increase in Cl^- levels (217). Furthermore, after 3 h of treatment of DRG cells with the inflammatory mediators, the Cl^- accumulation was further enhanced by an increased expression of the Na^+-K^+-$2Cl^-$ cotransporter and a downregulation of the main Cl^- extrusor, the K^+-Cl^- cotransporter. These data show that inflammatory mediators are able to further increase the intracellular Cl^- concentration in nociceptive sensory neurons which may contribute to increased nociceptor excitability by allowing for larger depolarizing Cl^- currents. Very recently, bradykinin has been shown to activate Ca^{2+}-activated Cl^- channels in cultured rat DRG neurons, and this effect was mediated by a B_2 receptor-PLC-IP_3-Ca^{2+} pathway (436). The bradykinin-induced inward current (actually an outward current of the negative charge carrier Cl^-) was abolished by either lowering intracellular Cl^- concentration or by niflumic acid, suggesting that Ca^{2+}-activated Cl^- channel opening played an essential role in the response. Furthermore, nocifensive behavior induced by intraplantar bradykinin injection was diminished by co-applied Cl^- channel blockers. The same authors provided evidence for reciprocal effects of bradykinin, inhibiting the M-type K^+ current and activating the Ca^{2+}-activated Cl^- channels that both contribute to the excitatory effect on rat sensory neurons and in combination appear to fully account for it (436). Also reciprocal, a combination of bradykinin, PGE_2, and serotonin increased the Ca^{2+}-activated Cl^- current and reduced the Ca^{2+}-activated K^+ current in rat trigeminal sensory neurons (743). According to the data presented, a limited amount of evidence supports the role Ca^{2+}-activated Cl^- channels in bradykinin signaling in peripheral endings of nociceptive sensory neurons.

D) VOLTAGE-GATED Na^+ CHANNELS. Two subtypes of tetrodotoxin-resistant (TTX-R) voltage-gated Na^+ channels termed SNS/PN3 (now known as $Na_v1.8$) and SNS2/NaN ($Na_v1.9$) are exclusively expressed in nociceptive primary afferent neurons (7, 150; for review, see Ref. 690). $Na_v1.8$ and $Na_v1.9$ mediate a slow-inactivating and a persisting TTX-R Na^+ current, respectively. The bradykinin-induced overt nociception (hindpaw licking/flinching) was shorter, mechanical hyperalgesia less pronounced and heat hyperalgesia missing in mice deficient of the gene for $Na_v1.9$ (22). The $Na_v1.9$-mediated current in mouse sensory neurons was not altered by bradykinin alone, but when it was combined with PGE_2, histamine, ATP, and norepinephrine, a facilitation was observed (459). A less ample combination of mediators including bradykinin, PGE_2, and serotonin increased the compound TTX-R Na^+ current (see sect. III*B2*A) and, surprisingly, decreased voltage-gated Ca^{2+} currents in rat trigeminal sensory neurons (743). As shown by the above-mentioned behavioral data, $Na_v1.9$ channels may play some role in sensory transduction in peripheral terminals of nociceptive sensory neurons.

E) THE cAMP-PKA PATHWAY. A sustained (3 h) pretreatment with bradykinin increased levels of cAMP in cultured rat DRG neurons (552), whereas a short exposure failed to do so (75). In a recent study, bradykinin applied for 1 min was able to elevate cAMP levels and cause translocation of protein kinase (and also PKC) to the plasma membrane in rat DRG neurons (762). As mentioned in section II*C3*B, the stimulatory effect of bradykinin on TRPA1 function involved activation of PKA. In accord with these data, B_2 receptors are also known to couple with the adenylate cyclase-stimulating G_s protein in addition to $G_{q/11}$ and G_i (243, 432). To date, no study has established a role for the cAMP-PKA signaling pathway in models reflecting the activity of peripheral nociceptors.

F) MITOGEN-ACTIVATED PROTEIN KINASES. The bradykinin-induced PGE_2 release from rat trigeminal sensory neurons was reduced by inhibition of mitogen-activated protein kinase kinase-1 (MEK-1; Ref. 315). Its targets, the serine/threonine protein kinases collectively called mitogen-activated protein kinase (MAPK) family, include extracellular signal-regulated kinase (ERK), p38 and c-*jun* NH_2-terminal kinase (JNK). Of them, bradykinin increased the phosphorylation of both ERK1 and ERK2, which was attenuated by MEK-1 inhibition (315). Bradykinin led to phosphorylation of ERK1 and ERK2 in sensory nerve terminals of the dentin and dental pulp complex as well (382). Two minutes after intraplantar bradykinin injection in mice, ERK phosphorylation in small-diameter DRG neurons was noted (595). Likewise, SP release from rat DRG neurons induced by a sustained (3 h) bradykinin application involved activation of MEK and ERK but not p38 or JNK (709). In human embryonic kidney cells expressing the bradykinin B_1 receptor, its agonist des-Arg^{10}-kallidin caused phosphorylation of p38 kinase (230). In addition, the mechanical hyperalgesia induced by the B_1 receptor agonist des-Arg^9-bradykinin following IL-1β pretreatment was shown to depend on activation of p38. The activation of MAPKs by bradykinin raises the possibility that the peptide can alter gene expression possibly leading to long-term alterations of the function of nociceptors. In accord, the B_2 receptor activation-induced increase in intracellular Ca^{2+} in cultured DRG neurons (partly via influx of extracellular Ca^{2+}, partly via IP_3-mediated release from endoplasmic reticulum) caused nuclear translocation of the transcription factor nuclear factor of activated T-cells (NFAT-4) with a resultant increase in COX-2 mRNA expression level (309).

G) SIGNALING MECHANISMS OF B_1 RECEPTORS. The signaling mechanisms of B_1 receptors were less extensively studied, but they appear to involve similar pathways as those revealed for B_2 receptors. However, as already mentioned, B_1 receptors, unlike B_2 receptors, do not exhibit notable internalization or tachyphylaxis. Revealed elements of B_1 receptor signaling include G_q and G_i protein, PKC and PLC activation, and accumulation of IP_3 and intracellular Ca^{2+} (27, 50, 187, 746). In addition, activation of the MAPK p38 was also shown to contribute to B_1 receptor signaling (187, 230). Intraplantar injection of the B_1 receptor agonist evoked ERK phosphorylation in large-diameter DRG neurons and satellite cells from nerve-injured mice but not from control animals (595).

5. *Molecular mechanisms of the tachyphylaxis to bradykinin*

The B_2 receptor-mediated neuronal excitatory actions of bradykinin, unlike those through B_1 receptor activation (27, 475), showed a rapid tachyphylaxis or desensitization in several studies (see, e.g., Refs. 35, 46, 76, 186, 298, 330, 393, 397, 403, 465, 719). The signal transduction pathways involved in the desensitization of B_2 receptors are different from those responsible for the excitatory actions of bradykinin. Bradykinin was shown to elevate cGMP levels in cultured rat DRG neurons (75). This response was dependent on Ca^{2+} influx and was ascribed to activation of guanylyl cyclase (GC), as it was not altered by phosphodiesterase (PDE) inhibition. A role for a cGMP increase in reducing the excitatory action of bradykinin was also proposed, as dibutyryl cGMP, a membrane-permeable analog of cGMP, or the NO donor sodium nitroprusside diminished the bradykinin-induced rise in IP_3. It is worth mentioning that the B_1 receptor agonist des-Arg^9-bradykinin failed to alter cGMP levels in sensory neurons (75). The bradykinin-induced elevation of the intracellular Ca^{2+} concentration in sensory neurons did not show tachyphylaxis when the first response was prevented by a Ca^{2+}-free extracellular medium or by a nonselective blocker of voltage-gated Ca^{2+} channels (434), reinforcing that Ca^{2+} influx is required for tachyphylaxis to occur. In cultured rat sensory neurons, B_2 receptor stimulation induced Ca^{2+} influx which activated NOS and the resulting NO stimulated the soluble GC to form cGMP which led to desensitization of the B_2 signaling at the level of the receptor or the G protein (44, 481). The role of the NO-cGMP pathway in reducing bradykinin sensitivity was also shown in the neonatal rat spinal cord-tail preparation in vitro (162, 617). In this model, after development of desensitization to bradykinin, phorbol ester was still capable of activating PKC, suggesting that tachyphylaxis took place upstream of PKC activation (162). In rat DRG neurons, repeated bradykinin applications led to a reduction in both bradykinin-induced IP_3 formation and the number of bradykinin binding sites, suggesting that bradykinin can evoke receptor downregulation (270). Activation of the NO-cGMP pathway also reduced bradykinin-induced IP_3 formation but failed to alter the number of binding sites for bradykinin, and inhibition of NO synthesis prevented the decrease in bradykinin-induced IP_3 formation. These results indicate that the NO-cGMP pathway is involved in the functional uncoupling of the B_2 receptors that occurs downstream of bradykinin binding and upstream of IP_3 formation, but NO does not contribute to receptor downregulation.

Consistent with the proposed role of NO to reduce bradykinin responsiveness, the mechanical hyperalgesia induced by intraplantar injection of bradykinin in the rat was potentiated by local pretreatment with an inhibitor of GC (124). L-Arginine, the precursor of NO, reduced the number of bradykinin-evoked spikes recorded from nociceptive articular C-fibers of the tibial nerve in both normal and arthritic rats (346). Conversely, NOS inhibition enhanced responsiveness to bradykinin, but only in arthritic rats, suggesting a role for endogenous NO to exert a tonic inhibition of bradykinin responsiveness in the inflamed joint. Bradykinin-induced firing of mesenteric afferents in the isolated murine jejunum was reduced in a model of indomethacin-evoked enteritis by a NOS-dependent mechanism (783). In accord with the above findings, NOS-like immunoreactivity was shown in small and medium-sized rat and monkey DRG neurons, and colocalization with CGRP or SP, markers of nociceptive neurons, was also revealed (see references in sect. VI*A*).

Concerning bradykinin receptor downregulation, a rapid internalization of the B_2 receptors dependent on phosphorylation has been described in B_2 receptor-transfected cells (27, 579). Bradykinin activation of heterologously expressed B_2 receptors induced colocalization of the agonist-bound receptor with β-arrestin in endosomes (657). Following agonist removal, β-arrestin rapidly dissociated from the receptor in the endosomes and the receptors recycled to the plasma membrane causing resensitization. It was shown that the COOH-terminal of the B_2 receptor was responsible for regulation of interaction with β-arrestin. Bradykinin is also capable of inducing heterologous desensitization, i.e., reduced responsiveness to other agents acting on nociceptors. A mutual cross-desensitization between bradykinin and neuropeptide Y was revealed in cultured primary sensory neurons (270). In cultured neuronal hybrid cells, bradykinin pretreatment diminished responsiveness to carbachol and ATP, and this interaction was shown to be due to depletion or alteration of intracellular Ca^{2+} stores from which Ca^{2+} is mobilized by these agonists (63).

D. The Pronociceptive Actions of Applied Bradykinin

Manifestations, mediating receptors, and signaling mechanisms of the excitatory/spike generating and the nociceptor-sensitizing/hyperalgesic actions of bradykinin are summarized in **TABLES 2** and **3**, respectively.

1. The neural excitatory action of bradykinin

A) MANIFESTATIONS AND SIGNALING MECHANISMS OF THE NEURAL EXCITATORY ACTION OF BRADYKININ (**TABLE 2**). The excitatory, i.e., spike-generating, effect of bradykinin on nociceptive primary sensory neurons has been studied in various experimental models including behavioral studies in humans and conscious animals as well as electrophysiological recordings in vivo and in vitro. Early studies demonstrating the algogenic effect of bradykinin include those in humans in which the substance was applied on the fresh blister base and the evoked pain was subjectively estimated (26, 345). In subsequent studies, bradykinin also induced pain in humans upon administration into the intact skin (172, 365, 465, 642, 771), skeletal muscle (32), or a vascularly isolated vein segment (364). In rats and mice, intraplantar injection of bradykinin induced overt nociception manifesting itself as paw lifting and licking (22, 186, 245, 293, 337, 400, 436). Intraperitoneal injection of bradykinin induced writhing in mice (757).

In anesthetized dogs, cats, rabbits, and rats, bradykinin applied into different vascular beds of various organs induced a stereotyped nocifensive response (674) or various reflex cardiorespiratory changes as a consequence of stimulation of nociceptors (114, 255, 292, 326, 667). In electrophysiological experiments in anesthetized rats, multifiber recordings revealed bradykinin's spike-generating action in cutaneous afferents (96). In the neonatal rat spinal cord-tail preparation in vitro, bradykinin applied to the tail activated peripheral fibers and evoked a concentration-dependent depolarization recorded from a spinal ventral root (162).

A more advanced method for the study of the neural excitatory effect of bradykinin is recording action potentials from single fibers of sensory nerves in vivo (in anesthetized animals) or in vitro which allows determination of the fiber types activated by the applied stimulus. There are two extensively used in vitro models that have provided a large amount of data about the features of bradykinin's effects on

Table 2. *Manifestations, receptor types, and signaling mechanisms of the neuronal excitatory effects of bradykinin*

Evoked Response	Receptor Type	Signaling Mechanism	Membrane Target	Reference Nos.
Pain in humans upon blister base or intradermal application	B_2			26, 172, 345, 365, 465, 642, 771
Pain in humans upon injection into muscle				32
Pain in humans upon injection into a vascularly isolated vein segment		NO-cGMP		291, 364
Nocifensive reaction in rats	B_2		Ca^{2+}-activated Cl^- channels M-type K^+ channels (K_v7 or KCNQ)	245, 293, 436
Nocifensive reaction in mice	B_2	PLC-PKC PLA_2-5-LOX COX	TRPV1 (low dose) TRPV1 (high dose) TRPA1 $Na_v1.9$	22, 186, 337, 400, 757
Pseudoaffective reaction in anesthetized dogs and cats		COX NO-cGMP		114, 255, 674
Cardiorespiratory reflex responses in anesthetized rats and rabbits	B_2			292, 667
Depolarization response in the rat spinal cord–tail preparation in vitro	B_2	COX		162, 616
Spike discharge in the isolated perfused rabbit ear		COX-PGE_1 (nonneuronal)		326, 327, 416, 417
Spike discharge in cutaneous afferents of the cat, rat, rabbit, monkey, and human (~50%)				46, 96, 350, 642, 693
Spike discharge in rat cutaneous polymodal nociceptors in vitro (~50%)	B_2	PLA_2-LOX COX	TRPV1	39, 258, 403, 576, 653
Spike discharge in mouse cutaneous nociceptors in vitro			TRPV1	337
Spike discharge in muscle afferents of the cat and dog (dog 87 %)				210, 286, 396, 398, 488, 489
Spike discharge in articular nociceptors of the cat and rat (76–96%)				54, 247, 346, 491, 330, 636, 706, 707
Spike discharge in testicular nociceptors of the dog (88%)				397
Spike discharge in dog testicular nociceptors in vitro (93%)	B_2	COX-PGE_2 PKC		395, 497, 500, 502
Spike discharge in dog tracheobronchial afferents				339
Spike discharge in guinea pig tracheobronchial afferents in vitro (84–100%)	B_2	5-LOX, 12-LOX COX	TRPV1 Ca^{2+}-activated Cl^- channels	88, 207, 329, 410, 735
Spike discharge in mouse bronchopulmonary afferents in vitro (42%)	B_2	COX		373
Spike discharge in cardiac "sympathetic" afferents of the cat, dog and ferret (cat 83%)	B_2	COX (cat) NO	TRPV1 (ferret)	35, 555, 556, 721, 763–765
Spike discharge in cardiac vagal afferents of the dog and rat (dog 83%)		COX (dog)		285, 338, 646
Spike discharge in guinea pig esophageal vagal C-fibers ex vivo (100%)	B_2		TRPA1	791
Spike discharge in abdominal afferents of the cat (73%)		COX PKC		252, 253, 440, 557
Spike discharge in colonic afferents of the mouse and cat (66–67%)	B_2		TRPA1	67, 68, 272
Spike discharge in jejunal afferents of the rat in vitro	B_2	COX-PGE_2 COX2		476
Spike discharge in mesenteric jejunal afferents in the mouse in vitro			TRPV1	279, 611

Table 2—*Continued*

Evoked Response	Receptor Type	Signaling Mechanism	Membrane Target	Reference Nos.
Spike discharge in renal afferents of the rat (53%)		COX-PGE$_2$ NK$_1$ receptors		379, 692
Spike discharge in urinary bladder afferents of the rat				738
Spike discharge in nociceptors of rat oral cavity and periodontium				722, 723
Spike discharge in rat and mouse sensory neurons	B$_2$	PLA$_2$-LOX 12-LOX IP$_3$ i.c. Ca^{2+} release	TRPV1	34, 313, 331, 534, 653, 781

Underline, lack of involvement; i.c., Intracellular. Percentage values in brackets refer to the incidence of bradykinin sensitivity among C-fibers. For abbreviations, see text.

nociceptors: the canine testis-spermatic nerve preparation and the rat skin-saphenous nerve preparation, suitable for studying the visceral and cutaneous nociceptors, respectively (395, 600). Of the various types of nociceptive fibers, bradykinin primarily acts on the mechano-heat-sensitive or polymodal nociceptors including both the unmyelinated C and thinly myelinated Aδ units as studied in the rabbit, rat, monkey, or human skin; dog and cat gastrocnemius muscle; dog testis; rat oral cavity; rat temporomandubular joint; and rat periodontium (see details in **TABLE 2**). Bradykinin-induced spike discharge was observed in several other studies in afferent fibers from various organs and also in cultured rat and mouse trigeminal and DRG neurons **(TABLE 2)**. It is worth mentioning that the incidence of bradykinin responsiveness varies among nociceptors in various tissues: while in the skin about half of the C-fibers were excited by bradykinin, the incidence of bradykinin sensitivity is significantly higher in most deep tissues (note percentage values in **TABLE 2**).

Regarding the mechanisms of bradykinin's excitatory actions, in all studies in which the receptor type mediating the excitatory effect of bradykinin was tested, the involvement of the B$_2$ subtype was revealed **(TABLE 2)**. In many studies, a strong tachyphylaxis to the excitatory effect of bradykinin was noted (see, e.g., Refs. 35, 46, 186, 298, 356, 393, 397, 398, 403, 431, 465). Concerning the intracellular signaling mechanisms underlying bradykinin's sensory stimulant effects, a role for PKC activation in the bradykinin-induced spike discharge was revealed in canine testicular nociceptors and cat abdominal ischemia-sensitive visceral afferents (252, 253, 497). In several studies the nociceptor-activating effect of bradykinin was shown to involve TRPV1 or TRPA1 channels. The bradykinin-evoked spiking of rat cutaneous nerve fibers, cardiac "sympathetic" afferents in the ferret, murine jejunal afferents, and guinea pig tracheobronchial afferents were diminished by TRPV1 receptor antagonists (88, 410, 556, 611, 653). The action potential-generating effect of bradykinin on rat cardiac DRG neurons also involved TRPV1 stimulation in addition to 12-LOX activation, IP$_3$ formation, and intracellular Ca^{2+} release but not influx of extracellular Ca^{2+} (781). The overt nociception evoked by intraplantar injection of bradykinin in mice was abolished by a selective PLC inhibitor and reduced by inhibitors of PKC, TRPV1, as well as PLA$_2$ and 5-LOX (186). In addition, the latter behavioral response was shorter lasting in Na$_v$1.9 null mutant mice lacking this intermediate amplifier ion channel (22). In TRPV1 knockout mice, the nocifensive effect of a lower dose of bradykinin was reduced while that of a higher one remained unaltered similarly to the spike discharge response in cutaneous C-fibers (337). In TRPA1 knockout mice, the bradykinin-induced nocifensive reaction was also reduced (400). In contrast, the bradykinin-induced spike discharges in guinea pig esophageal vagal C-fibers did not involve activation of TRPA1 channels. Action potential generation induced by bradykinin in tracheobronchial fibers depended on activation of Ca^{2+}-activated Cl$^-$ channels (410, 791).

B) CONTRIBUTION OF PROSTANOIDS, LIPOXYGENASE PRODUCTS, AND NO TO THE NEURAL EXCITATORY EFFECTS OF BRADYKININ **(TABLE 2)**.

There exists plenty of experimental data demonstrating that the excitatory effect of bradykinin involves formation and action of COX products, i.e., prostanoids. Although numerous studies revealed a role for secondary COX products, only a few of them identified the prostanoid(s) and their cellular source(s) which can be the sensory neurons and/or other, adjacent cells. In the isolated perfused, innervated rabbit ear, the paravascular nociceptor-activating effect of bradykinin was inhibited by co-applied indomethacin, a nonselective COX inhibitor (416). In this model, bradykinin evoked PGE$_1$ release which was abolished by indomethacin but not reduced following chronic denervation, suggesting that the bulk of prostanoids originated from nonneuronal cells (327, 417). The discharge-inducing effect of bradykinin in the in vitro dog testis-spermatic nerve preparation, in serosal afferents of the rat jejunum in vitro and the bradykinin-induced incapacitation in the rat knee joint were all diminished/abolished by nonselective COX inhibitors whose effects were largely reversed by exogenously applied PGE$_2$ supporting the involvement of endogenous prostanoids (476, 502, 727).

In the neonatal rat spinal cord-tail preparation in vitro, activation of nociceptors by low concentrations of bradykinin was diminished by nonselective COX inhibition, whereas the effect of high concentrations remained unaltered (162, 616). The nocifensive reflex response evoked by excitation of perivascular afferents by bradykinin in the dog was reduced by systemic COX inhibition (114). Bradykinin-induced spike discharge in renal pelvic afferents of the rat and in cardiac and abdominal visceral ischemia-sensitive afferents of the cat were reduced by COX inhibition (379, 557, 721). In the latter model, a more marked diminishment was observed by simultaneous inhibition of COX and PKC, demonstrating potential additive roles of prostanoids and PKC activation in ischemic pain (252, 253). The activation of cardiac "sympathetic" and vagal afferents by epicardial or intra-arterial administration of bradykinin in anesthetized dogs was enhanced in heart failure, and these facilitated responses, unlike those in normal dogs, were inhibited by indomethacin, demonstrating a potential role of secondary prostanoids in cardiac pain (646, 763–765). In contrast to the above whole animal models, a lack of involvement of prostanoids in the neuronal excitatory effect of bradykinin was revealed in several cellular and isolated organ models (186, 207, 209, 329, 373, 483, 514, 546, 576, 653).

LOX products are also involved in some excitatory actions of bradykinin. The spike discharge-inducing effect of bradykinin in rat cutaneous afferents and DRG neurons depended on PLA_2 activation and 12-LOX activity (653). The action potential-evoking effect of bradykinin in guinea pig tracheobronchial afferents in vitro was diminished by either 12- or 5-LOX inhibition (88). In acutely isolated cardiac DRG neurons, the bradykinin-induced increase in firing rate and decrease of the threshold for action potential generation were inhibited by a selective 12-LOX inhibitor (781). One may remember that bradykinin stimulated via the 12-LOX pathway the production of 12-HETE, a TRPV1 agonist, in sensory neurons in vitro and in rat skin in vivo (653, 761). The overt nociception evoked by intraplantar injection of bradykinin in mice was reduced by inhibitors of either PLA_2 or 5-LOX (186). In accord, intraplantar injection of bradykinin in mice increased the levels of LTB_4 in the injected paw via activation of LOX enzymes.

NO appears to be involved not only in B_2 receptor desensitization but also in some excitatory effects of bradykinin. Pain induced by bradykinin injected into a vascularly isolated vein segment in humans was reduced by inhibition of NOS or GC, pointing to an involvement of NO and cGMP in the algogenic action of bradykinin in this model (291, 364). Consistent with this, exogenously applied NO solutions also induced pain in this experimental arrangement (289). The reflex increase in renal sympathetic nerve activity induced by epicardial application of bradykinin was reduced by systemic NOS inhibition in dogs (765). The enhanced form of this response observed in heart failure, however, was not influenced by NOS inhibition. The reflex response induced by excitation of perivascular afferents in the occipital artery territory by bradykinin in the dog was reduced by systemically applied inhibitors of either NOS or GC (114). Although these studies show that NO can be a mediator of the neuroexcitatory effect of bradykinin, at least in certain models, they do not provide information about the source of NO which might be the bradykinin-responsive nociceptor or other, adjacent cells. Also suggested by these studies is that NO can act as a pronociceptive agent in the periphery. As mentioned previously in section II*C5*, the NO-cGMP pathway is involved in the desensitization of B_2 receptor-mediated actions pointing to a peripheral antinociceptive role of this signaling mechanism. There are numerous studies supporting either a pro- or an antinociceptive role for the NO-cGMP axis in the periphery, independently of the bradykinin action. The dual role of the NO-cGMP pathway in peripheral nociception is discussed in sections VI, *B* and *C*.

It is important to emphasize that prostanoids, LOX products, and NO are also involved in numerous nociceptor sensitizing/hyperalgesic actions of bradykinin; these data are described in section II*D*, *2–4* (see also **TABLE 3**).

2. The sensitizing action of bradykinin to heat stimuli

The features and molecular mechanisms of the heat-sensitizing effect of bradykinin have been studied in great detail, and the results obtained suggest that probably this action of bradykinin is of primary importance with regard to inflammatory pain (**TABLE 3**).

A) MANIFESTATIONS OF THE HEAT-SENSITIZING ACTION OF BRADYKININ. An early in vivo evidence for the heat-sensitizing effect of bradykinin was provided by Beck and Handwerker (46) who demonstrated that bradykinin applied by close arterial injection increased the number of spikes evoked by heat stimulation in cutaneous nociceptive unmyelinated afferents of the anesthetized cat. In vitro, the first evidence was obtained in the isolated rat skin-saphenous nerve preparation which also provided the reverse finding that heat stimulation could markedly facilitate a subsequent bradykinin response even when tachyphylaxis had developed (403). In this model the heat-sensitizing effect, similar to the spike-generating one, of bradykinin on mechano-heat-sensitive polymodal C-fibers was mediated by B_2 receptors and was characterized by a drop of the heat threshold, an increase in the number of spikes evoked by the heat stimulus as well as a leftward shift and increased slope of the stimulus-response function (258, 375). Furthermore, unequivocal evidence was provided that, unlike bradykinin-induced mechanical hyperalgesia in rats (see sect. II*D4*), the heat-sensitizing effect of the peptide did not involve activation of

Table 3. *Manifestations, receptor types, and signaling mechanisms of the nociceptor-sensitizing/hyperalgesic effects of bradykinin*

Sensitized Response	Receptor Type	Signaling Mechanism	Membrane Target	Reference Nos.
		Heat sensitization		
Heat-induced pain in human skin		Sympathetic system		465, 492
Heat-induced paw withdrawal in rats	B_2	COX 12-LOX		245, 644 653
Heat-induced paw withdrawal in mice		Sympathetic system	TRPV1 TRPA1 Na_v1.9	22, 45, 110
Heat-induced discharge of cat or monkey cutaneous noiceptors				46, 350
Heat-induced discharge of rat cutaneous nociceptors in vitro	B_2	Nonneuronal COX-1 and COX-2 Sympathetic system		258, 375, 403, 431, 478, 576, 630, 631
Heat-induced discharge of dog testicular nociceptors in vitro	B_2	COX PKC		392, 497
Heat response in the rat spinal cord–tail preparation in vitro		COX, LOX		616
Heat-induced CGRP release from isolated rat skin				348
Heat-induced CGRP release from isolated mouse trachea			TRPV1	356
Heat-induced CGRP release from isolated mouse sciatic nerve		PKC		200
Heat-induced current in rat DRG neurons		PKCε		94, 95
Heat-induced TRPV1 activation in HEK cells and rat DRG neurons		PKC	TRPV1	685
		Mechanical sensitization		
Mechanically-induced hindpaw withdrawal in the rat (Randall-Selitto method)	B_2	Sympathetic postganglionic fibers PLA$_2$-COX-PGE$_2$ NO-cGMP-PKG	P2X$_3$/P2X$_{2/3}$	142, 354, 423, 424, 525, 674, 695-697 354
The same response after CFA treatment	B_2 B_1	PKA		
Mechanically-induced hindpaw withdrawal in the rat (modified Randall-Selitto method)	B_2 B_2 B_1	TNF-α/IL-6/IL-1β/prostanoids TNF-α/CINC-1 (CXCL1)/ sympathetic amines Leukocyte infiltration	β_1 adrenoceptor	127, 189, 192, 581
Mechanically-induced hindpaw withdrawal in the mouse	B_2	COX + sympathetic amines (TNF-α, IL-1β)	TRPA1 Na_v1.9	22, 126, 400
The above response after LPS treatment	B_1	TNF-α/IL-1β/prostanoids KC (CXCL1)/prostanoids + sympathetic amines		126
Articular mechanical hyperalgesia in the rat	B_2			132, 133
Mechanically-induced spike discharge in cat muscle afferents, cat or rat knee joint afferents, rat ankle joint nociceptors, rat cardiac vagal polymodal nociceptors, dog testicular nociceptors in vitro				54, 247, 285, 370, 487, 533, 564
Mechanically-induced spike discharge in guinea pig esophagal vagal C-fibers ex vivo			TRPA1	791
Mechanically-induced spike discharge in mouse splanchnic colonic afferents in vitro			TRPA1	67, 68

Table 3—*Continued*

Sensitized Response	Receptor Type	Signaling Mechanism	Membrane Target	Reference Nos.
Chemical sensitization				
Capsaicin-induced response in the rat spinal cord–tail preparation in vitro		COX, LOX		616
Capsaicin-induced firing of vagal tracheal C-fibers in the guinea pig				208
Capsaicin- or low pH-induced nocifensive reaction in the mouse				186
Capsaicin-induced Ca^{2+} uptake in rat DRG neurons	B_2	PLC, PKC, COX-1 (COX-2)		85, 711
Anandamide-induced cobalt uptake in rat DRG neurons				658
Capsaicin-induced SP release from rat DRG neurons		IP_3-dependent Ca^{2+} release COX-2		552, 709
Incidence of capsaicin or proton responsiveness among rat DRG neurons		COX, LOX		679
Capsaicin- or low pH-induced TRPV1 gating				110
Capsaicin-induced current in TRPV1-transfected cells and rat DRG neurons	B_2	PKC-AKAP	TRPV1	436, 794
Low pH-induced current in DRG neurons				386
Formalin-induced nocifensive reaction in the mouse	B_2 B_1			140
Mustard oil-induced nocifensive reaction in the rat				762
Histamine-induced spike discharge in rat cutaneous nociceptors in vitro				380, 381
Histamine response in the human skin				381
Histamine or TXA_2 analog-induced spike discharge in cat cardiac C-fibers		COX		213, 214
Histamine-induced CGRP release in the guinea pig lung		COX		645
Noradrenaline-evoked spike discharge in rat cutaneous nociceptors in vitro following CFA treatment				40
Recruitment of noradrenaline-evoked spike discharge in rat cutaneous nociceptors				629
Serotonin-evoked inward current in rat trigeminal neurons		PKC		296

Underline, lack of involvement; i.c., Intracellular. For abbreviations, see text.

sympathetic postganglionic fibers as it was unaffected by surgical sympathectomy. This finding was reinforced by two other studies investigating bradykinin-induced heat hyperalgesia in humans and rats (492, 644). In the isolated rat skin-saphenous nerve preparation, the excitatory and heat-sensitizing actions of bradykinin were compared with regard to prevalence and susceptibility to tachyphylaxis (431). A 5-min exposure to 10 μM bradykinin sensitized to heat 85% of the mechano-heat-sensitive polymodal C-fibers, whereas only 40% of the units were excited by bradykinin. The heat sensitization could be washed out and repeated several times without any decrease in the magnitude of the sensitization, while the excitatory effect showed a profound tachyphylaxis. The high-threshold mechanosensitive C-fibers were not excited by bradykinin, but 50% of them gained transient heat sensitivity following bradykinin treatment, indicating a de novo recruitment of heat responsiveness by the agent.

In the isolated canine testis-spermatic nerve preparation, bradykinin enhanced the number of spikes evoked by heat stimulation of the Aδ polymodal nociceptors (392). This augmenting effect could be induced by 100 times lower concentration than required for inducing spike discharge of nociceptors, and it was short-lived, outlasting the bradykinin superfusion for no more than 10 min; it was suppressed by a B_2 receptor antagonist.

Injection of bradykinin into the human skin induced heat hyperalgesia (with a leftward shift in the stimulus-response function) in addition to overt pain observed at higher dosage as assessed by subjective ratings (465). No alteration of the mechanonociceptive threshold was noted and, while the algogenic effect exhibited a near-complete tachyphylaxis upon repeated applications, the heat-sensitizing action, albeit reduced, remained still significant. Bradykinin caused a sensitization to heat but not to mechanical stimuli in identified nociceptors in the hairy skin of the monkey (350). This action again comprised a decrease in the heat threshold and an augmentation of the heat response. Interestingly, both B_1 and B_2 receptor selective agonists had similar sensitizing effects. In the neonatal rat spinal cord-tail preparation in vitro, bradykinin also induced an augmentation of the heat response (a nocifensive ventral root reflex; Ref. 616). In a study employing the behavioral plantar test in the rat, heat sensitization induced by intraplantar bradykinin injection was strongly reduced by systemic COX blockade, indicating that prostanoids contribute to the response (644). In the same model, both the nocifensive (algogenic) and the heat-sensitizing effects of bradykinin were abolished by a non-peptide B_2 receptor antagonist (245). The heat-sensitizing effect of bradykinin also became evident when studying neuropeptide release from isolated rat skin or axons (200, 348). In addition to the above models reflecting the function of the peripheral terminals of nociceptive sensory neurons, bradykinin's heat-sensitizing effect was also revealed by measuring a novel heat-activated membrane current mediated by nonselective cation channels in somata of cultured DRG neurons (95). This current was sensitized by a short (20 s) exposure to bradykinin, and the sensitizing effect manifested itself as a drop of the heat threshold and an increase in the depolarizing cation conductance. Subsequently, following cloning of TRPV1, the heat-sensitizing action was also demonstrated in nonneuronal cells transfected with TRPV1 (685). These cellular models significantly contributed to the understanding of the molecular mechanisms of this effect of bradykinin (see sect. II*D2*B).

B) MECHANISMS OF THE HEAT-SENSITIZING ACTION OF BRADYKININ. A role for PKC activation, the heat-sensitizing action of bradykinin, was first suggested by findings that a heat-activated ionic current in the somata of rat DRG neurons *1*) was similarly sensitized by bradykinin or the PKC-activating phorbol ester PMA; *2*) it was prolonged by the phosphatase inhibitor calyculin A; and *3*) the PMA effect was blocked by the nonselective protein kinase inhibitor staurosporine (95). These results suggest that the heat-sensitizing effect of bradykinin was due to a PKC-mediated phosphorylation in this model. In a subsequent study, the Ca^{2+}-independent PKCε was identified as the molecular entity responsible for this action (94). The Ca^{2+}-independent nature of the heat-sensitizing effect of bradykinin was also evident from the lack of effect of BAPTA-AM, a membrane-permeant Ca^{2+} buffer, on the heat sensitization of polymodal nociceptors in the isolated rat skin-saphenous nerve preparation (S. Günther, M. Kress, and P. Reeh, unpublished data). Recording of action potentials from canine testicular nociceptors in vitro also revealed an involvement of PKC in the heat-sensitizing effect of bradykinin (497). Bradykinin massively lowered the threshold temperature of the noxious heat-sensitive TRPV1 channel in both transfected cells and rat DRG neurons in a PKC-dependent manner (685). A further confirmation of the role of PKC in heat sensitization was that PKC activation by phorbol esters facilitated the heat-induced activation of TRPV1 in transfected cells and DRG neurons (120, 745). Noxious heat was also shown to release CGRP from the isolated rat skin, and this heat response was facilitated by bradykinin or PMA, suggesting PKC involvement (348). In isolated desheathed nerves of the mouse, bradykinin applied for 10 min enhanced the heat-evoked axonal CGRP release, and this effect was abolished by PKC inhibition (200). It is worth mentioning that PKCε is also involved in the long-term modulation of nociception as it mediates a chronic hypersensitivity for inflammatory nociceptor sensitization (16).

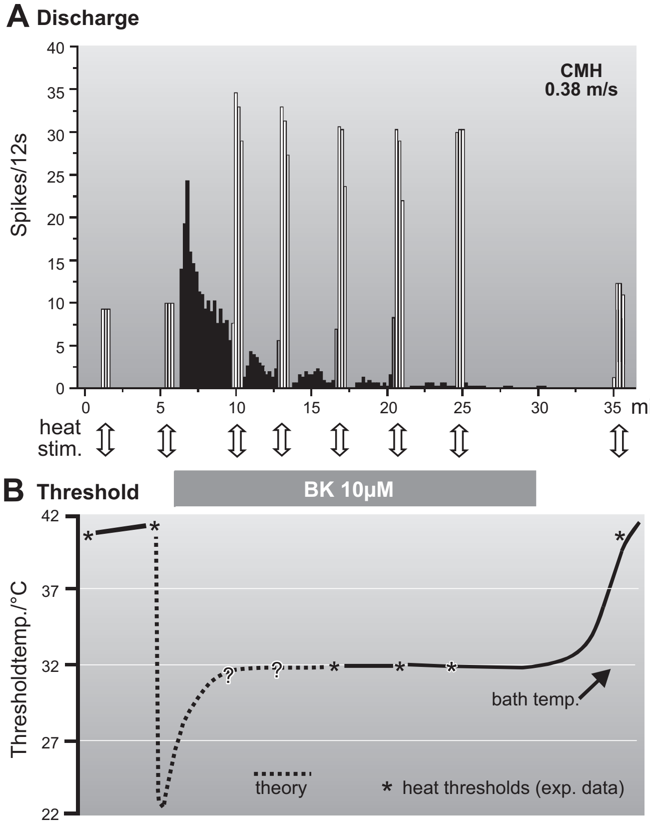
Although PKC activation is of crucial importance in the immediate heat sensitization induced by bradykinin, its role is not exclusive. In the isolated rat skin-saphenous nerve preparation, heat sensitization of polymodal C-fibers induced by a more sustained (5 min) application of bradykinin was abolished by the active, but not the inactive, enantiomer of the nonselective COX inhibitor flurbiprofen, and the effect of the active isomer was largely reversed by exogenously applied PGE_2 (576). These results were confirmed and extended by a subsequent study employing the same preparation, in which the heat-sensitizing effect of bradykinin was reduced by either COX-1 or COX-2 inhibition (478). Heat injury-induced sensitization to heat in cutaneous C polymodal units also involved formation of COX products as studied in an isolated perfused rabbit ear preparation (112). As the heat-sensitizing action of PGE_2 and related prostaglandins is predominantly mediated by the cAMP-PKA cascade, at least in the rat (see sect. III*C1*), these results suggest that PKA activation may also be involved in the heat-sensitizing effect of bradykinin. It was proposed that the early phase of heat sensitization by bradykinin predominantly depends on PKC activation, while in the sustained or after-effects the COX products and the cAMP-PKA signaling mechanism gain increasing importance (576). This hypothesis would explain why a major contribution of PKC activation was revealed in studies employing short bradykinin exposures, whereas a predominant role of COX products became evident after a prolonged bradykinin superfusion. It is worth mentioning that COX inhibition failed to diminish bradykinin-induced heat sensitization in rat testicular and tail nociceptors in vitro (392, 616). It should be kept in mind, however, that a cross-talk between the PKC and cAMP-PKA pathways has been revealed as phorbol ester-induced activation of PKC led to activation of adenylyl cyclase (AC) in various cell types including rat DRG neurons (662). The cutaneous source of prostaglandins sustaining the heat-sensitizing effect of bradykinin could be the nociceptors and/or various nonneuronal cell types in the vicinity of them as bradykinin receptors have been identified not only on nerve endings but also on endothelial, epidermal, and mast cells in the skin (261). However, the peripheral COX products mediating bradykinin-induced heat sensitization appear to originate mostly from nonneuronal cells because the bradykinin-induced cutaneous PGE_2 release remained unaltered following chronic denervation of the rat skin (630, 631). Local inhibition of the 12-LOX enzyme abolished the heat-sensitizing action of intradermally applied bradykinin in the rat indicating the contribution of LOX product(s) to the response (653).

The final step in the bradykinin-induced heat sensitization, not accompanied by mechanical hypersensitivity, is most probably a facilitation of heat-sensitive ion channels such as TRPV1 located in the peripheral nerve endings and/or a recruitment of heat transducers hardly activated by moderate heat stimuli under normal conditions. As mentioned above, bradykinin lowered the heat threshold of TRPV1 both in TRPV1-transfected cells and DRG neurons (685). The role of TRPV1 is also supported by a finding that in TRPV1 knockout mice intraplantar injection of bradykinin failed to evoke heat hyperalgesia, whereas it was effective in wild-type animals (110). The heat-sensitizing effect of the combination of bradykinin and PGE_2 on sciatic nerve axons was absent in TRPV1 knockout mice (200). The facilitatory effect of bradykinin on the heat (40°C)-induced CGRP release in the isolated mouse trachea proved to be TRPV1 dependent (356). A role of the other heat-activated ion channels TRPV2, TRPV3, and TRPV4 in the bradykinin-induced heat sensitization is not established. One must also consider that the bradykinin-induced heat hyperalgesia was absent in either TRPA1 or $Na_v1.9$-deficient mice compared with wild-type animals (22, 45). The former finding is difficult to interpret, possibly an interaction between TRPV1 and TRPA1 may be involved (see sect. II*C3*B).

C) THE RELATIONSHIP BETWEEN THE HEAT-SENSITIZING AND NEURONAL EXCITATORY ACTIONS OF BRADYKININ: A UNIFYING HYPOTHESIS.

As mentioned in section II*D2*A, apparently contradictory data have been obtained in the isolated rat skin by single-unit recording from C polymodal nociceptors. On the one hand, the heat-sensitizing effect of bradykinin was shown to be devoid of any notable tachyphylaxis, whereas the excitatory bradykinin effect exhibited the typical homologous desensitization (431). On the other hand, however, both effects were mediated by the same bradykinin receptor subtype, the B_2 receptor (258). As mentioned in section II*C5*, a rapid internalization of B_2 receptors occurs following bradykinin exposure (27, 579), which is likely to affect the sensitizing and the excitatory actions equally making it difficult to explain the difference in their tendency to desensitize. To resolve this contradiction, a hypothesis connecting the heat-sensitizing and excitatory (i.e., spike-generating) actions of bradykinin has been put forward **(FIGURE 2)**. This denies a direct neuronal excitatory action of bradykinin but assumes a rapid and profound sensitization of nociceptors to heat involving a drop of the heat threshold below the ambient temperature (599, 431). This major sensitization would enable the ambient or body temperature to function as a heat stimulus and thereby to produce an excitatory effect. In addition, it is assumed that the apparent excitatory effect of bradykinin fades with desensitization as the heat threshold exceeds ambient temperature, but this desensitization levels at low threshold temperatures so that bradykinin's heat-sensitizing action appears maintained **(FIGURE 2)**. Direct experimental support for the theory has been obtained. In rat skin-saphenous nerve preparations cooled down to 16–18°C, bradykinin failed to evoke discharge, but it lowered the heat threshold well below 32°C (599). In addition, bradykinin induced a drop of the heat threshold below normal skin temperature (32°C) in cells cotransfected with B_2 and TRPV1 receptors as well as in sensory neurons (685). In a recent study on an isolated mouse trachea preparation held at 37°C, bradykinin failed to enhance noxious heat (45°C)-induced CGRP release (356). When the preadaptation temperature was reduced from 37 to 22°C and the test stimulus was 40°C, a significant CGRP release was observed that was the same in wild-type and TRPV1 knockout animals. This heat response was markedly enhanced by bradykinin in wild-type but not TRPV1 knockout preparations. These results also show that bradykinin can lower the heat threshold below 40°C and recruit TRPV1 channels.

This hypothesis, unifying the heat-sensitizing and excitatory actions of bradykinin, can account for several earlier observations. The prevalence of bradykinin responsiveness among nociceptors is generally higher in deep tissues at core temperature than in the skin at surface temperature (see sect. II*D1*A). Lower concentrations of bradykinin are required for the heat-sensitizing effect than for the excitatory one (392). The heat-sensitizing effect of bradykinin was shown to be more sustained than the excitatory one (465)



modified from Reeh and Pethő, 2000 (599)

FIGURE 2. Hypothesis explaining the excitatory, i.e., spike-generating effect of bradykinin as a result of an extreme heat-sensitizing action leading to a drop of heat threshold below ambient temperature (see details in text in section II*D2*c). *A*: experimental results obtained by recording action potentials from a single C mechano-heat-sensitive nociceptor in the isolated rat skin. Black columns show histogram of action potential discharges evoked by bradykinin (BK) superfusion of the cutaneous receptive field; open columns indicate histogram of heat stimulation-induced discharges. Note the marked desensitization of the excitatory bradykinin response and simultaneous sustained sensitization to heat induced by bradykinin. *B*: theory assuming a drop of heat threshold of the unit far below temperature of the preparation (32°C) that enables the environment to evoke a "heat response" that appears as spike discharges of an apparent excitatory bradykinin action subject to sensory adaptation. Desensitization to bradykinin allows the heat threshold to rise with a tendency to level out. Question marks indicate hypothetical threshold values that could not be assessed due to vigorous discharge.

and could occur in the absence of bradykinin-induced discharge activity (46, 350, 392). A strong correlation was found between the degrees of heat sensitization and excitation by bradykinin both in the canine testis and rat skin preparations (392, 431). Heat thresholds of C-fibers excited by bradykinin were lower than those of the unresponsive ones (403).

It is worth recalling that a similar theory was previously applied to capsaicin that was thought to activate the TRPV1 channels by lowering their heat threshold below ambient temperature (725). Consistent with this, cooling of the skin abolished nociceptor discharge in the rat or pain in humans evoked by topical administration of capsaicin or low pH (357, 673, 691). In fact, it has been shown that capsaicin, bradykinin, and protons can decrease the activation energy required to operate the gates of heat-activated channels in sensory neurons so that room temperature becomes sufficient to activate currents (754). Other proinflammatory mediators including ATP and prostaglandins (PGE$_2$ and PGI$_2$) were also shown to lower the heat threshold of TRPV1 in transfected cells below ambient temperature through purinergic P2Y$_1$ and prostanoid EP$_1$ and IP receptors, respectively, and a similarly massive heat threshold-lowering action of serotonin in mouse sensory neurons was also reported (726,511, 684, 726). These results indicate that several agents are able to cause a major sensitization to heat (heat threshold drop below tissue temperature) predominantly by a facilitatory effect on the TRPV1 receptor.

3. The sensitizing action of bradykinin to mechanical stimuli (Table 3)

Bradykinin's sensitizing action to mechanical stimuli was extensively investigated in behavioral studies in which the decrease of the mechanonociceptive threshold of the rat was measured using the Randall-Selitto hindpaw withdrawal method (423, 424, 674, 695). In this model, the mechanical hyperalgesic effect of bradykinin applied by intradermal injection in the normal skin was mediated by B$_2$ receptors while in a sustained inflammatory state induced by CFA both B$_2$ and B$_1$ receptors were involved (354). The bradykinin response was *1*) reduced by depletion of polymorphonuclear leukocytes (423), *2*) diminished by a systemic COX blockade (423), and *3*) absent following chemical (induced by chronic guanethidine pretreatment) or surgical sympathectomy (353, 424). Likewise, the B$_1$ receptor agonist-evoked mechanical hyperalgesia observed following CFA pretreatment was prevented by surgical sympathectomy (354). In addition, *1*) bradykinin was reported to cause excitation of sympathetic postganglionic neurons (430, 732), *2*) the bradykinin-induced mechanical hyperalgesia was shown to be mediated by PLA$_2$ activation and PGE$_2$ formation (696, 697), *3*) production of PGE$_2$ and PGI$_2$ by sympathetic postganglionic neurons was reported (239), and 4) PGE$_2$ and PGI$_2$ can induce mechanical hyperalgesia and nociceptor sensitization (see sect. III*C2*) On the basis of these data, a hypothesis was put forward that bradykinin exerted its mechanical sensitizing effect indirectly by releasing from the sympathetic postganglionic fibers prostanoids that eventually sensitized nociceptors. The mechanical hyperalgesia induced by intradermal injection of bradykinin in the rat hindpaw was also diminished by local inhibiton of NOS, GC, or cGMP-dependent protein kinase (PKG) as well as by inhibition of PKA (525). NO precursor, NO donor compounds, or cGMP analogs applied alone all failed to lower the mechanonociceptive threshold but coadministered with a subthreshold dose of bradykinin induced mechanical hyperalgesia. These results suggest that activation of the NO-cGMP-PKG pathway is required together with the cAMP-PKA and possibly other pathway(s) for the bradykinin-evoked peripheral mechanical hyperalgesia.

The mechanical sensitizing effect of bradykinin applied intraplantarly was also revealed by a modified Randall-Selitto test in which the latency to the onset of the "freezing" reaction of the rat evoked by a constant mechanical stimulus applied to the hindpaw was measured (192). Support for a role of prostanoids in the bradykinin or kallidin-induced mechanical hyperalgesia in this model was provided as these responses were diminished by local or systemic COX blockade in both rats and mice (126, 192, 581). The bradykinin-induced hindpaw hyperalgesia (called hyperalgesia) in the rat and mouse was diminished by B_2, but not B_1, receptor antagonism (126, 192, 581); however, the B_1 receptor agonist des-Arg^9-bradykinin was also able to induce hyperalgesia (581). The bradykinin-induced mechanical hyperalgesia in the rat was also diminished by fucoidin, a leukocyte adhesion inhibitor, suggesting a role for leukocytes (127).

A significant contribution of cytokines to the bradykinin-induced mechanical hyperalgesia measured with the modified Randall-Selitto test was revealed using locally, i.e., intraplantarly administered antisera against cytokines, COX inhibitor (indomethacin,) and adrenergic β_1 receptor antagonist (atenolol). Evidence was provided that the bradykinin-induced mechanical hyperalgesia (similarly to that evoked by kallidin or des-Arg^9-bradykinin) involved initial release of TNF-α, supposedly from resident macrophages, which then initiated two pathways: production of IL-6, then IL-1β that induces formation of nociceptor-sensitizing prostanoids (see sect. IIIC2) as well as cytokine-induced neutrophil chemoattractant-1 (CINC-1, related to human IL-8) releasing nociceptor-sensitizing sympathetic amines (189, 192, 581). In accord, intraplantar injection of bradykinin in the rat increased local concentrations of TNF-α, IL-1β, IL-6, and CINC-1 (142). Local antagonism of $P2X_3$ or $P2X_{2/3}$ receptors strongly diminished bradykinin-induced mechanical hyperalgesia but failed to alter bradykinin-induced cytokine production, suggesting that P2X purinoceptor activation also contributes to the response in a cytokine-independent manner (142). In mice, activation of either B_2 or B_1 receptors can induce mechanical hyperalgesia via different mechanisms (126). In naive mice, B_2 receptors mediate the hyperalgesic effect of bradykinin dependent on prostanoids, sympathetic amines, but not cytokines. Conversely, in LPS-pretreated mice, B_1 receptors mediate the response depending on TNF-α and IL-1β which then induce production/release of prostanoids and sympathetic amines. In mice, cytokines act by somewhat different mechanisms: TNF-α stimulates only the prostanoid pathway to hyperalgesia, whereas keratinocyte-derived chemokine (KC, analogous to rat CINC-1 and human IL-8) stimulates both the prostanoid and sympathetic pathways. In addition, IL-6 induces prostanoid formation independently of IL-1β. As studied using von Frey filaments, the bradykinin-induced mechanical allodynia was abolished in mice lacking the gene for TRPA1 (400) and was diminished in mice deficient of the gene for $Na_v1.9$, the Na^+ channel mediating the noninactivating persistent tetrodotoxin-resistant Na^+ current and serving as an amplifier of generator potentials (22).

Bradykinin, but not the B_1 receptor agonist des-Arg^9-bradykinin, injected intra-articularly evoked mechanical hyperalgesia as assessed by measuring the load tolerated by the injected leg (133). This response was mediated by B_2 but not B_1 receptors (132, 133). Mechanical sensitization in deep tissues induced by bradykinin has been revealed also by single-unit recordings (see details in **TABLE 3**). Bradykinin increased the mechanical response of splanchnic, but not pelvic, colonic afferents in mice, in vitro, and this effect was lacking in preparations from TRPA1 knockout animals (67, 68). Bradykinin was shown to sensitize to esophageal distension C-fibers of vagal nodose and jugular ganglion neurons in an ex vivo guinea pig esophagus-vagus preparation, and this effect was reduced by a TRPA1 receptor antagonist (791).

In other, mostly cutaneous, models, however, bradykinin failed to sensitize nociceptors to mechanical stimuli. In the isolated rat skin-saphenous nerve preparation, bradykinin, even co-applied with histamine, serotonin, and PGE_2, was unable to produce a mechanical nociceptor sensitization (349, 375, 403). Intradermally applied bradykinin failed to sensitize cutaneous C polymodal nociceptors to mechanical stimuli in the monkey or to evoke mechanical hyperalgesia in the human skin (350, 465, 642). Also, no development of muscular mechanical hyperalgesia was found in humans by intramuscular injection of bradykinin alone, although the combination with serotonin was reported effective (31, 32, 316). The exact depth of bradykinin injection into the skin was reported to determine whether or not mechanical hyperalgesia develops, because intraepidermal but not subepidermal injection of bradykinin produced mechanical hyperalgesia in the rat (352). Recently, a significant differentiation of cutaneous sensory projection layers has been published with terminals in the superficial epidermis (stratum granulosum) being responsible for inflammatory mechanical hyperalgesia and nerve endings in deeper layers (stratum spinosum) competent for thermal hyperalgesia (93, 807). These nociceptor subpopulations also differ essentially with respect to neurochemical markers, the superficial endings expressing the sensory neuron-specific G protein-coupled receptor Mrgprd and IB_4 binding sites but no neuropeptides, while the deeper terminals express SP and CGRP, and TRPV1 occurs in both fiber types.

4. *The sensitizing action of bradykinin to chemical stimuli (Table 3)*

Several studies have revealed an enhancing effect of bradykinin on responses evoked by the TRPV1 receptor agonist capsaicin in reduced models in which the likely target for the facilitatory effect is TRPV1 itself (see sect. II*C3*A). Low concentrations/doses of bradykinin, lacking an excitatory/nocifensive action of their own, increased the capsaicin-induced *1*) nociceptive reflex in the neonatal rat spinal cord-tail preparation in vitro (616), *2*) firing response of single vagal sensory C-fibers innervating the guinea pig trachea (208), and *3*) Ca^{2+} uptake in rat DRG neurons (711) and enabled a subthreshold dose of capsaicin or low pH to induce a nocifensive reaction in the mouse (186). In host cells transfected with both B_2 and TRPV1 receptors, bradykinin potentiated the effect of capsaicin or low pH by modifying TRPV1 channel gating (110). The facilitatory action of bradykinin on the capsaicin-induced Ca^{2+} uptake was abolished by a B_2 or TRPV1 receptor antagonist and by inhibition of PLC or COX-1 but not COX-2 (711). Interestingly, the bradykinin-induced elevation of IP_3 levels was also inhibited by COX-1 blockade, suggesting that COX products are involved in the intracellular liberation of IP_3. Bradykinin restored the capsaicin-induced Ca^{2+} uptake response of rat DRG neurons that was diminished upon repeated exposures to capsaicin, and this "resensitizing" effect of bradykinin was PKC dependent (85). In rat DRG neurons, bradykinin enhanced the capsaicin-induced inward current via a B_2 receptor-PKC-AKAP-TRPV1 pathway (794; see details in sect. II*C3*A). Furthermore, the tachyphylaxis of the capsaicin-induced current observed upon repeated exposure was reduced by bradykinin (436). In cultured rat sensory neurons, bradykinin alone, or more effectively in combination with histamine and PGE_2, resulted in a significant enhancement of the sustained ionic current induced by low pH (386). A sustained (3 h) pretreatment with bradykinin enhanced SP release induced by capsaicin in rat DRG neurons, and this facilitatory effect depended on PKA activation (552). As COX-2 expression was also induced by bradykinin, its potentiating effect probably involved prostanoid formation with subsequent PKA-mediated sensitization of TRPV1 (see sect. III*B2*E).

Regarding agents acting on TRPA1 channels, a subnociceptive dose of bradykinin applied intraplantarly potentiated nociception evoked by the TRPA1 receptor agonist allyl isothiocyanate (mustard oil) in the rat, and coinjection of a B_1 or B_2 receptor agonist with a submaximal dose of formalin potentiated both phases of the nocifensive reaction in mice (140, 762).

In the isolated rat skin-saphenous nerve preparation, bradykinin pretreatment enhanced the histamine-induced discharge activity and recruited C-fibers that were previously unresponsive to histamine (380, 381). Consequently, following bradykinin pretreatment (at unperceptible concentration) of human skin, histamine induced burning pain, whereas in normal skin it caused a pure itch sensation when applied iontophoretically (381). Several other models revealed bradykinin's sensitizing effect on other chemical mediators (40, 96, 213, 214, 296, 629, 645; see details in **TABLE 3**).

5. *Stimuli and conditions that sensitize nociceptors to bradykinin*

A) PROSTANOIDS. Prostanoids have been shown to increase the sensitivity of sensory neurons to bradykinin in various models, and the biochemical mechanisms underlying this sensitization have been studied in great detail. These results are described in section III*C3* as own sensitizing effects of prostanoids.

B) OTHER MEDIATORS. Serotonin facilitated spike discharges evoked by bradykinin in canine testicular nociceptors, rat cutaneous nociceptors, canine perivascular afferents, and human muscular nociceptors (31, 114, 403, 502) as well as the bradykinin-induced overt nociception (293) but failed to sensitize to bradykinin visceral afferents of the rat jejunum (70). In the latter preparation, histamine or adenosine exerted a sensitizing effect to bradykinin, and these effects were mimicked by an analog of cAMP. In the isolated rat skin-saphenous nerve preparation, the bradykinin-induced discharge activity was enhanced following histamine superfusion (380). Combination of bradykinin with a mixture of serotonin, histamine, and PGE_2 ("inflammation soup") increased the proportion of polymodal C-fibers responding with spike discharge in the isolated rat skin (349). Serotonin or histamine enhanced the SP and CGRP-releasing effect of bradykinin in the isolated rat skin, and NGF, also an inflammatory mediator, enhanced the incidence of bradykinin sensitivity among rat DRG neurons (29, 334).

C) TISSUE INFLAMMATION. Tissue inflammation induced by carrageenan or CFA was shown to increase bradykinin sensitivity, decreasing tachyphylaxis as tested in vitro on identified nociceptors or cultured DRG neurons of the rat (39, 335, 367). In the latter model, the facilitatory effect of CFA-induced inflammation was reduced by an antibody raised against NGF (335). Ultraviolet-induced erythema of the rabbit ear enhanced the spike dicharge activity of C polymodal units evoked by bradykinin applied in close arterial injection (693). In accord, in ultraviolet-B-irradiated human skin, the pain induced by either B_1 or B_2 bradykinin receptor activation was enhanced (172). In a study examining the effect of kaolin/carrageenan-induced arthritis on articular nociceptor responsiveness to bradykinin in anesthetized cats, sensitization was found only in a small portion of fibers, on average a trend to decreased sensitivity was observed (491). Injection of the B_1 receptor agonist des-Arg^9-bradykinin into the mouse paw evoked no nocifensive response in naive animals, but following a low-dose PMA pretreatment it produced a marked nocifensive reaction (187). This sensitized response was diminished when PMA was co-applied with inhibitor of PKC or protein synthesis and also when des-Arg^9-bradykinin was co-applied with inhibitor of PKC or the MAPK p38. In accord, in HEK cells expressing the bradykinin B_1 receptor, its agonist des-Arg^{10}-kallidin resulted in a phosphorylation of p38 (230).

D) HEAT STIMULI. An elevated ambient temperature or conditioning noxious heat stimulation can also facilitate the effect of bradykinin. The discharge activity of canine testicular polymodal nociceptors induced by bradykinin was increased by a 6 or even 2°C rise in ambient temperature (393, 394). In the same preparation, noxious heat stimulation at 55°C potentiated the discharge-generating effect of a subsequent bradykinin exposure, and this facilitation was suppressed by acetylsalicylic acid (503, 504). Conditioning heat stimulation was found to sensitize polymodal nociceptors to bradykinin in the isolated rat skin as well (403). The membrane current induced by bradykinin in cultured rat DRG neurons was also increased at higher temperatures (754).

E) OTHER FACTORS. Heart failure was shown to increase the responses of both cardiac spinal and vagal afferent units to bradykinin, and these sensitizing actions were inhibited by COX blockade showing an involvement of prostanoids (646, 763–765). Urinary bladder afferents were sensitized to bradykinin by colonic irritation evoked by intraluminal administration of an irritant substance as a result of pelvic visceral cross-sensitization (738). Experimental ileus in mice enhanced the discharge-generating effect of bradykinin on mesenteric jejunal afferents in vitro, and this sensitization was abolished by selective COX-2 inhibition (514, 515). A short 17β-estradiol pretreatment (15 min) enhanced the inositol phosphate accumulation evoked by bradykinin in cultured rat trigeminal sensory neurons, and intraplantar administration of the hormone enhanced the bradykinin-induced heat hyperalgesia in awake rats (613). As a membrane-impermeable form of 17β-estradiol also evoked these effects, the likely mode of action of the hormone was nongenomic, possibly mediated by putative plasma membrane, but not intracellular, receptors.

E. Concluding Remarks and Open Questions

Findings from animal and, to a much lesser degree, human studies support the view that endogenous bradykinin is involved in the induction and maintenance of inflammatory and neuropathic pain. A huge array of neuronal excitatory and sensitizing effects to heat, mechanical, and chemical stimuli of exogenously applied bradykinin have been described. The lack of desensitization and tachyphylaxis together with the higher incidence, longer duration, and lower concentration need of the heat-sensitizing action of bradykinin compared with its transient excitatory effect indicates that it is the former action that corroborates a role for this peptide in inflammatory pain. The nondesensitizing B_1 receptors are upregulated under inflammatory and neuropathic conditions. Not only bradykinin can sensitize nociceptors to other inflammatory mediators, but the reverse interaction can augment the actions of the peptide, thereby establishing a self-reinforcing cycle. Experiments employing bradykinin receptor antagonists as well as bradykinin receptor knockout animals provided the most reliable information on pathophysiological functions of endogenous bradykinin. In experimental animal models of inflammatory or neuropathic pain, B_1 and/or B_2 bradykinin receptor antagonists exerted antihyperalgesic actions, and animals lacking B_1 and/or B_2 bradykinin receptor genes exhibited diminished nociceptive responses (see sect. II*B*, *3* and *4*). Relevant for visceral pain, activation of cardiac afferents by ischemia was shown to involve endogenous bradykinin via B_2 receptors raising the possibility that the peptide contributes to pain associated with angina pectoris (557, 721).

To what extent endogenous bradykinin contributes to inflammatory or ischemic pain in humans remains to be shown by clinical studies. According to phase II clinical trials, icatibant (HOE 140) does not appear to be efficacious in acute postoperative pain and in allergic rhinitis. Icatibant, however, was reported to be effective as an intra-articular analgesic in patients with osteoarthritis (666). The efficacy of this potent and selective B_2 receptor antagonist will be investigated in rheumatoid arthritis as well. Considering the induction of B_1 receptors under inflammatory and neuropathic conditions, it also seems reasonable to examine the clinical efficacy of B_1 receptor antagonists alone and in combination with B_2 receptor blockers (for review, see Refs. 81, 415, 508).

Regarding the molecular mechanisms of bradykinin actions, a finally unresolved issue is the identity of ion channel(s) underlying the membrane depolarization induced by bradykinin. Possible candidates are the nonselective cation channels TRPV1 and TRPA1 but also the Ca^{2+}-activated Cl^- channels. Some data indicate that these channels contribute to the depolarization in an additive fashion (see sect. IIC, *3*B and *4*C). Concerning the mechanisms of the excitatory/spike-generating and sensitizing actions of bradykinin, a great amount of data have been obtained from transfected nonneuronal cells and DRG or trigeminal ganglion neurons which are compatible with the view that the nociceptive heat threshold can fall below tissue temperature, thus inducing depolarizing ion currents and discharge activity. The mechanisms described in these models also seem to operate in the peripheral terminals of nociceptive primary sensory neurons where the neuron is actually exposed to the algogenic physical and chemical stimuli. Therefore, single-fiber recording, neuropeptide release-measuring, and behavioral studies allowing insight into the function of peripheral nociceptors are necessary complements. Of the numerous molecular targets and intracellular mechanisms of bradykinin, relatively few have been "validated" with these peripheral methods (see **FIGURE 4**). They include PKC activation, formation of prostanoids and 12- or 5-LOX products, activation of TRPV1 and TRPA1 channels, for which mechanisms consistent supportive data are available from various experimental arrangements. In other models, however, conflicting evidence has been obtained suggesting that differential mechanisms may operate in different tissues. For a peripheral nociceptive role of Ca^{2+}-activated Cl^- channels and of $Na_v1.9$ channels, much less data are available. Finally, no such supportive data have yet been reported for Ca^{2+}-activated K^+ channels, M-type K^+ channels, and the cAMP-PKA pathway. Therefore, further studies are required to reveal a possible role of these structures in the function of peripheral nociceptors.

III. ROLE OF PROSTANOIDS IN PERIPHERAL MECHANISMS OF NOCICEPTION

A. Biosynthesis and General Features of Pronociceptive Prostanoids

Prostanoids including prostaglandins (major members are PGE_2, PGI_2, PGD_2, and $PGF_{2\alpha}$) and thromboxanes (e.g., TXA_2) are those derivatives of arachidonic acid whose synthesis depends on COX enzymes. COX has two major isoforms: COX-1 being predominantly a constitutive enzyme and COX-2 that is mainly inducible. Prostanoids are short-lived "tissue hormones" requiring continuous formation for sustained effects. In inflammation, levels of PGE_2 and PGI_2 are typically elevated, while those of $PGF_{2\alpha}$ appear not to rise. Prostanoids act on G protein-coupled prostanoid receptors for which several types, subtypes, and splice variants have been identified (for a review, see Refs. 11, 284). Various prostanoid receptor types have been distinguished on the basis of relative agonist preference: EP receptors preferring PGE_2, IP (for PGI_2), DP (for PGD_2), and TP (for TXA_2) receptors. Among DP receptors, G_s-coupled DP_1 and G_i-coupled DP_2 receptors have been identified. Of EP receptor subtypes, EP_1 receptors are likely coupled to G_q, EP_2, EP_{3B}, EP_{3C} and EP_4 receptors to G_s, EP_{3A} receptors to G_i. IP and TP receptors are coupled to G_s and G_q, respectively. The most widely used pharmacological tools for studying the roles of endogenous prostanoids are the nonselective or COX-2-selective inhibitors as most prostanoid receptor antagonists available lack sufficient specificity. Of the prostaglandins, most data regarding a pronociceptive role have been obtained with PGE_2 and PGI_2, while $PGF_{2\alpha}$ was ineffective in most models.

Different classes of nonconventional prostaglandins have also been identified. COX-2 was shown to convert 2-arachidonoylglycerol, an endogenous agonist of cannabinoid receptors, to a variety of prostaglandin glyceryl esters including PGE_2 glyceryl ester (PGE_2-G), PGD_2-G, $PGF_{2\alpha}$-G, and PGI_2-G, in vitro (383, 384). PGE_2-G does not bind to cannabinoid receptors and has only a very low affinity for some subtypes of prostanoid receptors (538, 612). It is rapidly hydrolyzed to PGE_2 in plasma (383), but data suggest that PGE_2-G can act without being converted to PGE_2 (295, 538, 626). In addition, PGE_2-G occurs in the rat hindpaw, and its level depends on COX-2 activity (295). Isoprostanes are steroisomers of prostaglandins formed nonenzymatically (i.e., independent of COX enzymes) by peroxidation of arachidonic acid induced by reactive oxygen species associated with oxidative stress. Of them, 8-iso PGE_2 and 8-iso $PGF_{2\alpha}$ have been studied in detail. The cyclopentenone prostanoids are also formed from common prostaglandins during oxidative stress and contain one or two electrophilic carbons unlike their precursors. They include, e.g., 15-deoxy-$\Delta^{12,14}$-PGJ_2 ($15dPGJ_2$), a derivative of PGD_2.

B. Prostanoid Receptors and Their Signal Transduction Mechanisms in Sensory Neurons

1. *Prostanoid receptors and their intracellular signaling mechanisms*

This section deals with those aspects of prostanoid receptors and their signal transduction mechanisms that have been examined predominantly in somata of sensory neurons or in transfected host cells, when the readout was a change on (sub)-cellular levels of intracellular cAMP, IP_3, or Ca^{2+}, or when membrane current or neuropeptide release was measured. These data are summarized in **FIGURE 3**. Signaling mechanisms revealed during analysis of the typical sensitizing actions of prostanoids, when the readout was an increase in heat, mechanical, or chemical responsiveness of nociceptive neurons or the whole animal, are discussed in section III, *C* and *D*.

With regard to the receptor subtypes mediating the sensory actions of prostaglandins, EP_1, EP_2, EP_{3B}, EP_{3C}, EP_4, DP_1, DP_2, and IP receptors are all possible candidates since the mRNAs and/or proteins of these subtypes (but not that of EP_{3A}) were revealed in mouse and/or rat DRG neurons (25, 156, 167, 180, 433, 447, 523, 547, 668, 683). Later, EP_{3A} receptors were cloned from dog DRG neurons and shown to couple with G_i and G_q protein (385). In rat trigeminal ganglion, significant EP_2, EP_3, and EP_4 receptor expression was observed along with a lower density of EP_1 receptors (521).

Regarding signal transduction mechanisms of prostanoids in sensory neurons, both PGI_2 analogs and PGE_2 stimulated cAMP accumulation in cultured rat DRG neurons (523, 526, 662, 668, 776). The PGE_2-induced cAMP accumulation was mediated by EP_{3C} and EP_4 receptors (526, 668, 776). Furthermore, both PGI_2 analogs and PGE_2 caused inositol phosphate accumulation in cultured rat sensory neurons, suggesting that

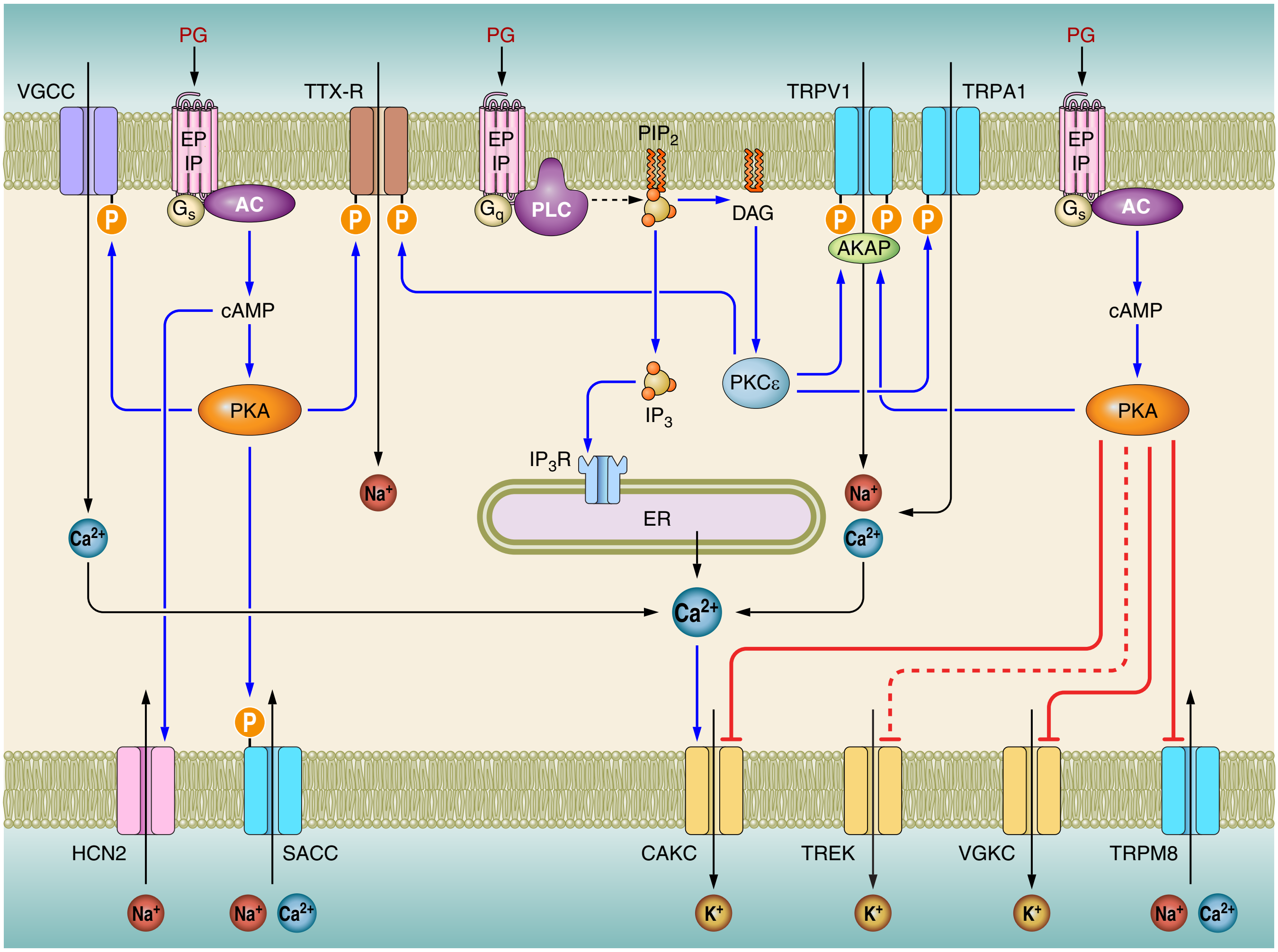


FIGURE 3. Schematic representation of the most important signal transduction mechanisms of prostaglandins (PG) in nociceptive sensory neurons. Blue arrows: activation of a target or stimulation of synthesis of a substance; red lines: inhibition of a target (dashed line indicates a likely inhibitory effect); dashed black arrow: cleavage of a substance. To avoid confusion, the subtype(s) of prostanoid receptors are not shown. Also not shown are the minor outward K^+ currents in case of TRPV1, TRPM8, TRPA1, HCN2, and SAAC channels. VGCC, voltage-gated Ca^{2+} channels; TTX-R, tetrodotoxin-resistant Na^+ channels ($Na_v1.8$, $Na_v1.9$); HCN2, hyperpolarization-activated cyclic nucleotide-gated channel; SACC, stretch-activated cation channel; CAKC, calcium-activated K^+ channel; TREK, mechanosensitive K^+ channel; VGKC, voltage-gated K^+ channels; ER, endoplasmic reticulum; IP_3R, IP_3 receptor. For other abbreviations, see text. AKAP is only shown when its involvement was directly revealed.

they are able to stimulate PLC (662). This means that EP and IP receptor activation is linked to both cAMP and inositol phosphate accumulation. In accord, the PGI_2-evoked depolarization of the isolated vagus nerve was mediated by both the cAMP-PKA pathway and PKC (662). PGE_2 was shown to cause translocation of PKCε in cultured DRG neurons, suggesting that DAG formation is induced by PLC activation in response to PGE_2 (746). Vice versa, while AC stimulation by forskolin had no effect on inositol phosphate levels, PKC stimulation by a phorbol ester led to cAMP accumulation and potentiated such effect of a PGI_2 analog, suggesting that PKC can stimulate the AC-cAMP-PKA pathway in sensory neurons (662). PGE_2 increased the intracellular Ca^{2+} concentration in sensory neurons fully depending on extracellular Ca^{2+} (434, 435, 663), although in one other study the same concentration of PGE_2 (10 μM) failed to alter the intracellular Ca^{2+} level (537). The PGE_2-evoked increase in intracellular Ca^{2+} was shown to involve cAMP and activation of PKA (663). As the response depended on extracellular Ca^{2+} and PGE_2 failed to induce IP_3 accumulation in this study (unlike that of Ref. 662), a PKA-mediated phosphorylation and consequent facilitation of voltage-gated Ca^{2+} channels is a likely mechanism involved. Also TP receptor activation by a selective agonist increased intracellular Ca^{2+} concentration in mouse DRG neurons, suggesting that TP receptors are coupled to G_q (25).

As a result of elevating the intracellular Ca^{2+} concentration, prostaglandins (PGE_1, PGE_2, and PGI_2) can evoke release of SP and/or CGRP from sensory nerves in isolated preparations of various organs including the guinea pig heart (209, 231, 297, 376–378). In the latter preparation, the PGE_2-induced SP release involved EP_4 receptors, activation of the AC-cAMP-PKA pathway and influx of Ca^{2+} through N-type voltage-gated Ca^{2+} channels. Similarly, PGE_1, PGE_2, or PGI_2, but not $PGF_{2\alpha}$, induced SP and/or CGRP release from cultured adult, neonatal, or embryonic rat sensory neurons involving activation of the AC-cAMP-PKA pathway and Ca^{2+} influx through N-type voltage-gated Ca^{2+} channels (280, 524, 663, 774). In addition, 8-iso PGE_2, but not 8-iso $PGF_{2\alpha}$, also induced SP and CGRP release from cultured rat DRG neurons (176). In cultured rat trigeminal sensory neurons, PGE_2, PGD_2, a PGI_2 analog, but not $PGF_{2\alpha}$ or a TP receptor agonist, evoked CGRP release dependent on extracellular Ca^{2+} (314). In addition to plasma membrane Ca^{2+} channels (see above), possible target structures involved in prostanoid-evoked neuropeptide release are the vesicular proteins required for exocytosis. Of them, rabphilin was shown to be modulated by phosphorylation by either PKA or Ca^{2+}/calmodulin-dependent protein kinase (221). In addition, in cultured sensory ganglion explants, a stable PGE_2 analog applied for 3–72 h increased mRNA and protein levels of both SP and CGRP through activation of EP_1 and EP_4 receptors as well as by stimulation of PKA and PKC and by NGF production (455).

Western blot analysis of the mouse paw tissue confirmed that PGE_2 treatment activated PKA, PKCα, and all examined members of the MAPK family: ERK, p38, and JNK (336). The PGE_2-induced PKA activation was reduced by EP_3 or EP_4 receptor antagonism, whereas activation of ERK was only inhibited by EP_3 receptor blockade (PKCα activation was independent of these two prostanoid receptor subtypes). In an ex vivo culture of DRG explants, a PGE_2 analog increased IL-6 mRNA and protein levels through EP_4 receptors involving activation of PKA, PKC and ERK (676).

2. *Membrane structures targeted by prostanoid-induced signaling*

The known membrane currents and ion channels in primary afferent neurons targeted by prostanoid-induced signaling mechanisms are the following.

A) TETRODOTOXIN-RESISTANT AND SENSITIVE VOLTAGE-GATED Na^+ CHANNELS. In capsaicin-sensitive small rat DRG neurons considered nociceptive, PGE_2 positively modulated the TTX-R, but not the TTX-S, Na^+ current by increasing the magnitude of the current and inducing a small leftward (hyperpolarizing) shift in its current-voltage relationship (175, 235). In the presence of TTX, PGE_2 reduced the current threshold required for action potential firing (175). Activation of the cAMP-PKA signaling pathway both increased TTX-R Na^+ current and induced a leftward shift in its conductance-voltage relationship, while PKC activation only enhanced TTX-R Na^+ current (175, 234). Although the PGE_2-induced modulation of TTX-R current appeared more likely PKA- than PKC-mediated, PGE_2 effects were reduced by inhibitors of either PKA or PKC (175, 234). The facilitatory effect of PGE_2 and of activation of the cAMP-PKA pathway on $Na_v1.8$-mediated TTX-R current was confirmed in later studies on rat and mouse DRG neurons as well as on rat capsaicin-sensitive vagal pulmonary sensory neurons (36, 402). A cAMP-dependent phosphorylation of $Na_v1.8$ has been shown, and the molecular site of phosphorylation has been identified as well (201).

Recently, complex effects of PGD_2 on TTX-R currents have been described in cultured rat DRG neurons: a shift of the voltage-dependent conductance toward hyperpolarization was observed in most neurons, whereas an increase in peak amplitude of the currents was noted in one third of neurons (167). Selective DP_1 receptor activation mimicked both effects while DP_2 receptor agonism reduced the peak amplitude suggesting that G_s-coupled DP_1 receptors are functionally antagonized by G_i-coupled DP_2 receptors. Furthermore, NO synthesis inhibition was reported to reduce the augmenting effect of PGE_2 on the TTX-R Na^+ current in small DRG neurons, suggesting a contribution of NO to the facilitatory action of PGE_2 (15). In rat DRG neurons, a prolonged (1 h) treatment with PGE_2 has been shown to stimulate $Na_v1.8$ trafficking from the endoplasmic reticulum to the cell surface through activation of the cAMP-PKA pathway, and an RRR motif in the first intracellular loop of the channel α-subunit was indispensable for this effect (437, 798).

Contradictory results were obtained in mouse DRG neurons: an acute PGE_2 treatment failed to alter the $Na_v1.8$ (and also the $Na_v1.9$)-mediated component of the TTX-R current (800). This open contradiction becomes less important, however, if one considers that the PGE_2-induced hyperpolarizing shift of the TTX-R activation curve corresponds to only a few millivolts and is measured at room temperature, as usual when patch-clamping cultured DRG neurons. Among real cutaneous nerve endings, however, TTX resistance is temperature-dependent, being frequent at cold and rare at body temperature (802). A subpopulation of dorsal root nerve fibers conducts action potentials in the presence of TTX at room temperature but ceases to do so upon warming up to 35°C (577). In addition, $Na_v1.8$ knockout mice exhibited only subtle differences in models of inflammatory hyperalgesia and no deficit in neuropathic hyperalgesia (8, 347, 418). Thus sensitization of $Na_v1.8$ by PGE_2 is not likely an important pathophysiological mechanism of inflammatory or neuropathic pain. Further behavioral data regarding the possible role of $Na_v1.8$ in the sensitizing actions of prostaglandins are mentioned in section III*C1*.

The $Na_v1.9$-mediated persistent TTX-R Na^+ current in rat and mouse sensory neurons was not altered by a short exposure to PGE_2 or activators of PKA (36, 459, 800). In contrast, in mouse DRG neurons, a prolonged (1 h) exposure to PGE_2 caused an increase in the $Na_v1.9$-mediated TTX-R Na^+ current and shifted the steady-state voltage dependence of activation in a hyperpolarizing direction, with the former effect occurring via activation of the $G_{i/o}$ but not the G_s protein (620). However, $Na_v1.9$ knockout mice, similarly to $Na_v1.8$ knockouts, showed no deficit in neuropathic hyperalgesia and only slight phenotypic differences in models of inflammatory hyperalgesia arguing against a major role for PGE_2-induced $Na_v1.9$ sensitization in neuropathic or inflammatory pain (22, 418, 588). Further behavioral data supporting a role of $Na_v1.9$ in the sensitizing actions of prostaglandins are mentioned in section IIIC, *1* and *2*.

Concerning the possible involvement of tetrodotoxin-sensitive (TTX-S) Na^+ channels in prostanoid effects, indirect evidence was provided for $Na_v1.7$, a TTX-S channel occurring in sympathetic and sensory ganglia (627). COX-2 blockade prevented the upregulation of $Na_v1.7$ immunoreactivity in large (presumably non-nociceptive) DRG neurons in response to CFA-induced inflammation in the rat (242). Remarkably, kinetics of this $Na_v1.7$ upregulation closely correlated with that of the measured heat and mechanical hyperalgesia. On the other hand, conditional $Na_v1.7$ knockout in the small $Na_v1.8$-expressing mouse DRG neurons abrogated inflammation-induced pain and hyperalgesia (529). Although PGE_2 failed to enhance the TTX-S current in capsaicin-sensitive, i.e., nociceptive, DRG neurons (235), in certain capsaicin-insensitive medium-sized DRG neurons PGE_2 upregulated the TTX-S current via activation of the AC-cAMP-PKA pathway (87, 733). Interestingly, this facilitation was preceded by a transient inhibitory effect on these channels possibly mediated by PKC activation.

B) TRP CHANNELS. In early studies on cultured sensory neurons of the rat, PGE_2 or PGI_2 enhanced the capsaicin-induced, i.e., TRPV1-mediated, membrane current through activation of the cAMP-PKA signaling pathway (401, 433, 441, 442, 578). Single-channel studies revealed that PGE_2 increased the overall channel activity evoked by capsaicin without affecting unitary conductance, pointing to a function of PGE_2 to facilitate gating of the TRPV1 channel. In subsequent studies conflicting results were reported on whether activation of PKA can enhance the capsaicin-evoked inward current observed in heterologous expression systems of TRPV1 (414, 753). The lack of PKA-evoked facilitation of recombinant TRPV1 could be explained by assuming that phosphorylation not of TRPV1 itself but of regulatory proteins possibly associated with native TRPV1 may be responsible for the sensitizing effect of PGE_2 in cultured DRG neurons. Unequivocal evidence for the participation of TRPV1 in the actions of prostaglandins was provided by a study in which PGE_2 or PGI_2 enhanced responses of the TRPV1 channel to its activators heat, capsaicin, or protons in both transfected cells and mouse DRG neurons predominantly by activation of PKC (induced through EP_1 and IP receptors) with only a minor contribution of PKA (activated through EP_4 and IP receptors) (323, 511). These data suggest that in mouse sensory neurons PKC is predominantly responsible for the sensitizing effects of PGE_2/PGI_2, and IP receptors can initiate not only the classical G_s-AC-cAMP-PKA signaling pathway but also the G_q-PLC-DAG-PKC pathway (see also Ref. 662).

An essential role has been revealed for the PKA-anchoring protein AKAP79/150 in the facilitatory effect of PGE_2 on TRPV1 function. In rat DRG neurons, the PGE_2-induced enhancement of the capsaicin-induced, TRPV1-mediated inward current was abolished by either a peptide inhibiting the interaction of AKAP with TRPV1 or by decreasing the synthesis of AKAP using a siRNA (794). In HEK293 cells coexpressing TRPV1 and the EP_1 receptor, PGE_2 induced sensitization was abolished by additional expression of AKAP with the PKC binding site deleted or by the use of siRNA for AKAP, indicating an EP_1-PKC-AKAP-TRPV1 pathway in this PGE_2 signaling. Similar experiments revealed an additional role for the EP_4-PKA-AKAP-TRPV1 pathway. In mouse DRG neurons, desensitization of TRPV1 was decreased by PGE_2 via activation of PKA, and this effect was shown to require AKAP150 (643). In addition, the PGE_2-evoked facilitation of noxious heat-induced currents in mouse sensory neurons also depended on AKAP150. Concordant results were obtained in a parallel study on rat trigeminal neurons: siRNA-mediated knock-down of AKAP150 expression reduced PKA-evoked phosphorylation and sensitization of TRPV1 (318). The role of AKAP150 in PKA-mediated sensitization of TRPV1 was already noted in previous studies on TRPV-transfected HEK cells and on mouse sensory neurons (596, 684). Importantly, considerable behavioral data support the role of both TRPV1 and AKAP in the heat-sensitizing effect of prostaglandins on peripheral terminals of nociceptive sensory neurons (see details in sect. IIIC*1*).

Regarding cold-activated TRP channels, PGE_2 inhibited the effect of cooling from 32 to 18°C in cultured sensory neurons by reducing the response to cooling and decreasing the threshold temperature for activation via PKA (435). These actions suggest an inhibitory effect of PGE_2 on the TRPM8 channel activated by innocuous cooling (485, 565). It is worth mentioning that activation by menthol of cutaneous cold-sensitive nerve fibers exerted an antinociceptive effect by a spinal inhibitory mechanism (589). The PGE_2-induced facilitatory effect on the acetaldehyde-evoked, i.e., TRPA1-mediated, increase of intracellular Ca^{2+} concentration in cultured mouse trigeminal sensory neurons and on acetaldehyde-induced acute nocifensive behavior in mice are compatible with the view that TRPA1, a noxious cold-sensitive channel, is another target for the sensitizing actions of PGE_2 (38). The osmotically sensitive TRPV4 channel was also shown to play a role in some sensitizing actions of PGE_2 (9, 10; see details in sect. IIIC2).

Recently a novel, prostaglandin receptor-independent mechanism for cyclopentenone prostaglandins has been revealed. $15dPGJ_2$, a derivative of PGD_2 presenting with an electrophilic moiety, was shown to activate the TRPA1 channel in both transfected HEK cells (where PGD_2 and PGJ_2 were ineffective) and sensory neurons as well as to induce a nocifensive reaction in mice that was absent in TRPA1 knockout animals (23, 122, 458, 474, 703, 715). Like other activators of TRPA1 (cinnamaldehyde, allyl isothiocyanate, acrolein), $15dPGJ_2$ reacts covalently with cysteine residues of TRPA1 (283, 456, 703). Other cyclopentenone prostanoids that contain one or two electrophilic carbons (12-PGJ_2, 8-iso PGA_2, PGA_2, PGA_1) mimicked the TRPA1-activating effect of $15dPGJ_2$, whereas their structurally related precursors lacking electrophilic carbons (PGD_2, PGE_2) failed to do so, indicating the importance of electrophilic moieties for TRPA1 activation (474, 715). It is worth mentioning, however, that $15dPGJ_2$ has been reported to inhibit carrageenan- or PGE_2-induced mechanical hyperalgesia and formalin-evoked overt nociception (528, 566). It was proposed that $15dPGJ_2$ activated opioid peptide-expressing macrophages via peroxisome proliferator-activated receptor gamma receptors and that the released opioids then produced local antinociception. However, as allyl isothiocyanate is known to activate and desensitize TRPA1, an antinociceptive action could also result from a possible sustained desensitization of TRPA1 by $15dPGJ_2$.

C) HYPERPOLARIZATION-ACTIVATED CYCLIC NUCLEOTIDE-GATED CHANNELS. A further ionic conductance modulated by prostaglandins is a hyperpolarization-activated current (I_h) which passes through cation channels permeable to both Na^+ and K^+ in embryonic mouse DRG neurons (477). Later this current was observed in cultured guinea pig nodose and trigeminal neurons as well (306). PGE_2, forskolin, cAMP, and cGMP analogs all positively modulated this current by shifting the activation curve in the depolarizing direction and increasing the maximum amplitude (306). Evidence was provided that cAMP (and also cGMP) modulates this current directly, i.e., without activation of PKA or PKG. For this reason, the channels mediating this type of current are termed hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Cyclic nucleotide sensitivity applies in particular to HCN2 and HCN4 channels that are expressed in small sensory neurons, whereas HCN1 channels dominate in the larger-size neurons and are not facilitated by cyclic nucleotides but by low extracellular pH (443, 507, 675). In cultured rat DRG neurons, PGE_2 increased action potential frequency elicited by a current pulse and induced a depolarizing shift in the resting potential with both effects being antagonized by a specific I_h blocker but remaining largely unaltered by a PKA inhibitor suggesting a role for HCN2 and HCN4 channels (506). A contribution of I_h to various nociceptive manifestations of neuropathic pain and also mild heat injury has been established raising the possibility that HCN channels may have a significant role in actions of prostanglandins on peripheral nociceptive nerve terminals (97, 174, 445, 506).

D) STRETCH-ACTIVATED NONSELECTIVE CATION CURRENTS. PGE_2 was shown to sensitize the high-threshold, but not the low-threshold, type of stretch-activated ion currents carried by Na^+, K^+, and Ca^{2+} in small cultured DRG neurons of neonatal rats (106). Sensitization manifested itself as a decrease in activation threshold and a leftward shift of the pressure-activity curve, and it was mediated by the cAMP-PKA pathway. Recently, a further mechanically activated current displaying the same cationic preferences but different electrophysiological properties has been identified in small sensory neurons, described as low-threshold small mechanosensitive conductance, and PGE_2 sensitized this, too, via the cAMP-PKA pathway (105). No data are available about the role of the channels mediating the abovementioned currents in the pronociceptive effects of prostaglandins in the peripheral nociceptive terminals.

E) VOLTAGE-GATED K^+ CHANNELS. PGE_1, similarly to forskolin (a direct activator of AC) and cAMP analogs, prolonged a Ca^{2+} component in action potentials and caused a decrease of afterhyperpolarization in cultured mouse sensory neurons by reducing a voltage-dependent K^+ conductance (244). PGE_2 caused suppression of the total K^+ current in mouse sensory neurons, and forskolin, membrane-permeant analogs of cAMP, and PDE inhibitors all produced an inhibition of the outward K^+ conductance in adult rat sensory neurons (5, 321). In embryonic rat DRG neurons, PGE_2 lowered the action potential firing threshold to brief steps of depolarizing current without altering the resting membrane potential (534). In the same model, PGE_2 increased the number of action potentials elicited by a ramp of depolarizing current without affecting the resting potential and the slow afterhyperpolarization (535). This study also revealed that PGE_2 or PGI_2, but not $PGF_{2\alpha}$, inhibited a sustained or delayed rectifier-like outward K^+ current. These results led to a hypothesis that a voltage-dependent K^+ conductance modulates the firing threshold for spike generation, and its inhibition is involved in the enhanced excitability evoked by PGE_2. A membrane-permeant analog of cAMP mimicked the K^+ current-inhibiting action of PGE_2 while an inhibitor of PKA prevented it, showing the involvement of the cAMP-PKA pathway in this effect of PGE_2 (178). All these findings corroborate a role for the PGE_2-cAMP-PKA axis in blockade of outward K^+ currents and subsequent enhancement of the excitability of primary sensory neurons, in culture, at least. In mice lacking the delayed rectifier $K_v1.1$ channel, PGE_2 still caused suppression of the total K^+ current in sensory neurons together with reducing heat and mechanical hyperalgesia, suggesting that this type of K^+ channel is not a target of the sensitizing actions of PGE_2 (321). No data are available about the role of voltage-gated K^+ channels in the pronociceptive effects of prostaglandins in models reflecting the function of peripheral nociceptive nerve terminals.

F) Ca^{2+}-ACTIVATED K^+ CHANNELS. The slow afterhyperpolarization (AHP), revealed in nodose ganglion neurons of the rabbit in vitro and mediated by a Ca^{2+}-dependent K^+ current, was inhibited by PGE_1, PGE_2, PGD_2, or PGI_2, but not $PGF_{2\alpha}$, independently of their effects on Ca^{2+} influx, suggesting that Ca^{2+}-activated K^+ channels were blocked by these prostaglandins (203, 204, 736, 768). Subsequently it was shown that, following a brief burst of spikes, the slow AHP produced a relative refractory period, and its elimination by PGD_2 or an activator of AC increased excitability of the nodose sensory neurons in a way that brief depolarizing current pulses became able to induce repetitive action potentials occurring at higher frequency (768). It is worth mentioning that PGE_1 and forskolin decreased AHP also in cultured mouse DRG neurons (244). In another study on embryonic rat DRG neurons, however, PGE_2 failed to affect the slow AHP (535). The slow AHP was not detected in acutely dissociated DRG neurons in vitro (269, 479) and in DRG neurons in vivo (494, 607, 789), suggesting that slow AHP may only develop under culture conditions. However, in a study that utilized a high-frequency repetitive stimulation protocol, a slow AHP restricted to putative nociceptive rat DRG neurons was revealed, and PGE_2 exerted an inhibitory effect on it resulting in an increase in the number of action potentials evoked by depolarizing current injection (236). The sensitizing effect of PGE_2 on rat DRG cells also includes a decrease in the action potential firing threshold (236, 534). Inhibition of the slow AHP by PGE_2, however, had little effect on the action potential threshold, indicating that inhibition of the slow AHP mediated by Ca^{2+}-activated K^+ channels is not the sole mechanism involved in the sensitizing effect of PGE_2 (236). No study assessed the possible role of Ca^{2+}-activated K^+ channels in the pronociceptive effects of prostaglandins on the peripheral nociceptive nerve terminals.

G) MECHANOSENSITIVE/OSMOSENSITIVE K^+ CHANNELS. TREK-1, a member of the two-pore domain K^+ channel family, and the first mammalian mechanosensitive K^+ channel identified at the molecular level (198, 560), has been revealed as a target molecule for some sensitizing actions of PGE_2. The TREK-1 channel has been localized in sensory neurons coexpressed with TRPV1 (18). The arachidonic acid-evoked activation of TREK-1-mediated hyperpolarizing K^+ current was inhibited by PGE_2 in mouse DRG neurons, and this action of PGE_2 was mimicked by activation of the cAMP-PKA pathway (18, 560). TREK-1 gene-deficient mice proved more sensitive than wild-types to low-intensity noxious heat stimuli and to low-intensity mechanical stimulation, and displayed increased thermal and mechanical hyperalgesia in conditions of inflammation. Conversely, these transgenic animals showed diminished behavioral responses to strong hyperosmotic (but not to hyposmotic or weak hyperosmotic) stimulation, suggesting TREK-1 involvement in this transduction. In wild-types, the nociceptive response evoked by intraplantar administration of either hyper- or hypotonic solution was facilitated by coapplied PGE_2, and this response was reduced in TREK-1-deficient mice. The revealed inhibitory effect of PGE_2 on TREK-1-mediated outward (hyperpolarizing) K^+ current is expected to facilitate depolarization of nociceptive nerve endings which could contribute to the pronociceptive action of PGE_2. As mentioned above, some data indicate that these channels might contribute to the pronociceptive effects of prostaglandins in the periphery, i.e., on peripheral nociceptive nerve terminals.

H) OTHER TARGETS. Further membrane target structures proposed to be involved in some nociceptor-sensitizing actions of prostanoids include voltage-gated Ca^{2+} channels, the IP_3 receptor of the endoplasmic reticulum, and vesicular proteins involved in exocytosis (these are dealt with in more detail in section III, *B1* and *C3*A).

C. Manifestations and Mechanisms of Peripheral Nociceptor-Sensitizing and Hyperalgesic Effects of Applied Prostanoids

As discussed in section II*D*, *2–4*, the sensitizing actions of bradykinin to heat, mechanical, or chemical stimuli have been shown to involve COX products in several studies, implicating that prostanoids are able to sensitize nociceptors. Indeed, in a variety of experimental models, prostanoids (predominantly PGE_2 and PGI_2) were shown to induce nociceptor sensitization or hyperalgesia to heat, mechanical, or chemical stimuli **(TABLE 4)**. This section summarizes data obtained in experimental paradigms reflecting prostaglandin effects on the peripheral endings of nociceptive sensory neurons.

1. *Prostanoid-induced sensitization to heat stimuli*

PGE_2 enhanced the number of discharges evoked by heat stimulation in dog testicular nociceptors in vitro, and this effect was reported to be mediated by EP_2 receptors (389, 391, 499). In this model, forskolin or dibutyryl cAMP (an analog of cAMP) combined with a PDE inhibitor, also enhanced heat-induced discharge activity (496, 499). Also in vitro, in the neonatal rat spinal cord-tail preparation, PGE_1, PGE_2, $PGF_{2\alpha}$ and PGI_2, but not PGD_2, augmented the heat response of nociceptors (616). In the isolated rat skin-saphenous nerve preparation, a very high concentration (100 μM) of PGE_2 or PGI_2 was required to induce sensitization of polymodal nociceptors to heat which included an in-

Table 4. *Manifestations, receptor types, and signaling mechanisms of the nociceptor-sensitizing/hyperalgesic effects of prostanoids*

Prostanoid	Sensitized Response	Receptor Type	Signaling Mechanism	Membrane Target	Reference Nos.
			Heat sensitization		
PGE_2, PGI_2, PGE_1, $PGF_{2\alpha}$	Heat response in the rat spinal cord-tail preparation in vitro				616
PGE_2, PGI_2	Heat-induced spike discharge in dog testicular and rat cutaneous nociceptors in vitro	EP_2 (dog)	cAMP (dog)		146, 389, 391, 496, 499
PGE_2, PGE_2-G	Heat-induced hindpaw withdrawal in rats		PKA-AKAP		176, 295, 318, 644
8-iso PGE_2					
PGE_2 PGI_2	Heat-induced hindpaw withdrawal in mice	EP_1 IP	PKA-AKAP	TRPV1 $Na_v1.9$ $Na_v1.8$	22, 202, 323, 347, 463, 511, 643, 787
PGE_2	Prolongation of heat hyperalgesia	EP_4	cAMP–Epac–Rap1-PKCε; ERK		171
PGE_2	Heat-induced tail withdrawal in the monkey				531
PGE_2	Heat threshold in human skin (1°C decrease)				618
PGE_2	Heat-induced TRPV1 activation in HEK cells and mouse DRG neurons	EP_1 EP_4	**PKC**-AKAP PKA-AKAP	TRPV1	511, 643
PGI_2		IP IP	**PKC** PKA		
PGE_2	Reduction of cooling-induced Ca^{2+} transients in rat DRG neurons		cAMP-PKA	TRPM8	435
PGE_2	Heat-induced CGRP release from isolated mouse sciatic axons		cAMP-PKA	TRPV1	200
			Mechanical sensitization		
PGE_1	Mechanically-induced vocalization in the rat				593
PGE_2, PGI_2 PGE_2-G, 8-iso PGE_2, 8-iso $GF_{2\alpha}$	Mechanically-induced hindpaw withdrawal in the rat (Randall-Selitto test)		G_s–cAMP–PKA–5-LOX NO	$Na_v1.8$	14, 15, 176, 295, 351, 355, 423, 553, 694, 695, 697–700
PGE_2	The above response in carrageenan-induced primed state in the rat skin		PKCε; ERK		16, 154, 325, 558, 559
PGE_2	The above response in carrageenan- or vibration-induced primed state in rat skeletal muscle		PKCε		151, 153
PGE_2, PGI_2	Mechanically-induced hindpaw withdrawal in the rat (modified Randall-Selitto test)		cAMP-PKA-PDE4 PKCε		124, 193, 194, 202, 278, 623, 751
PGE_2 (repeated)	Mechanically-induced hindpaw withdrawal in the rat (modified Randall-Selitto test): persistent hyperalgesia		PKA PKCε	$Na_v1.8$	190, 748–750

Table 4—*Continued*

Prostanoid	Sensitized Response	Receptor Type	Signaling Mechanism	Membrane Target	Reference Nos.
PGE_2	Mechanically-induced hindpaw withdrawal in the mouse	EP_3	cAMP-PKA PKC(ε) ERK, ERK CXCR2 receptor	$Na_v1.9$	22, 336, 464, 748
PGE_2	Mechanically-induced spike discharge in rat cutaneous nociceptors		G_s-cAMP-PKA NO		1, 101, 760, 773 100
PGE_2 and/or PGI_2	Mechanically-induced spike discharge in cat (knee joint) or rat (ankle, knee, tarsal joint) articular or meningeal nociceptors or pulmonary C-fibers, in canine testicular nociceptors in vitro		cAMP (dog)		53, 54, 247, 287, 370, 564, 636, 638, 796
PGE_2	Hyposmotic stimulation-induced spike discharge in rat cutaneous C-fibers			TRPV4	10
PGE_2	Hyperosmotic or hyposmotic stimulation-induced nocifensive reaction in the rat or mouse			TRPV4 TREK-1	9, 10, 18
	Chemical sensitization				
PGE_2, PGE_1	Bradykinin-induced pain in human skin and veins, low pH-induced pain in human skin and muscle				195, 366, 618
PGE_1	Bradykinin, histamine or serotonin-induced spike discharge and reflex hypotension in the isolated perfused rabbit ear				326
PGE_2	Bradykinin, capsaicin or phenyl-biguanide-induced cardiorespiratory reflex in dogs or rats				114, 409
PGE_2, PGI_2	Bradykinin or acetate-induced writhing in the mouse, formalin (both phases), capsaicin, α,β-methylene-ATP or acetaldehyde-induced nocifensive reaction in rat or mouse paw				38, 49, 104, 262, 634, 757
PGE_2, PGI_2, PGE_1, $PGF_{2\alpha}$	Bradykinin or capsaicin-induced reflex response in the rat spinal cord-tail preparation in vitro		cAMP		162, 616, 786
PGE_2, PGE_1, PGI_2	Bradykinin-induced spike discharge in cat and rat cutaneous, cat muscle, cat knee joint, rat ankle joint, cat cardiac, rat jejunal (in vitro), dog testicular (in vitro) nociceptors	EP_3 (dog testis)			54, 55, 70, 96, 213, 247, 265, 389, 390, 490, 502, 636, 638,
TXA_2 analog					
PGE_2	Bradykinin-induced spike discharge in rat DRG neurons K^+-induced spike discharge in rat trigeminal and DRG neurons		cAMP-PKA		34, 123, 534
PGE_2, PGI_2	Bradykinin-induced increase in Ca^{2+} in rat DRG neurons		cAMP-PKA		663, 680
PGE_2, PGD_2, PGI_2	Bradykinin or kallidin-induced SP and/or CGRP release from rat DRG neurons, bovine dental pulp or rat skin (in vitro)		cAMP-PKA	L, N, P-type Ca^{2+} channels	29, 177, 240, 280, 282, 524, 663, 742
8-iso PGE_2					176

Table 4—*Continued*

Prostanoid	Sensitized Response	Receptor Type	Signaling Mechanism	Membrane Target	Reference Nos.
PGE_2	Capsaicin-, lactate- or adenosine-induced spike discharge in rat pulmonary C-fibers				287
PGE_2, PGI_2	Capsaicin-induced membrane current in rat DRG neurons	EP_4	cAMP–PKA AKAP	TRPV1	433, 441, 442, 578, 794
PGE_2	Capsaicin or phenylbiguanide induced membrane current in rat vagal sensory neurons	EP_2		TRPV1	401
PGE_2	Anandamide-induced cobalt uptake in rat DRG neurons			TRPV1	658
PGE_2 (short)	Capsaicin- or proton-induced TRPV1 activation in HEK cells and mouse DRG neurons	EP_1	**PKC**	TRPV1	511, 643
PGE_2 (long)		EP_4	PKA-AKAP	TRPV1	
PGI_2 (short)		IP	**PKC**	TRPV1	
PGI_2 (long)		IP	PKA	TRPV1	
PGE_2, PGI_2	Capsaicin-induced SP and CGRP release from rat DRG neurons	EP_{3C} EP_4	cAMP–PKA	L, N, P-type Ca^{2+} channels	177, 280, 282, 668
8-iso PGE_2					176
PGE_2, PGI_2	Capsaicin- or anandamide-induced CGRP release in rat trigeminal, capsaicin, ATP, K^+-induced SP release from rat DRG neurons, proton-induced CGRP release in rat dura mater (in vitro)	EP_2 (trig.)		TRPV1	523, 563, 587, 804
PGE_2	Acetaldehyde-induced Ca^{2+} accumulation in mouse trigeminal neurons	EP_1	PLC–PKC cAMP-PKA	TRPA1	38
PGE_2	α,β-Methylene-ATP-induced mechanical and heat hyperalgesia in rats		cAMP–PKA		759
PGE_2	ATP-induced membrane current in rat DRG neurons The same response in neurons from CFA-treated rats	EP_3	cAMP–PKA PKA + $PKC\varepsilon$		759 758
PGE_2	Capsaicin, ATP, phenylbiguanide, histamine, SP or K^+-induced increase of Ca^{2+} in rat vagal sensory or DRG neurons		cAMP–PKA–Ca^{2+} entry		248, 537, 649

Short and long refer to duration of PG exposure; trig, trigeminal; bold, predominant signaling mechanism; underline, lack of involvement. For other abbreviations, see text.

crease in the number of spikes evoked by heat stimulation but no drop of the heat threshold (146). In accord with this, stable analogs of cAMP increased the magnitude of heat response of rat cutaneous nociceptors in the same preparation but failed to decrease their heat threshold, and pharmacological stimulation of the cAMP-PKA pathway also failed to reduce the temperature threshold of heat-activated TRPV1 in transfected cells (388, 511). All these findings raise the possibility that the two major components of heat sensitization, enhancement of the response to suprathreshold stimuli and drop of the threshold, are differently regulated.

Intraplantar injection of PGE_2, similarly to the isoprostane 8-iso PGE_2, but not 8-iso $PGF_{2\alpha}$, evoked heat hyperalgesia in rats as measured by a decrease in the paw withdrawal latency (176, 644). PGE_2 injected into the tail of rhesus monkeys also induced heat hyperalgesia with slight differences between females and males as well as between follicular and luteal phase females showing a minor role for sex and gonadal hormone levels (531). In humans, intradermal injection of PGE_2 led to heat hyperalgesia measured as a reduced heat pain threshold (618). However, PGE_2 may increase local skin temperature which lowers heat pain threshold, an effect also reported from the rat tail (for review, see Ref. 407). Intraplantar injection of PGE_2-G, derived from the endocannabinoid 2-arachidonoylglycerol by COX-2, evoked heat hyperalgesia in the rat similarly to PGE_2 administration (295). While a combination of EP_1, EP_2, EP_3, and EP_4 receptor antagonists abolished PGE_2-evoked heat hyperalgesia, the effect of PGE_2-G was only partially reversed showing that formed PGE_2 is only partially responsible for the pronociceptive effects of PGE_2-G. All these data suggest that COX-2 serves as an enzymatic switch, converting a potentially antinociceptive endocannabinoid into a pronociceptive prostaglandin-like mediator (PGE_2-G) whose action involves prostanoid receptor-dependent and -independent mechanisms. Accordingly, COX-2 inhibitors may produce their analgesic effect not only by reducing the production of pronociceptive prostanoids but also by reducing the breakdown of antinociceptive endocannabinoids.

In mice, intraplantar injection of PGE_2 into the hindpaw increased the response evoked by noxious heat stimulation as measured with the hot plate test (202). PGE_2 also reduced paw withdrawal latency to radiant heat stimulation, and this response was slightly reduced in mice with a targeted mutation of the type I regulatory subunit of PKA (463, 787). In addition, the PGE_2-induced heat hyperalgesia was shown to depend on AKAP150 in both rats and mice (318, 643). The PGE_2- and PGI_2-induced heat hyperalgesia was mediated by EP_1 and IP receptors, respectively, with both responses being diminished in TRPV1 receptor knockout mice compared with wild-type littermates (323, 511). PGE_2-induced heat hyperalgesia was not reduced in $Na_v1.8$ knockout mice, but it was lacking in $Na_v1.9$ gene-deficient animals showing the importance of the persistent TTX-R Na^+ current in the effect (22, 347, 588).

In the isolated desheathed sciatic nerve preparation of the mouse, PGE_2 enhanced the heat-induced CGRP release, and this effect was mimicked by forskolin and abolished by PKA inhibition, emphasizing the surprisingly similar heat sensitivity of peripheral terminals and axons of primary sensory neurons (200). In this study, the heat-sensitizing effect of PGE_2 combined with bradykinin was absent in TRPV1 knockout mice.

2. *Prostanoid-induced sensitization to mechanical stimuli*

Prostaglandin-induced mechanical sensitization was noted from early studies in which PGE_2, PGE_1, or PGI_2 was applied intraplantarly in the rat, into the knee joint of the dog, or into the isolated perfused rabbit ear (193, 278, 328, 593). As studied by the modified Randall-Selitto test, PGE_2 or PGI_2 injected intraplantarly in the rat evoked mechanical hyperalgesia (193, 202). The PGE_2 effect depended on activation of the cAMP-PKA pathway, and the PDE4 isoenzyme was shown to be involved in cAMP breakdown (124, 194). In contrast, elevated cGMP levels were shown to have antihyperalgesic actions in this model (see sect. VIC*1*). In a recent study, the role of the cAMP-PKA pathway in the early phase (30 min) of PGE_2-induced mechanical hyperalgesia was confirmed, and evidence for an involvement of PKCε, most probably activated by PKA, in the late phase (90 min) of hyperalgesia was also provided (623). PGE_2, PGI_2, but not PGD_2, $PGF_{2\alpha}$, or TXB_2, also induced mechanical hyperalgesia in the classical Randall-Selitto test which was not influenced by chemical sympathectomy or COX blockade (423, 695, 697, 698, 700). The mediator role of the G_s-cAMP-PKA pathway was revealed, and it was shown that 5 min after its induction, PGE_2-evoked mechanical hyperalgesia is maintained by PKA activity and that AC activity is no longer required (14, 355, 553, 694, 699).

Endogenous NO was also reported to play a role in the PGE_2-evoked mechanical hyperalgesia of rats but not through activation of GC, instead, most probably at a point prior to activation of PKA (15). In accord, PGE_2-induced (minor) sensitization to mechanical stimuli of C-fibers in the saphenous nerve was reported to be reduced by a NOS inhibitor applied adjacent to the receptive field in anesthetized rats (100). Data supporting prostaglandin-induced NO production are also known (for review, see Ref. 149). An involvement of 5-LOX products in the PGE_2-induced hyperalgesia at or downstream of PKA was also revealed (12). Intrathecal administration of an antisense oligodeoxynucleotide to $Na_v1.8$ reduced the mechanical hyperalgesia induced by PGE_2 (351). As measured with the electronic von Frey method, the time course of mechanical hyperalgesia evoked by intraplantar injection of PGE_2 in the rat depended on the route of administration: upon intradermal injection the effect was immediate, peaking within 15–30 min and lasting for 45–60 min, whereas upon subcutaneous administration it was delayed by 1 h, peaking at 3 h, and lasting for further 3 h (751). The isoprostane 8-iso PGE_2 or 8-iso $PGF_{2\alpha}$ induced mechanical hyperalgesia and decreased the mechanical thresholds of cutaneous C nociceptors in rats (176). Intraplantar injection of PGE_2-G also evoked mechanical allodynia in the rat (295).

In mice, intraplantarly applied PGE_2 induced mechanical allodynia that appeared to be mediated by EP_3 receptors, PKA, PKC, and ERK but not p38 or JNK (336). In a subsequent study, the PGE_2-induced mechanical hyperalgesia (as tested by dynamic plantar esthesiometry) depended on both the cAMP-PKA pathway and PKCε, but not ERK (748). The PGE_2-induced mechanical allodynia/hyperalgesia was reduced in $Na_v1.9$ gene-deficient mice and by systemic antagonism of the CXCR2 chemokine receptors (22, 464).

A mechanical sensitizing effect of prostanoids was also reported from single-fiber recording experiments. In one lab, PGE_2 applied intradermally to anesthetized rats moderately decreased the mechanonociceptive threshold of cutaneous nociceptors including C polymodal, C mechano-cold, and Aδ high-threshold mechanonociceptor units (1, 101, 471, 773). The PGE_2-evoked decrease in the mechanonociceptive threshold and increase in the number of spikes evoked by mechanical stimulation were mediated by the G_s-cAMP-PKA pathway (760). In accord, stable analogs of cAMP slightly decreased the mechanical thresholds of polymodal nociceptors in the rat skin-saphenous nerve preparation in vitro (388). However, in the same preparation, neither PGE_2 nor the "inflammatory soup" (composed of PGE_2, bradykinin, histamine, and serotonin) caused a decrease in the mechanical von Frey threshold of the mechanosensitive C-fibers, and the responses to suprathreshold mechanical stimuli remained unaltered as well (403, 639). In a study on polymodal nociceptors of the isolated dog testis, PGE_2 did not alter the von Frey threshold but increased the number of spike discharges evoked by mechanical stimulation and this effect was mimicked by forskolin (370). PGE_2 and/or PGI_2 caused a mechanical sensitization of articular afferents of the cat knee joint, rat ankle and knee joint (53, 54, 564, 636, 638). In the case of joint nociceptors, it cannot be excluded that the sensitization of nociceptors was secondary to prostaglandin-induced elevation of the intraarticular pressure. In addition, these joint preparations are the only model in which prostaglandins by close-arterial injection caused spike discharge with short delay. In the anesthetized rat, PGE_2 potentiated the response of pulmonary C-fibers to lung inflation and PGI_2, but not PGD_2, increased responses of meningeal nociceptors to mechanical stimulation (287, 796).

Intradermal injection of PGE_2 was reported to enhance the firing activity of C-fibers of the saphenous nerve induced by a hypotonic solution, considered to mimic mechanical stimulation, applied to the cutaneous receptive field in anesthetized rats (10). Likewise, intraplantarly applied PGE_2 enabled an otherwise innocuous hypotonic stimulus to induce nocifensive reaction. PGE_2 also facilitated the nocifensive action of an intraplantarly applied hypertonic NaCl solution in mice (9). It was suggested that TRPV4 plays a role in responses to osmotic stimulation both under physiological conditions and in the PGE_2-sensitized state (9, 10). In addition, the enhancement of the nocifensive response to intraplantar administration of either a hypertonic or hypotonic NaCl solution induced by coapplied PGE_2 was reduced in TREK-1-deficient mice compared with wild-types (18).

3. *Prostanoid-induced sensitization to chemical stimuli*

Locally applied PGE_2 or PGE_1 enhanced bradykinin-evoked pain in the human skin or isolated perfused venous segments on the back of the hand (195, 366). PGE_1 potentiated the discharge-producing effect of bradykinin, histamine, or serotonin as well as the reflex fall of blood pressure evoked by intra-arterial administration to the isolated perfused rabbit ear (326). PGE_2 enhanced the nociceptive reflex evoked by intracarotid injection of bradykinin in the anesthetized dog (114). In mice, intraperitoneal injection of PGE_2 (which by itself was inactive) enabled a subnociceptive dose of bradykinin to induce the writhing response (757). In addition, PGE_2 enhanced acetic acid-induced writhing as well (49). It is worth mentioning that the writhing test performed with any chemical stimulus applied intraperitoneally proved very sensitive to COX blockade, suggesting a contribution of endogenous prostanoids to visceral chemonociception (see Ref. 407). PGE_2, PGE_1, or PGI_2 were shown to enhance bradykinin-induced discharge activity recorded from cutaneous nociceptors of the anesthetized cat and rat (96, 265). However, in the isolated rat skin-nerve preparation, pretreatment with PGE_2 failed to enhance the bradykinin-induced discharge activity in contrast to serotonin and foregoing heat stimulation which were very effective (403). PGE_2 and/or PGI_2 increased the spike-generating effect of bradykinin in muscle nociceptors of cats (409), afferents of the medial articular nerve of the cat knee joint (636, 638), nociceptors in the rat ankle joint (54, 55, 247), afferents of the rat jejunum in vitro (70), and canine testicular polymodal nociceptors in vitro (at a 100-fold lower concentration than that needed for augmentation of the heat response; Ref. 502). In the latter model, the facilitatory effect of PGE_2 on bradykinin responsiveness depended on EP_3 receptors (389, 390) possibly coupled to G_i (see Ref. 385 in sect. III*D2*). In the isolated neonatal rat spinal cord-tail preparation, PGE_1, PGE_2, $PGF_{2\alpha}$, or PGI_2, but not PGD_2, augmented the response of nociceptors to bradykinin and capsaicin, and cAMP was also effective (162, 616, 786). PGE_2 augmented CGRP release induced by bradykinin in the bovine dental pulp and isolated rat skin (29, 240). PGE_2 applied intraplantarly augmented the capsaicin-evoked nocifensive reaction in the rat hindpaw (634). In anesthetized rats, PGE_2 increased the discharge activity of single pulmonary C-fibers evoked by capsaicin, lactate, or adenosine (287). Another COX product, an analog of TXA_2, enhanced the bradykinin-induced activation of ischemia-sensitive cardiac afferent C-fibers in anesthetized cats, whereas a TP receptor antagonist diminished the bradykinin response, suggesting a facilitatory role for endogenous TXA_2 (213). PGE_2 augmented CGRP release induced by bradykinin in the bovine dental pulp and isolated rat skin (29, 240).

A few studies revealed facilitatory effects of prostanoids on TRPA1-mediated responses. Locally applied PGE_2 enhanced both phases of the nocifensive reaction induced by injection of formalin (in lower concentration an established activator of TRPA1) into the upper lip of the rat (104). PGE_2 facilitated the acute nocifensive reaction in mice evoked by the TRPA1 agonist acetaldehyde (38).

PGE_2 enhanced the nocifensive reaction as well as the thermal and mechanical hyperalgesia in rats induced by α,β-methylene-ATP, an activator of fast inactivating P2X receptor channels for extracellular ATP (262, 759). However, ATP- (or UTP-) induced thermal hyperalgesia also depends essentially on metabotropic P2Y receptors that stimulate secondary PGE_2 formation which finally is responsible for the nociceptor sensitization, most likely achieved through sensitizing TRPV1 (462).

In this and the previous sections (III*C*, *1–3*) discrepancies have emerged between different stimulus modalities, tissues, and species (and finally labs) as to whether prostanoids, in particular PGE_2, exert sensitizing effects on nociceptors. The time-testing analgesic and antihyperalgesic effects of COX blockers in many painful human conditions seem to leave no doubt. However, exogenously introduced PGE_2 is a surprisingly poor algogenic agent, at least in human skin. High concentrations caused a minimal heat hyperalgesia, lowering the threshold by <1°C, and a marginal increase in painfulness of mild-acid injections; the sensation induced by 100 μM PGE_2 was one of mild itch, not pain, which corresponded to a moderate excitatory effect on few histamine-sensitive C-fibers in human microneurography recordings (532, 618, 642). On the other hand, the pain induced by intradermal acid buffer infusion or by topical (transdermal) capsaicin was most effectively reduced by either topical or systemic administration of acetylsalicylic acid and other prostaglandin synthesis inhibitors (640, 671, 672). Thus there is a remarkable asymmetry between the effects of augmenting versus reducing prostaglandins in the tissue. This was addressed by experiments stimulating isolated rat skin with an "inflammatory soup" and measuring CGRP release as an index of nociceptor activation. In inflammation PGE_2 does not appear alone but together, at least, with bradykinin, serotonin, and histamine which were all combined in an equimolar stimulus solution (1 μM). Whether or not PGE_2 was contained did not make any difference in stimulated CGRP release, which did not take wonder as each of the other three inflammatory mediators contributed to inducing endogenous PGE_2 formation in the skin (29). However, if this was prevented by flurbiprofen, the combination of three mediators lost its ability to activate nociceptors, regaining it if exogenous PGE_2 was added (28). Classical inflammatory mediators are not the only cause of increased PGE_2 formation, rather most noxious stimuli such as heat and even distension (of the colon) boost its synthesis (614), and virtually all cell types potentially neighboring nociceptors, including these nerve endings themselves (478), are known to liberate PGE_2 from their plasma membranes. In conclusion, one may hypothesize that tissues originally rich in PGE_2 or reactively and amply forming it show little nociceptor sensitization upon exogenous PGE_2, due to occlusion, whereas less reactive tissues gain in sensitivity. In any case, depriving tissues of PGE_2 by COX block exposes the prostaglandin sensitivity of their nociceptors (576).

D. Sensitizing Effects of Prostanoids on Cultured Sensory Neurons

Facilitation of heat or chemical stimuli-induced responses in somata of primary afferent neurons has been described in various models **(TABLE 4)**. Although these data provide valuable insight into the molecular details of prostanoid-induced intracellular signaling, the mechanisms revealed do not necessarily operate the same way in peripheral nerve endings due to ultrastructural and functional differences between the cell body and peripheral terminal (see sect. I).

1. Prostanoid-induced sensitization to heat stimuli

PGE_2 or PGI_2 enhanced the response of the TRPV1 channel to heat in both transfected cells and mouse DRG neurons predominantly by activation of PKC (induced through EP_1 and IP receptors) with only a minor contribution of PKA (activated through EP_4 and IP receptors) (511). In the presence of PGE_2 or PGI_2, the temperature threshold for activation of TRPV1 was decreased from 43 to 35°C, i.e., below normal body temperature, in HEK cells expressing TRPV1 plus the EP_1 and IP receptors, respectively. The results indicate that prostanoids are, thus, able to cause a major sensitization to heat (heat threshold drop below tissue temperature), meaning that normal body temperatures would be sufficient to activate TRPV1 in nociceptors, potentially inducing "spontaneous" pain that could be alleviated by cooling. Note that a similar effect was achieved with bradykinin (see sect. II*D3*). The PGE_2-evoked facilitation of noxious heat-induced currents in mouse sensory neurons depended on AKAP150, confirming the importance of this scaffolding protein in the PGE_2-PKC/PKA-AKAP-TRPV1 pathway (643).

2. *Prostanoid-induced sensitization to chemical stimuli*

In embryonic rat DRG neurons, PGE_2 increased the number of action potentials evoked by bradykinin most probably by lowering the firing threshold through activation of the AC-cAMP-PKA pathway (123, 534). In a heterologous expression system containing the EP_3 (EP_{3A} or EP_{3B}) plus B_2 receptors, an agonist of EP_3 receptors restored the second, desensitized, bradykinin response (mobilization of intracellular Ca^{2+}) without modifying the greater first response, i.e., it diminished B_2 receptor tachyphylaxis (385). Surprisingly, this effect of EP_3 receptor stimulation was mediated by the G_i protein and consequent reduction of PKA activity in case of both EP_{3A} or EP_{3B} receptors. A possible explanation could be a putative facilitatory effect of PKA-mediated phosphorylation on B_2 receptor desensitization/internalization. In this reduced model lacking targets (e.g., TRPV1, TRPA1) of bradykinin action other than the IP_3 receptor in the endoplasmic reticulum, the desensitization-promoting action of PKA may remain unopposed and therefore predominate. PGE_2 or PGI_2, but not $PGF_{2\alpha}$, acutely increased the proportion of cultured sensory neurons that respond to bradykinin with an elevation of intracellular Ca^{2+} level and also enhanced the response evoked by a low concentration of bradykinin (680, 663). PGE_2, PGI_2, or the isoprostane 8-iso PGE_2 increased the bradykinin, kallidin, or capsaicin-induced SP and/or CGRP release from rat DRG neurons and evidence for a role of cAMP was provided (176, 177, 280, 282, 524, 663, 742). The PGE_2-induced sensitization of the bradykinin-evoked increase in both intracellular Ca^{2+} levels and SP release also involved activation of the AC-cAMP-PKA pathway (663). These data suggest that PGE_2-induced sensitization to bradykinin depends on PKA-mediated phosphorylation of proteins resulting in increased Ca^{2+} levels, whereby the role of protein phosphorylation is supported by the finding that okadaic acid, a phosphatase inhibitor, also sensitized sensory neurons to bradykinin (281). Theoretically, such proteins could be voltage-gated Ca^{2+} channels, TRP channels, or the IP_3-gated Ca^{2+} channels in the endoplasmic reticulum. However, the sensitizing effect of PGE_2 on the bradykinin (or capsaicin) induced SP and CGRP release from rat DRG neurons was not altered by blockers of L-, N-, or P-type voltage-gated Ca^{2+} channels (177).

PGE_2 and/or PGI_2 increased the capsaicin-induced, i.e., TRPV1-mediated, membrane current in rat DRG and pulmonary vagal sensory neurons, and this effect was mediated by the cAMP-AKAP-PKA pathway (401, 441, 442, 433, 578, 794). In the latter response, a role for EP_4 receptors was revealed by using a selective antagonist, whereas the facilitatory action of PGE_2 in pulmonary vagal sensory neurons was mimicked by a selective EP_2 receptor agonist (401, 433). It was also revealed that the termination of this sensitization involved massive Ca^{2+} entry through TRPV1 channels that stimulated NOS (441). The resulting NO is assumed to activate sGC, and the formed cGMP could stimulate PKG that terminates sensitization by an as yet unidentified mechanism. These results suggest that PKA and PKG have opposing effects on TRPV1 channels: PKA evokes facilitation and PKG terminates this facilitation. It is worth mentioning that other studies also revealed opposing effects of cAMP and cGMP on nociceptor sensitivity (124, 162). PGE_2 and PGI_2 enhanced the responses of the TRPV1 channel to capsaicin and protons in both transfected cells and mouse DRG neurons predominantly by activation of PKC (evoked by EP_1 and IP receptors, respectively) with only a minor contribution of PKA (evoked by EP_4 and IP receptors, respectively; Ref. 511). Upon sustained exposure, however, the sensitizing effects of PGE_2 involved EP_4 receptors only. The facilitating effect of PGE_2 on the capsaicin-induced peptide release from DRG neurons was mediated by EP_{3C} and EP_4 receptors (668). In rat trigeminal sensory neurons, CGRP release evoked by anandamide or capsaicin was enhanced by PGE_2 or a selective EP_2 receptor agonist (563, 587). PGE_2 facilitated acetaldehyde-induced, i.e., TRPA1-mediated, increase in intracellular Ca^{2+} in cultured mouse trigeminal neurons (38). This response was mediated by EP_1 receptors and involved the PLC-PKC pathway but not the cAMP-PKA axis confirming that EP_1 receptors are coupled with G_q, at least in the mouse.

PGE_2 administration potentiated ATP-evoked currents mediated by homomeric $P2X_3$ receptors in DRG neurons, and this response was mediated by the cAMP-PKA pathway and mimicked by selective EP_3 receptor activation (759). It is worth mentioning that ATP enhanced the proton-induced dural CGRP release through P2Y, but not P2X, receptors, and this effect was suppressed by COX inhibition and mimicked by applied PGE_2 (804). A PGI_2 analog increased SP or KCl-induced SP release from DRG neurons without altering Ca^{2+} accumulation or membrane depolarization caused by these agents (523), suggesting an effect for the prostanoid on a regulatory molecule involved in exocytosis (see sect. III*B1*). PGE_2 enhanced various responses induced by diverse chemical agents (see details in **TABLE 4**).

E. Plastic Changes of Prostanoid Signaling Induced by Inflammatory States

Evidence has been provided that in inflammatory states the signaling mechanisms of prostanoid-induced sensitization may undergo alterations in a way that pathways not activated under physiological conditions are opened. For up to a month after recovery from carrageenan-induced mechanical hyperalgesia, intraplantarly applied PGE_2 evoked a prolonged (from <4 to >24 h), not intensified, mechanical hyperalgesia (30% weight reduction in Randall-Selitto test) compared with naive rats, a phenomenon called hyperalgesic priming (16). Moreover, 10 times lower doses of PGE_2 were reported to induce hyperalgesia in the primed state, whereas adrenaline/epinephrine-evoked hyperalgesia was not prolonged by priming, suggesting a PGE_2-selective extension of hyperalgesia in the primed state (559). In naive rats, the PGE_2-induced hyperalgesia was reported to be mediated in parallel by peripheral NO formation and PKA activation (see sect. IIIC2), and also the carrageenan-induced hyperalgesia seemed to depend on PKA and PKG activity. In the primed state, however, only the early period of PGE_2 hyperalgesia involved the NO and PKA pathways with the late, prolonged, phase being mediated by PKCε and ERK (16, 154). Furthermore, chemical activation of PKCε induced mechanical hyperalgesia, and when this had faded, the hyperalgesic effect of PGE_2 appeared prolonged, similar to the condition after carrageenan pretreatment. With the use of an antisense oligodeoxynucleotide against PKCε, it was demonstrated that PKCε is necessary for the induction of carrageenan-evoked priming and for the maintenance of the primed state, because even a temporary interruption of PKCε activity could terminate the primed state (559). A lower dose of the peptidic PKCε activator that failed to evoke hyperalgesia was also able to induce priming (see also Ref. 325). Evidence was obtained that in the primed state the prolongation of PGE_2-activated signaling mechanisms occurs downstream to AC and upstream to PKA, suggesting that cAMP gains power to activate PKCε which means that a novel cAMP-PKCε side-track in addition to the usual cAMP-PKA pathway may be opened (558). The cross-talk between cAMP and PKCε was shown to depend on the exchange protein activated by cAMP, Epac, that acts through a small G protein (143). Epac can activate both PLC and PLD producing DAG that in turn activates PKCε (300). Interestingly, this signaling pathway was restricted to IB_4-positive nonpeptidergic DRG neurons. Recently, a similar PKC-dependent priming has been demonstrated in the skeletal muscle of rats induced by either carrageenan or mechanical vibration (152, 153).

Another model namely for modulation of PGE_2-induced heat hyperalgesia has been described which is based on nociceptor-specific deficiency of the G protein-coupled receptor kinase 2 (GRK2, Ref. 171). GRK2 can regulate G protein-coupled receptors at various levels of signaling including receptor desensitization and interaction with intracellular kinases such as MEK1/2 and p38, leading to inhibition of their activity. Mice heterozygous for a conditional deletion of the GRK2 gene in $Na_v1.8$-expressing sensory neurons exhibited a 50% deficiency of GRK2 protein along with a marked increase and prolongation (from normally 6 h to 3 days) of PGE_2-induced behavioral heat hyperalgesia as well as carrageenan-evoked heat hyperalgesia and mechanical allodynia (170, 171). Pharmacological evidence has been provided that prolongation of PGE_2-induced heat hyperalgesia occurs as a result of a switch of EP_4-AC-cAMP signaling from stimulation of PKA to activation of Epac leading, via Rap1, a small Ras-like GTPase, to increased activity of PKCε and ERK (171). In addition, GRK2 was shown to directly bind to Epac1 which is suggested to prevent Epac-stimulated development of hyperalgesia. Intraplantar carrageenan treatment resulted in a 35% reduction of GRK2 levels in small-diameter mouse DRG neurons, suggesting that proinflammatory stimuli may remove this GRK2-mediated desensitizing "brake" thereby allowing Epac to contribute to hyperalgesia.

Daily intraplantar injections of PGE_2 for 14 consecutive days in the rat or mouse induced a persistent mechanical hyperalgesia lasting for more than a month afterwards (190, 748). After reversal of this hyperalgesia by means of peripherally acting analgesics such as dipyrone (metamizole) or *N*-methyl-morphine, a small dose of PGE_2 (that produced only a transient hyperalgesia in control animals) was sufficient to restore the persistent hyperalgesic state. In this model of persistent mechanical hyperalgesia, $Na_v1.8$ mRNA was upregulated in DRG neurons (749, 750). Inhibition of PKA and/or PKCε reduced both $Na_v1.8$ upregulation and persistent hyperalgesia (750). As AC inhibition failed to diminish persistent hyperalgesia, it appears that elevated cAMP levels are not required, but the chronic condition depends on ongoing PKA (and PKCε) activity (see also Ref. 14). The roles of both PKA and PKCε as well as the lack of AC involvement in the persistent behavioral hyperalgesia were confirmed in the mouse (748).

While the PGE_2-induced potentiation of $P2X_3$ receptor-mediated currents evoked by ATP was mediated by the cAMP-PKA pathway in DRG neurons from uninflamed rats, in sensory neurons from rats with CFA-induced inflammation, the same effect involved both PKA and PKCε activation indicating recruitment of an additional signaling pathway (758, 759).

F. Prostanoid-Induced Nociceptor Activation and Overt Pain

In several studies the excitatory action of bradykinin was diminished by COX inhibitors, suggesting that prostanoids may contribute also to the spike discharge-inducing effect of the peptide (see sect. II*D1*B). Exogenously applied prostanoids (mostly PGE_2, PGE_1, and PGI_2), however, typically failed to cause neuronal spike discharges (70, 96, 247, 287, 326, 403, 499, 501, 502, 616) or pain (121, 294, 366, 512, 618).

In some studies, however, higher concentrations of PGE_2 or PGI_2 were reported effective to induce spike discharges mostly in joint afferents (490, 34, 636, 53, 638, 54, 55, 539, 796, 786,), nocifensive reaction in animals (23, 30, 38, 122, 155, 262, 293, 336, 519; see however Ref. 49), or pain in humans (654). Of the isoprostanes, both 8-iso PGE_2 and 8-iso $PGF_{2\alpha}$ augmented the number of action potentials elicited by a ramp-depolarizing current in cultured rat DRG neurons (176).

The receptor subtypes and signal transduction mechanisms involved in prostaglandin-induced neuronal discharges or nocifensive reaction/pain have not been studied in detail. The PGE_2-evoked afferent renal nerve activity recorded in anesthetized rats was shown to be mediated by EP_4 receptors, whereas PGE_2-induced paw licking in the mouse was dependent on EP_3 and EP_4, but not EP_1 or EP_2, receptors (336, 376). The latter nocifensive action of PGE_2 seemed to be predominantly mediated by PKA activation with a minor contribution of ERK (PKC involvement was excluded). TP receptor activation increased discharge activity of cardiac spinal afferents through activation of PKC (212). Selective activation of intraperitoneal IP receptors induced a writhing response in mice (30).

It is unlikely that in inflamed tissues prostanoids reach the high micromolar concentrations necessary for causing neuronal discharges. Therefore, their involvement in the excitatory effect of bradykinin, suggested by experiments employing COX inhibitors, represents an apparent contradiction that can theoretically be resolved in the following ways: *1*) exogenously administered prostanoids such as PGE_2 or PGI_2 cannot mimic the action of endogenously synthesized ones, e.g., for accessibility reasons; *2*) COX products other than these prostanoids, e.g., reactive oxygen species, prostaglandin glycerol esters, or cyclopentenone prostaglandins are also involved in the excitatory effect of bradykinin; and *3*) prostanoids have only a permissive or complementary role in neuronal excitation by bradykinin, i.e., they can cause excitation only in concert with other excitatory mechanisms triggered by bradykinin.

G. Role of Endogenous Prostanoids in Peripheral Mechanisms of Inflammatory Pain and Hyperalgesia

A contribution of locally produced prostanoids to the second phase of nocifensive behavior induced by formalin injection into the orofacial area of rats was inferred from antinociceptive effects of coinjected diclofenac (104, 554). The phorbol ester (PMA)-induced nociceptive behavior in mice was reduced by either COX-1 or COX-2 inhibitor pretreatment performed intraplantarly (188). A monoclonal PGE_2 antibody (raised against a PGE_2-thyroglobulin conjugate) applied intraperitoneally reduced the phenylbenzoquinone-induced writhing reaction in mice and the carrageenan-evoked heat hyperalgesia in rats (505, 583, 797). The latter response was also diminished by either nonselective or COX-2-selective inhibitors applied systemically (583, 797). Carrageenan-induced paw inflammation in the rat was accompanied by increased levels of PGE_2, metabolites of PGI_2 and of TXA_2 along with an upregulation of both COX-1 and COX-2 in the treated paw (109, 527, 583, 624, 632, 729). The increase in PGE_2/PGI_2 levels was mediated by COX-1 and COX-2 activity in the early and late phase of inflammation, respectively (729). The inflammatory upregulation of both prostaglandin production and COX-1/2 expression were suppressed after chronic denervation of the hindpaw, indicating a contribution of sensory nerves to these responses. Carrageenan or CFA-induced mechanical hyperalgesia in the rat and the same condition in a guinea pig model of osteoarthrits were reversed by systemically applied EP_4 receptor antagonists (111, 517, 526). In CFA-induced inflammation, EP_4 receptor protein and mRNA expression in rat DRG neurons were increased, whereas mRNA levels of EP_1, EP_2, or EP_3 receptors were not altered (433). CFA-induced paw inflammation in the rat and impacted third molar extraction-evoked injury in humans were associated with an increased local PGE_2 formation as well as COX-2 mRNA/protein expression (218, 230, 413, 457). A topically applied COX inhibitor reduced both heat and mechanical hyperalgesia induced by ultraviolet B irradiation in the rat hindpaw (57). In accord, increased COX-2 expression and PGE_2 production evoked by ultraviolet B irradiation were reported from the human and mouse skin (71, 322). Local treatment with a nonselective COX inhibitor reduced the drop of the behavioral noxious heat threshold evoked by either a mild heat injury or surgical incision applied to the hindpaw in rats (64, 220). In the latter model, the COX-2-selective inhibitor celecoxib reduced mechanical hyperalgesia, the increase in TTX-R Na^+ current in DRG neurons, and elevation of both PGE_2 and CGRP content in incisional paw tissue and DRG neurons (447). In a novel inflammatory model, the ceramide-induced mechanical and heat hyperalgesia of rats was shown to be mediated by a peripheral p38-NF-κB-COX-2-PGE_2 pathway (159). A role for prostanoids in the zymosan-induced joint mechanical hyperalgesia was proposed (250, 251).

In the acetic acid-induced acute inflammation of the rat urinary bladder, the increase in ongoing discharge of the bladder afferents was shown to be mediated by EP_1 receptor activation (302). However, a peripherally acting EP_3 receptor antagonist reduced the increased discharge activity of urinary bladder afferents evoked by bladder distension (681). A TP receptor antagonist reduced ischemia-induced activation of cardiac spinal afferents in anesthetized cats and TP receptor expression was revealed in thoracic DRGs, suggesting that TXA_2 from activated thrombocytes may contribute to excitation of cardiac afferents during myocardial ischemia (212).

H. Role of Endogenous Prostanoids in Peripheral Mechanisms of Neuropathic Pain and Hyperalgesia

Following partial transection of the sciatic nerve in the rat, hindpaw injection of either a nonselective or a COX-2-selective inhibitor on the injury side (6–42 days after injury) strongly diminished mechanical, and to a lesser extent, heat hyperalgesia (689). An EP_1 receptor antagonist applied similarly was also effective. These agents acted locally, i.e., in the injured paw, as they had no effect on administration into the contralateral paw. In the same model, COX-2 induction was revealed 2 and 4 wk following nerve injury at the injury site and adjacent region, partly in identified macrophages but not Schwann cells (453). Preexisting COX-1 expression was upregulated in the epidermis of the partly denervated footpad. Local injection of a nonselective COX inhibitor into the ipsilateral plantar side or into the injury site reversed the established tactile allodynia but just reduced its development. Two and 4 wk after partial nerve ligation, a strong upregulation of EP_1, EP_2, EP_3, and EP_4 receptors in the injured nerve in macrophages and other types of inflammatory cells was revealed (452). Perineural injection of a nonselective COX inhibitor reversed tactile allodynia and suppressed upregulation of EP_1 and EP_4 receptors in macrophages. Eighteen months after partial sciatic nerve ligation, tactile allodynia and thermal hyperalgesia were still observed (450). At this advanced age, COX-2 and PGE_2 upregulation were still observed in injured nerve, mainly in invading macrophages, together with an increased expression in the ipsilateral DRG of EP_1 and EP_4 (but not EP_2 and EP_3) receptors as well as TRPV1 channels. Interestingly, nerve injury induced translocation of EP_1 receptors from the cytoplasm to the plasma membrane of the neurons. Perineural application of a COX-2 inhibitor inhibited all these changes (except COX-2 upregulation) and also decreased SP and CGRP content in DRG neurons.

In other types of peripheral nerve injury such as chronic constriction injury (CCI) or transection of the sciatic nerve and L5/L6 spinal nerve ligation, COX-2 was upregulated at the injury site for more than 6 mo, mainly in infiltrating macrophages, and a COX inhibitor applied locally 2–4 wk after injury reduced tactile allodynia (451). COX-2 upregulation (peaking 4–6 wk after injury) in the injured nerve was shown following CCI in the rat sciatic nerve or after surgical injury of human nerves (166). In these two models, an early EP_1 receptor upregulation (appearing from day 5 on) in the injured nerve fibers and the corresponding DRGs was revealed together with an early macrophage infiltration into the injured nerve that was followed by COX-2 upregulation only later (165). In the CCI model, EP_1 and EP_4 receptor upregulation was revealed in infiltrating macrophages and the Schwann cells of the injured sciatic nerve (778). CCI also led to increased PGE_2 levels (as assessed 10 days after injury) in injured nerves and DRG that were reduced by systemic nonselective COX inhibition (635). COX-2-specific inhibition was effective only when injured nerves were directly treated. In the same model, a novel COX-2 inhibitor decreased the number of fibers in the sural nerve showing spontaneous activity as well as their firing rate (799). Heat hyperalgesia, mechanical allodynia, and $P2X_3$ receptor upregulation in the DRGs of rats with CCI of the sciatic nerve were diminished by either a nonselective or a COX-2-selective inhibitor applied systemically (766). One day after L5 spinal nerve ligation, an early upregulation of COX-2 in Schwann cells of the affected sciatic nerve was revealed, and local administration of a nonselective COX inhibitor 3 or 24 h after injury prevented development of tactile allodynia (701, 702). A second, delayed increase in COX-2 upregulation in macrophages 1–2 wk after injury was also noted in this model.

Mechanical hyperalgesia induced by application of autologous nucleus pulposus to a lumbar nerve root in the rat was reduced by an inhibitor of TXA_2 synthetase injected into the epidural space 3 and 7 days thereafter, suggesting a role for platelet TXA_2 in radiculopathy due to lumbar disc herniation (342). In cultured sciatic nerve explants modeling injured nerves, COX-2-dependent production of PGE_2 and PGI_2 increased after 18 h and remained elevated for up to 4 days, while cultured macrophages produced large amounts of PGE_2 and PGI_2 in response to soluble factors eluted from the injured nerve explant (516). In mice with streptozotocin-induced diabetes, the COX-2-selective inhibitor meloxicam reduced mechanical allodynia upon either systemic or perineural (i.e., around the sciatic nerve) but not intrathecal administration, suggesting a contribution of peripheral, COX-2-derived prostanoids to allodynia (363). In a mouse model of type 2 diabetes, COX-2 upregulation in lumbar DRG neurons of all sizes was revealed (103). Accordingly, streptozotocin-diabetic rat skin showed excessive PGE_2 formation (and CGRP/SP release) upon stimulation with bradykinin, and this chronic condition was associated with thermal and mechanical hyperalgesia in vivo as well as with exaggerated nociceptor responsiveness to (hyperglycemic) hypoxia and tissue acidosis in vitro (215). Inoculation of mice with herpes simplex virus type 1 induced early mechanical allodynia and hyperalgesia (on days 5–8) that was followed, after a symptom-free period, by a delayed (30 days) reappearance of the same phenomena (704). The early, but not the late, symptoms were associated with induction of COX-2 and an increase in PGE_2 content in the affected DRGs. COX-2 was upregulated by virus infection for days 5–7, but the increased levels returned to baseline by day 30. In a model based on DRG inflammation mimicked by application of an inflammatory soup (consisting of bradykinin, histamine, serotonin, and PGE_2) into the L5 intervertebral foramen, both heat hyperalgesia and mechanical allodynia in the ipsilateral hindpaw were diminished by ibuprofen, a nonselective COX inhibitor, applied repeatedly for 5 consecutive days onto the skin covering the inflamed DRG (299). This treatment also suppressed hyperexcitability of sensory neurons from the inflamed DRG along with diminishment of $Na_v1.7$ and $Na_v1.8$ protein upregulation. In addition, the inflammatory soup-induced increase in expression of NF-κB, COX-2, and IL-1β was also reduced by ibuprofen.

With regard to direct effects of exogenous prostanoids on neural activity in neuropathic states, in anesthetized rats with a neuroma following sciatic nerve transection, PGI_2 activated C-fiber sprouts and enhanced ectopic activity of DRG neurons both upon local or systemic administration of the prostanoid (147, 549). In the latter study, PGE_2, PGD_2, $PGF_{2\alpha}$, and TXA_2 were ineffective, and a nonselective, but not a COX-2 specific, inhibitor reduced the PGI_2 effect, suggesting increased endogenous prostanoid formation.

The above data lend support to the hypothesis that COX-2 upregulation predominantly in invading macrophages is a common consequence of peripheral nerve injury associated with and outlasting the Wallerian degeneration; the formed prostanoids including PGE_2 and PGI_2 contribute to development of hyperalgesia and allodynia, acting especially through EP_1 and EP_4 as well as IP receptors. Possible sources of prostaglandins are endothelial and mast cells, macrophages, neutrophils known to be recruited upon peripheral nerve transection, and also Schwann cells (for review, see Ref. 454). In addition, COX-2 upregulation also contributes to development of hyperalgesia and allodynia in forms of neuropathy that are not necessarily associated with Wallerian degeneration.

I. Concluding Remarks and Open Questions

Although PGE_2 has been studied more extensively, PGI_2 may be of equal, if not greater, importance in inflammatory hyperalgesia while $PGF_{2\alpha}$ proved ineffective in most models studied. This view is supported by data regarding maximal stimulation of either cAMP or inositol phosphate formation in rat sensory neurons, in which respect PGI_2 analogs displayed greater efficacy than PGE_2, PGD_2, or $PGF_{2\alpha}$ (523, 662, 776). Likewise, PGI_2 produced a greater maximal depolarization of the isolated vagus nerve than PGE_2 (662). The maximum enhancing effect of a PGI_2 analog on the kallidin-evoked SP release from DRG neurons was higher than that of PGE_2 or PGD_2 (523). PGI_2 sensitized to mechanical stimuli a larger proportion of articular afferents in the cat knee joint and had a more pronounced sensitizing effect in rat articular nociceptors than PGE_2 (53, 54, 636, 638). In most studies, PGI_2 was found at least equipotent with PGE_2 at exciting sensory neurons (53, 55, 147, 328, 501, 638) and evoking nocifensive behavior (155, 193, 278, 697). Furthermore, the major metabolite of the unstable PGI_2, 6-keto-$PGF_{1\alpha}$, was found in higher concentrations in inflammatory exudates than PGE_2 (49, 59, 69).

Concerning the molecular mechanisms of prostanoid-induced actions, a predominant role for the G_s-AC-cAMP-PKA signaling pathway has been suggested from various models in the rat. Recent studies, however, indicate that in mouse sensory neurons PKC activation is of greater importance than PKA activity. Regarding the relative significance of membrane targets of prostanoid receptor signaling, one must consider that sensitization of voltage-gated Na^+ channels or hyperpolarization-activated cation channels and inhibition of Ca^{2+}-activated or voltage-gated K^+ channels would enhance the electrical excitability and, thus, facilitate the transformation of receptor potentials into trains of propagated action potentials in nociceptors. These mechanisms should lead to an indiscriminate sensitization of the polymodal nociceptive terminals to heat, mechanical, and chemical stimuli. Therefore, these mechanisms are unlikely to be responsible for any selective sensitization of primary afferent neurons, in the typical case enhancing responsiveness to one kind of stimulation but not to another. In contrast, facilitation of thermosensitive or mechanosensitive channels is likely to cause an isolated sensitization to heat or mechanical stimuli. Regarding the spike-generating or spontaneous pain-producing action of prostanoids, theoretically all mechanisms could contribute either by inducing spontaneous, even ectopic, action potential generation or by major sensitization to heat or possibly mechanical stress enabling ambient temperature or resting mechanical tension to elicit action potentials.

Recently, evidence has been presented for an almost exclusive contribution of HCN channels to the PGE_2-induced facilitation of the electrical excitability of cultured sensory neurons in a cAMP-dependent but PKA-independent fashion (studied as increased action potential frequency in response to current injection). In this model, evidence against a major role of voltage-gated Na^+ or K^+ channels in the PGE_2-evoked increased neuronal excitability was also obtained. While a number of membrane channels/currents have been shown to be modified by PGE_2 and PGI_2 in somata of sensory neurons, only some of them have been tested in models depending on the function of the peripheral terminals **(FIGURE 4)**. Considerable amount of data points to a role in peripheral nociceptors for TRPV1 and $Na_v1.9$ and also for AKAP, PKA, and PKC. However, the evidence for TRPA1, $Na_v1.8$, TRPV4, HCN, and TREK-1/TRAAK is incomplete, and that for voltage-gated or Ca^{2+}-activated K^+ channels is essentially lacking meaning that further studies are required in this direction.

Animal studies employing COX inhibitors strongly support a significant role for prostanoids in heat, mechanical, or chemical hyperalgesia under various conditions. Of the prostanoid receptor subtypes, the EP_4 was shown to significantly contribute to mechanical hyperalgesia in a variety of models as studied by recently developed selective antagonists. The role of prostanoids in human inflammatory hyperalgesia/pain is firmly established as COX inhibitors are extensively used and efficacious therapeutic agents in many types of inflammatory diseases. One must keep in mind that

COX inhibitors possess remarkable anti-inflammatory activity that might lead to an indirect antihyperalgesic effect via inhibition of inflammatory processes. Regarding animal models of neuropathic conditions, increasing amount of evidence exists for a significant peripheral role of prostanoids. This is, however, in sharp contrast to the prevalent belief that COX inhibitors hardly have clinical efficacy in neuropathic pain in humans (for review, see Ref. 752).

IV. ROLE OF LIPOXYGENASE PRODUCTS IN PERIPHERAL MECHANISMS OF NOCICEPTION

A. Biosynthesis and General Features of Lipoxygenase Products

Various types of LOX enzymes are known including 5-, 12-, and 15-LOX which synthesize from arachidonic acid 5-, 12-, and 15-HPETEs, respectively, that can further be converted to the corresponding HETEs. 5-LOX acts in concert with the five-lipoxygenase activating protein (FLAP), and the formed 5-HPETE is a starting substance for the synthesis of leukotrienes. The latter include LTB_4 as well as a series of LTC_4, LTD_4, and LTE_4 termed collectively as cysteinyl or peptidoleukotrienes. Leukotrienes act through their G_q protein-coupled receptors including BLT_1 and BLT_2 for LTB_4 as well as $CysLT_1$ and $CysLT_2$ for cysteinyl leukotrienes. The role of LOX products can be studied by using nonselective (e.g., nordihydroguaiaretic, NDGA) or isoform-selective LOX inhibitors (e.g., zileuton acting on 5-LOX), leukotriene receptor antagonists (e.g., zafirlukast or montelukast selective for $CysLT_1$ receptors), and 5-LOX knockout mice.

B. Pronociceptive Effects of Applied Lipoxygenase Products

Administration of LTB_4 in the rat hindpaw decreased the threshold of the vocalization response evoked by pressure (593). In addition, LTD_4 enhanced the hyperalgesic effect of PGE_1 in this model. LTB_4, but not LTD_4, applied intradermally to the rat hindpaw was shown to diminish the tolerance to noxious pressure in the Randall-Selitto test (423). Unlike the mechanical hyperalgesia evoked by bradykinin or PGE_2 in this model, the LTB_4 effect depended on the presence of polymorphonuclear leukocytes but not on COX activity. The former finding is not surprising as LTB_4 is known to have a strong chemotactic effect on neutrophils and macrophages. In vitro treatment of polymorphonuclear leukocytes with LTB_4 led to release of a factor that produced hyperalgesia in rats depleted of these cells and appeared to be identical with the 15-LOX product, 8(*R*),15(*S*)-di-HETE (421). Subsequently, 8(*R*),15(*S*)-di-HETE was also shown to induce mechanical hyperalgesia with a short onset latency similar to PGE_2 (422, 695). The hyperalgesic effect of LTB_4 had a markedly longer onset latency, suggesting that it acts by stimulating leukocytes to release 8(*R*),15(*S*)-di-HETE which eventually sensitizes nociceptors. LTB_4 applied intracutaneously in humans evoked heat hyperalgesia (56). In anesthetized rats, LTB_4 applied intradermally was reported to decrease the mechanonociceptive thresholds of cutaneous nociceptors including C polymodal (C mechano-heat), C mechano-cold, and Aδ high-threshold mechanonociceptor units (471). Likewise, LTB_4 also decreased the heat threshold of C polymodal nociceptors (470). Similarly, 8(*R*),15(*S*)-di-HETE was also reported to sensitize rat cutaneous C-nociceptors to both mechanical and heat stimulation (772). In mice, intra-articular injection of LTB_4 induced mechanical hyperalgesia depending on neutrophils and production of prostanoids and leukotrienes (see more details in sect. IVC; Ref. 251). Intraplantar injection of LTC_4 in the rat failed to evoke overt nociception, but it potentiated the acute nocifensive reaction induced by α,β-methylene-ATP with a bell-shaped concentration-response curve (548).

With regard to effects revealed in somata of cultured sensory neurons, LTC_4 caused a membrane depolarization in guinea pig nodose ganglion neurons and almost abolished the slow AHP in a subset of these cells (736). In mouse DRG neurons, the mRNA of BLT_1, but not BLT_2, receptor was revealed, largely colocalized with TRPV1, and LTB_4 evoked a Ca^{2+} influx (24). In contrast, in rat DRGs mRNA of the $CysLT_2$ receptor was revealed in small and medium-sized neurons colocalized with TRPV1 and $P2X_3$ channels but not CGRP (548). BLT_1 receptor mRNA was detected in nonneuronal cells, while BLT_2 and $CysLT_1$ receptor mRNA were not found in DRG. In capsaicin-sensitive guinea pig trigeminal neurons projecting to the nasal mucosa, LTD_4 increased through $CysLT_1$ receptors the intracellular Ca^{2+} concentration and the electrical excitability as measured by increased number of action potentials elicited by current pulses (714). In accord, mRNA of $CysLT_1$, but not $CysLT_2$, receptor was revealed in these neurons, and LTD_4 enhanced histamine-induced responses of capsaicin-sensitive neurons as measured by increased action potential discharge and peak frequency. The above data show that functional BLT_1, $CysLT_1$, and $CysLT_2$ receptors are expressed in the cell bodies of nociceptive neurons of different species which raises the possibility that leukotrienes may act directly on peripheral nociceptive nerve endings.

C. Role of Endogenous Lipoxygenase Products in Inflammatory and Neuropathic Hyperalgesia

In rats with zymosan-induced arthritis, local or systemic inhibition of 5-LOX either prophylactically or as a posttreatment inhibited articular incapacitation and cellular invasion (129). The zymosan-induced articular mechanical hyperalgesia in mice was diminished by a systemically applied FLAP inhibitor or a BLT receptor antagonist as well as by an inhibitor of neutrophil migration, by an antineutrophil antibody, and also in 5-LOX gene-deficient animals

(251). In accord, intra-articular injection of LTB_4 evoked hyperalgesia that was diminished by a nonselective or COX-2-selective inhibitor, by a FLAP inhibitor, the above-mentioned antineutrophil agents and also in 5-LOX knockout animals. Moreover, LTB_4 injected intra-articularly evoked PGE_2 production in the joint that was abolished by the inhibitor of neutrophil migration (cf. LTB_4-stimulated leukocytes released PGE_2, Ref. 173). On the basis of these data, it was proposed that LTB_4 was produced in response to zymosan and acted by recruiting neutrophils that produced additional LTB_4, inducing formation of prostanoids by neutrophils, and finally the prostanoids and/or LTB_4 sensitized the nociceptors. It may be recalled, however, that in a previous study the hyperalgesic action of LTB_4 in the rat skin was independent of prostanoid formation (423).

The mechanical hyperalgesia induced by ovalbumin in presensitized rats was reduced by a systemically applied inhibitor of FLAP or by a nonselective LTB_4 receptor antagonist, and increased levels of LTB_4 were detected in paw skin of ovalbumin-challenged rats (125). At 5 h post ovalbumin challenge, LTB_4 appeared to be the major mediator of hyperalgesia. Intraplantar pretreatment with inhibitors of PLA_2, 5- or 12-LOX reduced the carrageenan-induced heat and mechanical hyperalgesia, suggesting an involvement of peripheral LOX products in this model of inflammatory hyperalgesia (788). Orally applied zileuton or other 5-LOX inhibitors reduced mechanical hyperalgesia induced by CFA (116, 473). Higher doses of 5-LOX inhibitors diminished the elevated levels of LTB_4 in the exudate of CFA-treated paws, in which 5-LOX expression was also increased, suggesting a peripheral site of action for the LOX inhibitors. A systemically applied 5-LOX inhibitor reduced *1*) phenylbenzoquinone- or acetic acid-induced writhing in mice (246, 659), *2*) NGF-evoked heat hyperalgesia and carrageenan-induced mechanical hyperalgesia in the rat (21, 116, 659), *3*) carrageenan- or TNF-α-induced incapacitation in primed (i.e., previously inflamed with carrageenan) knee joint of the rat (728), and *4*) tactile allodynia in a model of osteoarthritis (116).

The nonselective LOX inhibitor NDGA reduced the second phase of formalin-induced nociception and the bradykinin-evoked action potential discharge in sensory fibers (653, 731). Similarly, intraplantar pretreatment with NDGA also diminished heat hyperalgesia (measured as a drop of the behavioral heat threshold) in rats induced by either a mild heat injury or plantar incision (219). The results obtained with NDGA, however, should be treated with caution as NDGA has been shown to block both TTX-S and TTX-R Na^+ channels in rat DRG neurons, raising the possibility that some part of its antihyperalgesic effects may be unrelated to LOX inhibition (362).

Systemically applied Cys-LT_1 receptor antagonists reduced acetic acid-induced writhing in mice and carrageenan-evoked heat hyperalgesia along with anti-inflammatory actions in rats, but failed to affect noxious heat responsiveness under basal conditions (311, 659). Furthermore, zafirlukast increased the antinociceptive effects of nimesulide, a relatively selective COX-2 inhibitor, in these models. These data suggest that cysteinyl leukotrienes may also contribute to nociception, and their effect appears to be additive to that of prostanoids.

LOX products appear to be involved in the pronociceptive actions of other proinflammatory mediators. Mechanical hyperalgesia in the rat induced by intradermally applied PGE_2 was reduced by local inhibition of the 5-LOX, whereas adrenaline (epinephrine) induced hyperalgesia was diminished by either 5- or 12-LOX inhibition (12). In accord, injection of 5-LOX or 12-LOX protein into rat hindpaw resulted in hyperalgesia that was not reduced by inhibitors of PKA, PKCε, or MAPKs. Hyperalgesia induced by activation of PKA or PKCε, but not MAPK, was attenuated by LOX inhibitors. These data suggest that products of 5- and/or 12-LOX can function as signaling molecules contributing to PGE_2 or adrenaline-induced mechanical hyperalgesia at or downstream of PKA and PKCε (12). A role for 5-LOX product(s) in the mechanical hyperalgesia induced by platelet-activating factor (PAF) in rats was also suggested (47, 131). As examined by measuring the histamine-induced increase in intracellular Ca^{2+} in DRG neurons, evidence was provided that H_1 receptor activation can lead to excitation of sensory neurons by an intracellular PLA_2-LOX-TRPV1 signaling pathway in addition to the conventional PLC-DAG-PKCε pathway (358, 498, 536). It is important to recall that various LOX products such as 12 and 15-(*S*)-HPETE, 5 and 15-(*S*)-HETE as well as LTB_4 were shown to directly activate TRPV1 channels, and a PLA_2-LOX-TRPV1 pathway has been revealed in bradykinin's excitatory and sensitizing actions (see details in sect. II, *C3*A and *D6*B). In accord, a bradykinin-stimulated production of 12-HETE in rat DRG neurons and skin was demonstrated (653, 761).

Relevant for neuropathic pain, mechanical hyperalgesia induced by application of autologous nucleus pulposus to a lumbar nerve root in the rat was reduced by a LTB_4 receptor antagonist injected into the epidural space 3 and 7 days after administration, suggesting a role for LTB_4 in radiculopathy due to lumbar disc herniation (342). Similarly, both heat and mechanical hyperalgesia evoked by application of autologous nucleus pulposus to the rat sciatic nerve was diminished by systemically applied zileuton on postoperative days 3, 5, and 7 (660). In addition, zileuton increased the antihyperalgesic effect of systemically administered indomethacin. Mice lacking the 12/15-LOX gene exhibited the same degree of streptozotocin-induced hyperglycemia as wild-types but a decrease in heat hypoalgesia, tactile allodynia, and nerve conduction velocity deficit characteristic after 14 wk of experimental diabetes (545). In addition, diabetic mice had increased levels in their sciatic nerve of 12/15-LOX and 12(*S*)-HETE.

D. Concluding Remarks

As LOX products have been shown to contribute to nociception also at the spinal level (540, 731), results obtained employing either systemically administered LOX inhibitors/leukotriene receptor antagonists with possible brain penetration or LOX knockout animals may reflect leukotriene effects exerted not necessarily in the periphery but possibly in the central nervous system. For this reason, several of the above-mentioned studies must be treated with caution when considering the peripheral pronociceptive effects of leukotrienes. A contribution of LOX products to various pronociceptive and sensory neuron-stimulant actions of other inflammatory mediators such as PGE_2, bradykinin, histamine, PAF, and adrenaline has also been revealed. Therefore, it appears that LOX products, similarly to prostaglandins, are not only pronociceptive mediators on their own right but also subserve a secondary mediator function in the effects of other algogens. It is also worth mentioning that an additive interaction between COX inhibitors and LOX inhibitors was demonstrated in various models of inflammatory and neuropathic hyperalgesia raising the possibility that a combination of nonsteroidal anti-inflammatory analgesics with anti-leukotriene drugs may lead to enhanced analgesic efficacy in humans.

V. ROLE OF PLATELET-ACTIVATING FACTOR IN PERIPHERAL MECHANISMS OF NOCICEPTION

A. Biosynthesis and General Features of Platelet-Activating Factor

The production of PAF, similarly to that of prostanoids and leukotrienes, depends on activity of PLA_2, the enzyme that cleaves arachidonic acid from membrane phospholipids. While prostanoids and leukotrienes are synthesized from arachidonate, PAF is formed from the rest of the phospholipid molecule remaining after the action of PLA_2, called lyso-PAF, by esterification with acetate. PAF acts through specific PAF receptors that are coupled with $G_{q/11}$-protein and which can lead to activation of the IP_3-Ca^{2+}/DAG-PKC and AC-cAMP-PKA signaling pathways (145).

B. Pronociceptive Effects of Applied Platelet-Activating Factor

Intraplantar injection of PAF in the rat was shown to decrease the mechanonociceptive threshold measured with either the conventional or the modified Randall-Selitto test, and the response measured with the latter, but not the former, method was reduced by systemic COX blockade (60, 741). The mechanical pressure applied to the hindpaw and required to induce vocalization was also reduced by intraplantarly applied PAF (131). This hyperalgesic effect was resistant to COX blockade, but it was diminished by a systemically injected 5-LOX inhibitor. The contribution of 5-LOX product(s) to the mechanical hyperalgesia induced by PAF was confirmed (47). Intraplantar injection of PAF in the rat evoked an acute nocifensive behavior that was shown to involve IL-1β, TRPV1 receptor, and mast cell degranulation (469). The same treatment with PAF also induced mechanical hyperalgesia lasting for at least 8 h that depended on IL-1β, neutrophils, CXCR2 chemokine receptors, COX-2 activity, and β_1 adrenoreceptor activation. PAF injection near the L5 DRG of rats induced mechanical allodynia in the hindpaw and an increase in TNF-α and IL-1β mRNA expression in the L5 DRG (271). The above studies demonstrate that pronociceptive PAF responses are mediated by secondary mediators including prostanoids, LOX products, cytokines, along with a contribution of inflammatory cells. A stable PAF receptor agonist injected intradermally in the hindpaw of mice resulted in mechanical, but not heat, hyperalgesia (793). Intradermal injection of PAF into human skin was reported to induce mechanical hyperalgesia (43).

In cultured mouse DRG neurons, a stable PAF receptor agonist induced an elevation of intracellular Ca^{2+} (734). Most of the responding neurons were capsaicin-sensitive, suggesting a colocalization of the PAF receptor and TRPV1. It is worth mentioning that PAF is able to upregulate B_1 bradykinin receptors in the rat skin, and this action depends on recruitment of neutrophils, protein synthesis, NF-κB activation, and consequent cytokine (TNF-α and IL-1β) production (181, 182). The eponymous action of PAF, activating thrombocytes in injured or inflamed tissues, is worth considering, because activated human platelets have been shown to excite and sensitize to heat nociceptors in the rat skin-nerve preparation in a very sustained manner and to induce acute pain and delayed hyperalgesia in human skin (606, 641). Although the soluble factor released and responsible for the pain induction has not been identified, sphingosine-1-phosphate (S1P) is a conceivable candidate, i.e., generated and released from platelats, sensitizing nociceptors, and inducing heat hyperalgesia through a sensory neuronal S1P receptor that facilitates the heat-activated TRPV1 channel (460).

C. Role of Endogenous Platelet-Activating Factor in Inflammatory and Neuropathic Hyperalgesia

The initial phase of mechanical hyperalgesia induced by intraplantar injection of *Bothrops jararaca* venom into the rat hindpaw was reduced by systemically applied PAF antagonists similarly to COX or LOX inhibitors (717). Systemically applied PAF antagonists reduced the late, but not early, phase of the formalin-induced nocifensive behavior (716). In PAF receptor knockout mice showing normal responses to acute heat and mechanical stimuli, both phases of formalin-induced nocifensive behavior were reduced compared with wild-types (734). In these knockout animals, the number of DRG neurons showing ERK activation in response to formalin was diminished, and the acute nocifensive reaction induced by capsaicin injected intraplantarly or acetic acid applied intraperitoneally was reduced compared with wild-types. Ultraviolet B irradiation of the mouse paw skin induced mechanical and heat hyperalgesia that were both absent in PAF receptor knockout animals (793). In accord, ultraviolet B irradiation has been shown to induce the synthesis of PAF and PAF-mimetic species in epidermal cells (41, 466).

Regarding the possible role of endogenous PAF in neuropathic pain, a PAF receptor antagonist applied near the L5 DRG in rats with L5 spinal nerve transection inhibited the development of nerve injury-induced tactile allodynia (271). Likewise, in mice lacking the gene for PAF receptor, the injury-evoked tactile allodynia was reduced compared with wild-type animals. In accord with the above results, PAF receptor mRNA was upregulated 3–14 days after spinal nerve transection in macrophages infiltrating the L5 DRG of rats. In addition, the upregulation of mRNAs for TNF-α and IL-1β in ipsilateral L5 DRG of injured wild-type mice was absent in PAF receptor knockout animals.

The above data show that the PAF receptor is not involved in noxious heat and mechanical responsiveness under basal conditions, but its activation plays a role in both inflammatory and neuropathic hyperalgesia. As intrathecally administered PAF also induced tactile allodynia and heat hyperalgesia (510), central site(s) of action for the mediator are also likely. This raises the possibility that the above-mentioned results obtained with systemically applied PAF receptor antagonists or PAF receptor-deficient mice at least partly reflect central actions of PAF. Therefore, further studies with locally applied PAF receptor antagonists are needed to establish a firm role for PAF in peripheral mechanisms of nociception.

VI. ROLES OF NITRIC OXIDE IN PERIPHERAL MECHANISMS OF NOCICEPTION

A. Biosynthesis and General Features of Nitric Oxide

NO, a free radical gas, is formed from L-arginine and molecular oxygen by the enzymes called NO synthase (NOS). There are three isoforms of NOS: the endothelial (eNOS) and neuronal (nNOS) forms act constitutively, while the inducible form (iNOS) is upregulated under pathological conditions, e.g., in inflammation. The activity of the constitutive isoforms of NOS is stimulated by intracellular Ca^{2+}-calmodulin, whereas the function of iNOS is independent of Ca^{2+}. NO activates the soluble guanylate cyclase (GC) that produces the second messenger cGMP. The latter activates PKG that can phosphorylate various proteins similarly to PKA and PKC. cGMP is inactivated by PDE enzymes, especially by PDE5. Also relevant for some actions of NO, the radical can combine with superoxide anion to yield the cytotoxic peroxynitrite anion. Pharmacological tools for studying the role of NO include the NO precursor L-arginine, NO donor compounds (e.g., glyceryl trinitrate, sodium nitroprusside), NOS inhibitors (either nonselective or specific for a given isoform), and GC inhibitors.

nNOS-like immunoreactivity was shown in small and medium-sized rat, mouse, monkey, and human DRG neurons, and its colocalization with CGRP, SP, TRPV1, and PKG was also revealed (3, 66, 461, 513, 590, 670, 708, 718, 747, 795). NOS expression was also detected in small and medium-sized neurons of the rat, cat, and rabbit trigeminal ganglion (179, 371, 406, 678). In human trigeminal ganglia, nNOS immunorectivity was revealed predominantly in large neurons with practically no iNOS staining in any neuronal population (62). This does not contradict a role in nociception, because most nerve fibers innervating teeth get myelinated, even reaching large A-fiber conduction velocity, when they leave the tooth pulp. Nonetheless, pain is the only sensation that these trigeminal "algoneurons" mediate (211). NOS-containing axons were also revealed in dental pulp and gingiva of cats and dogs (439). Subcutaneous formalin injection in the facial area enhanced NOS expression in the trigeminal ganglia of mice (61). Unlike other inflammatory mediators, NO has been reported to have both pronociceptive and antinociceptive actions in the periphery (see sect. VI, *B* and *C*) that are summarized in **TABLE 5**. It must also be emphasized that strong evidence exists for an involvement of NO in central processing of noxious stimuli, e.g., in the dorsal horn of the spinal cord (for review, see Ref. 128), only data reflecting a peripheral contribution of NO to nociception or antinociception are considered in the following sections.

B. Data Supporting a Pronociceptive Role of Nitric Oxide in the Periphery (Table 5)

1. Pronociceptive effects/roles of nitric oxide under basal or inflammatory conditions

As mentioned in section IID, *1B* and *4*, NO was shown to participate in some excitatory and sensitizing actions of bradykinin, which suggests a pronociceptive role for the agent. In accord, aqueous NO solutions either injected intracutaneously, paravascularly, or perfused through a vascularly isolated hand vein segment in humans evoked overt

Table 5. *Roles of NO in peripheral mechanisms of nociception and antinociception*

Experimental Model	Effect of Applied NO, NO Donor, or Precursor	NO Production	NOS Expression or Activity	nNOS Expression or Activity	iNOS Expression or Activity	Role of Endogenous NO in Nociception	Role of Endogenous NO in Antinociception
Evoked nocifensive reaction							
Formalin-induced nociception (2nd phase)	341 (m), 372 (m) **341 (m), 372 (m)**		61			260, 341 (m)	
Acetic acid-induced writhing							**312 (m)**
Third molar extraction-evoked pain in human		264					**264**
Inflammatory states associated with hyperalgesia							
Carrageenan-induced inflammation	**164**	624, 550, 109, 729		266, 550	624, 266, 109, 550, 72		**124 (M)** **561 (M)** **312 (M)** **72 (M)**
CFA-induced hind paw inflammation		137		98 (mRNA, DRG) 102 (mRNA, m)	137 102 (mRNA, m)	137 (M) 102 (H, m)	
CFA-induced arthritis	237 **237**						**346** (ongoing discharges)
Kaolin-induced arthritis				404	609	404 (H)	
						609 (M)	
Zymosan-induced inflammation	256 (H) **609 (M)**	51			256 (mRNA), 609	51 (M) 609 (M)	
Heat injury-induced heat hyperalgesia						219 (H)	
Plantar incision-induced heat hyperalgesia						219 (H)	
Indomethacin-induced intestinal inflammation					783 (m)		**783 (m)**
PGE$_2$-induced mechanical hyperalgesia or sensitization	**194, 164, 191, 124, 665, 664,** **622, 751** 751					100 (M) 15 (M)	
Bradykinin-induced mechanical hyperalgesia						525 (M)	**124 (M)**
TNF-α, IL-1β, IL-6 or IL-8-induced mechanical hyperalgesia							**124 (M)**

Table 5—*Continued*

Experimental Model	Effect of Applied NO, NO Donor, or Precursor	NO Production	NOS Expression or Activity	nNOS Expression or Activity	iNOS Expression or Activity	Role of Endogenous NO in Nociception	Role of Endogenous NO in Anti-nociception
Acrolein-induced hypersensitivity of bladder afferents							**4 (M)**
Neuropathic states associated with hyperalgesia							
CCI to the sciatic nerve		117, 118, 522	427 (eNOS only)	429 (eNOS also), 117, 118, 472 (m), 522, 274 (m)	429, 425, 137, 472 (m), 522, 274 (m)	720 (H) 522 (H+M) 427 (H+M) 137 (M) 274 (H+M, m)	
Transection of the sciatic nerve			747 (DRG) 795 (DRG) 196 (DRG) 604 (DRG)	805 (eNOS also)	805	747 (ongoing discharges) 775 (ongoing discharges)	
Partial ligation of the sciatic nerve		438				438 (H)	
Crush injury to the sciatic nerve				98 (mRNA, DRG)			
Spinal nerve ligation			670 (DRG) 107	446, 361 (DRG, sciatic nerve, skin) 249 (DRG, m)		361 (M)	
Cauda equina compression	551						
Experimental autoimmune neuritis					141		
Experimental diabetes			806 (DRG, activity only) 806 (eNOS transiently)	628 (decrease in DRG) 360 (decrease in DRG)	806 (decrease) 103 (DRG, m)	13 (M)	**562 (M)**
Vincristine-induced neuropathy						13 (M)	

Data regarding nociception and antinociception are marked with normal and bold typesetting, respectively. H, heat sensitization or hyperalgesia; M, mechanical sensitization or hyperalgesia; C, chemical sensitization or hyperalgesia. If species is not mentioned, rats were used. m, Mouse. For other abbreviations, see text.

pain in a concentration-dependent manner; no hyperalgesia, edema, or inflammation was reported to result from these experiments (289, 290). Exogenous NO delivered as a locally applied NO precursor or NO donor induced mechanical hyperalgesia in rats that was diminished by inhibition of GC (15, 361). In mice, NO donors applied by intraplantar injection induced heat hyperalgesia (495). Gaseous NO was shown to cause a weak excitation and a slight mechanical (but not heat) sensitization of unmyelinated nociceptors in the isolated rat skin (387). Such responses were not evoked by membrane-permeable analogs of cGMP, suggesting that these NO effects were independent of cGMP. In human volunteers, a topically applied NO donor enhanced pain evoked by intracutaneously applied acidic solution leaving the heat and mechanical pain thresholds unaltered (79).

In CFA-treated hindpaws of rats, the increased level of iNOS immunoreactivity (mainly in macrophages) correlated with the late phase of mechanical hyperalgesia (137). A selective iNOS inhibitor suppressed accumulation of the NO metabolite nitrite in the inflamed paw and partially reversed mechanical hyperalgesia and edema, indicating an involvement of iNOS in both inflammation and hyperalgesia. In the same model, an upregulation of nNOS mRNA was revealed in ipsilateral DRGs (98). In the CFA-inflamed plantar skin of mice, an upregulation of mRNAs of all three forms of NOS was noted, and a systemic inhibition of nNOS or iNOS, but not eNOS, diminished heat hyperalgesia (102).

In rats with kaolin-induced arthritis, intra-articular injection of a nonselective NOS inhibitor reduced secondary heat hyperalgesia and displayed anti-inflammatory effects, whereas a selective nNOS inhibitor exerted only an antihyperalgesic effect without influencing inflammation (404). These results suggest that neuronal-derived NO can exert a peripheral pronociceptive effect independent of proinflammatory actions in this model. Heat hyperalgesia in rats induced by either a mild heat injury or plantar incision was diminished by intraplantar pretreatment with a nonselective NOS inhibitor (219).

Following intraplantar injection of zymosan, iNOS was upregulated in the inflamed rat paw, and an NO donor enhanced both inflammation and heat hyperalgesia whereas an iNOS inhibitor reduced paw inflammation but not hyperalgesia (256). In zymosan-induced arthritis in rats, neutrophils were shown to be involved in generation of NO and its active metabolite peroxynitrite, in synovial fluid thereby contributing to mechanical hyperalgesia (51). iNOS mRNA was induced in LPS-induced pulpitis of the rat in macrophages and neutrophils, and a NOS inhibitor decreased LPS-evoked local upregulation of COX-2 and pronociceptive cytokines suggesting a pronociceptive role for NO (344).

Some pronociceptive effects of PGE_2 in the rat such as sensitization to mechanical stimuli of cutaneous C-fibers, enhancement of the TTX-R Na^+ current in cultured DRG neurons, and mechanical hyperalgesia upon intraplantar administration were shown to be diminished by NOS inhibition suggesting a contribution of endogenous NO (15, 100; see more details in sect. III*C2*). Further examples for a peripheral pronociceptive action of NO in inflammatory states are given in section VI*D1*.

2. Pronociceptive roles of nitric oxide in neuropathic states

In the CCI model of the rat, 2 days after injury (but not later) eNOS accumulation was revealed in damaged axons without appearance of nNOS or iNOS (427). At this early time point, local nonselective NOS inhibition (unlike selective nNOS or iNOS inhibition) reduced heat hyperalgesia and ectopic mechanosensitivity of injured A-fibers in a teased fiber preparation. At later time points (7 or 14 days after injury), increased expression of both nNOS and iNOS was detected at the injury site both in the rat and mouse (429, 472). In the rat, iNOS expression was induced in macrophages and Schwann cells in the injured nerve but not in DRGs (137, 425). In mouse DRGs, increased nNOS staining was observed in small dark neurons, nerve fibers, and Schwann cells, whereas enhanced iNOS staining was revealed in fibers (472). On days 7 and 14, increased levels of NO, nNOS, and NO metabolites were detected in the injured paw and sciatic nerve (117, 118, 522). Nonselective, nNOS or iNOS-specific inhibitors of NOS acting locally caused an alleviation of heat and mechanical hyperalgesia/allodynia both in the rat and mouse (137, 274, 522, 720; see, however, Ref. 13). As studied in mice, intraplantarly injected inhibitor of GC or PKG also diminished heat and mechanical hyperalgesia (274).

Transection of the sciatic nerve in the rat led to an upregulation of NOS for several weeks predominantly in small and medium-sized lumbar DRG neurons (196, 604, 747, 795). The same injury resulted in both eNOS and nNOS appearance in axonal endbulbs 2 days after surgery and iNOS expression in some endoneurial and epineurial macrophages observed on day 14 (805). NO has been implicated in generation of ongoing discharges of A-fibers of rat DRG neurons in this model (747, 775). In a related model based on partial sciatic nerve ligation, peroxynitrite was shown to be produced by macrophages and Schwann cells in the injured nerve, and a scavenger of peroxynitrite reduced concomitant heat hyperalgesia (438). However, a lack of involvement of peripheral NO in mechanical hyperalgesia in this model was also reported (13). Following crush injury to the sciatic nerve of rats, an upregulation of the mRNA for nNOS was revealed in ipsilateral DRGs (98). In rats with cauda equina compression, an NO donor was reported to induce ectopic firing in lumbar dorsal roots (551).

L5 and/or L6 spinal nerve ligation in rats resulted in NOS upregulation in the deafferentiated DRGs on days 3, 7, and 14 after injury (107, 670). nNOS upregulation was revealed for several weeks in small and medium-sized neurons (in colocalization with TRPV1, IB_4, and CGRP) and in glial cells of the affected DRGs as well as in the sciatic nerve (mainly in Schwann cells) and in the glabrous skin of the hindpaw (361, 446). However, conflicting results have been obtained regarding the effect of selective (for nNOS or iNOS) or nonselective NOS inhibitors on mechanical allodynia in this model, and even in studies in which NOS inhibition proved effective, its effect could not be reversed by L-arginine pretreatment or this option was not tested (361, 408, 446). In a murine model of L5 spinal nerve injury, the expression of nNOS, but not iNOS and eNOS, was increased in DRG 7 days after injury, and nNOS knockout mice failed to display injury-induced mechanical hyperalgesia in contrast to their wild-type littermates (249). In an experimental autoimmune neuritis of rats, iNOS-expressing macrophages and neutrophils infiltrated the DRGs, spinal roots, and the sciatic nerve (141).

With regard to diabetic neuropathy, a contribution of peripheral NO to streptozotocin-induced mechanical hyperalgesia in the rat was suggested (13). In diabetic rats and mice, signs of peroxynitrite injury were revealed in peripheral nerves (Schwann cells) and DRG neurons together with diminished conduction velocities in A-fibers and small-fiber sensory neuropathy (163, 543, 544, 740). In iNOS-deficient diabetic mice, all these phenomena were reduced (except for peroxynitrite injury in DRGs) compared with wild-type animals, suggesting that iNOS-dependent peroxynitrite formation in axons and Schwann cells, rather than cell bodies, of peripheral nerves plays a role in diabetic neuropathy (740). In nNOS-deficient mice made diabetic, peroxynitrite accumulation in DRG, but not peripheral nerve, was diminished compared with wild-type diabetic animals, but nerve conduction deficit and sensory neuropathy were only slightly reduced (739). In a mouse model of type 2 diabetes, iNOS mRNA/protein upregulation in lumbar DRG neurons of all sizes was revealed, and an inhibitor of the p38 MAPK applied intrathecally decreased both iNOS upregulation and mechanical hyperalgesia (103).

3. *Mechanisms of peripheral pronociceptive actions of nitric oxide*

The molecular mechanisms of peripheral pronociceptive actions of NO at the level of nociceptors are largely obscure. Some of them appear not to involve the GC-cGMP-PKG pathway. An indirect mechanism could be NO-induced synthesis of nociceptor-sensitizing prostanoids by stimulating/upregulating COX enzymes independently of cGMP (for review, see Ref. 149). Indeed, in the rat skin endogenous NO was shown to stimulate COX-1 in the early phase of carrageenan-evoked inflammation and upregulate COX-2 in the late phase, leading to increased production of PGE_2 and PGI_2 in both phases (729). Treatment with an NO donor for 6–12 h increased PGE_2 production in rat trigeminal satellite cells as a result of increased COX-1 activity (86). The molecular mechanism(s) of COX activation by NO remains to be elucidated.

NO donor compounds have been shown to activate various TRP channels including TRPC5, TRPV1, TRPV3, TRPV4, and TRPA1 in transfected host cells independently of GC through *S*-nitrosylation of identified cysteine residues (495, 633, 703, 790). Furthermore, an NO donor caused an elevation of intracellular Ca^{2+} in cultured mouse DRG neurons and this effect was mediated by both TRPV1 and TRPA1 (495). NO donors applied by intraplantar injection in mice failed to cause acute nocifensive behavior but induced TRPV1-dependent heat hyperalgesia. Following injection of forskolin together with a PLC activator, however, the NO donor became able to induce a nocifensive reaction that was diminished only when both TRPV1 and TRPA1 were genetically ablated. An NO donor compound potentiated proton-gated currents in DRG neurons and in CHO cells expressing an acid-sensing ion channel (ASIC; Ref. 79). This response was also independent of the GC-cGMP-PKG axis but reversed by application of reducing agents, suggesting that NO had a direct effect on ASIC probably through oxidation of cysteine residues. With respect to the GC and cGMP-independent pronociceptive actions of NO donors, one must consider that the mitochondrial superoxide dismutase also acts as NO dismutase producing the highly reactive nitroxyl anion (NO^-) which nitrosylates thiol groups in proteins (e.g., critical cysteines in TRPA1) creating posttranslational modifications of potentially sustained functional effect (197).

Possibly relevant for the pronociceptive actions of NO, this radical is known to react with superoxide anion to produce peroxynitrite that was shown to evoke heat hyperalgesia accompanied by signs of inflammation upon intraplantar administration in rats (530). Moreover, applied superoxide alone induced hyperalgesia that was blocked by NOS inhibition, suggesting that endogenous NO was required to form hyperalgesic peroxynitrite. Nitrooleic acid, a highly reactive cysteine-modifying agent formed through nitration of oleic acid by peroxynitrite and nitrogen dioxide, was also shown to activate TRPA1, but not TRPV1, in transfected host cells via covalent modification of cysteine residues (713). Nitrooleic acid caused an elevation of intracellular Ca^{2+} in cultured trigeminal and vagal sensory neurons, and induced action potential discharges from vagal pulmonary C-fibers in an ex vivo mouse lung preparation, with both responses being mediated by TRPA1.

On the basis of results obtained from posterior pituitary nerve terminals, activation of the NO-cGMP-PKG axis can enhance the activity of Ca^{2+}-activated K^+ channels but only at depolarized membrane potentials (368). Thereby the spike threshold remains unaltered, but AHP is augmented which accelerates recovery of Na^+ channels from inactivation leading to increased firing rate. Whether this mechanism operates in nociceptive sensory neurons is unknown. It is puzzling that the reverse mechanism, i.e., blockade of the Ca^{2+}-activated K^+ channels was proposed as one of the mechanisms underlying PGE_2-induced nociceptor sensitization (see sect. III*B2*c).

C. Data Supporting an Antinociceptive Role of Nitric Oxide in the Periphery (Table 5)

1. *Peripheral antinociceptive effects/roles of nitric oxide*

The involvement of the NO-GC-cGMP axis in bradykinin tachyphylaxis (see sect. II*C4*) is compatible with a peripheral antinociceptive role of NO. Support for a more general, peripheral antinociceptive role of NO was first obtained when locally applied NO donors and the NO precursor L-arginine inhibited mechanical hyperalgesia in the rat hindpaw induced by PGE_2 and carrageenan, respectively, and these actions were diminished by a GC inhibitor and potentiated by a blocker of the cGMP-specific isoform of PDE (124, 164, 191). Intraplantar injection of an iNOS inhibitor enhanced the mechanical hyperalgesia induced by carrageenan (72). In carrageenan-induced rat paw inflammation, an increase in nNOS activity (in both phases) and appearance of iNOS activity (in the late phase) were revealed along with enhanced levels of NO metabolites (nitrite/nitrate) in the paw exudate (109, 266, 550, 624, 729). Carrageenan-induced heat hyperalgesia was not altered in iNOS-deficient mice (712). GC inhibition enhanced mechanical hyperalgesia induced by bradykinin or cytokines (TNF-α, IL-1β, IL-6, IL-8) but not PGE_2 (124).

In rats with streptozotocin-induced diabetes, intraplantarly applied sildenafil, a selective inhibitor of PDE5 responsible for cGMP breakdown, exerted a mechanical antihyperalgesic effect that was antagonized by local administration of either a NOS inhibitor or a GC inhibitor (562). Inhibition of NOS or GC without sildenafil augmented the diabetes-induced hyperalgesia while in healthy animals sildenafil, NOS, or GC inhibitor failed to alter mechanonociception. In humans having undergone extraction of impacted third molar, NO levels at the surgical site gradually increased over the first 80 min compared with the rest of the 180-min observation period, and an inverse relationship between NO levels and pain intensity scores was revealed (264). All these data argue for a local antihyperalgesic effect of the NO-GC-cGMP pathway that is not tonically active but stimulated by diverse hyperalgesia-inducing agents/conditions including PGE_2, bradykinin, cytokines, carrageenan, diabetes, and oral surgery. Further data regarding a peripheral antinociceptive action of NO in inflammatory states are mentioned in section VI*D1*.

In anesthetized rats, a NOS inhibitor increased discharge activity of articular nociceptive C-fibers in both normal and arthritic (CFA-treated) ankle joints as well as the firing response of C and Aδ afferents of the urinary bladder evoked by distension (4, 346). These effects were reduced by coapplied L-arginine. L-Arginine also decreased the firing activity of urinary afferent units under normal conditions and also the enhanced firing induced by acrolein, a TRPA1 activator (4). The bradykinin-, serotonin-, or distension-induced firing of mesenteric afferents in the isolated jejunum taken from mice with indomethacin-induced intestinal inflammation was reduced by an iNOS-dependent mechanism (783). These data argue for a tonic NO production in the periphery that reduces nociceptor excitability.

2. *Mechanisms of the antinociceptive effects of nitric oxide*

A possible mode of action of NO as a peripheral antinociceptive agent is opening of ATP-sensitive K^+ channels ($K^+{}_{ATP}$) in the membrane of nociceptors which leads to hyperpolarization and thereby reduces excitability. As the first experimental support for this, it was demonstrated that glibenclamide or tolbutamide, selective blockers of the $K^+{}_{ATP}$ channels, dose-dependently inhibited the peripheral antihyperalgesic effect of the NO donor sodium nitroprusside exerted on the PGE_2-induced mechanical hyperalgesia in the rat hindpaw (665). In the same model, $K^+{}_{ATP}$ channels proved responsible for the peripheral antihyperalgesic action of dibutyryl-cGMP, a membrane-permeable analog of cGMP (664). In the latter study, glibenclamide or tolbutamide failed to alter mechanonociception in control animals, suggesting that $K^+{}_{ATP}$ channels are not tonically active. Diazoxide, an activator of $K^+{}_{ATP}$ channels, was also shown to diminish PGE_2-induced mechanical hyperalgesia by a local action that was prevented by glibenclamide (19). A specific PKG inhibitor reduced the peripheral antihyperalgesic effects of an NO donor and a cGMP analog, respectively, in both acute hyperalgesia evoked by a single intraplantar injection of PGE_2 and persistent hyperalgesia induced by 14 consecutive daily PGE_2 injections (622). In the latter model, the role of the closure of $K^+{}_{ATP}$ channels in maintenance of hyperalgesia was revealed by the inhibitory effect of glibenclamide on the antihyperalgesic action of the NO donor or a cGMP analog together with demonstration of the antihyperalgesic action of diazoxide. These data suggest that a GC-cGMP-PKG-$K^+{}_{ATP}$ channel pathway is involved in the peripheral antinociceptive action of NO. Somewhat discordant results have been obtained recently when an NO donor has been shown to activate $K^+{}_{ATP}$ channels in large rat DRG neurons independently of the cGMP-PKG pathway, although the cGMP analog-induced channel activation depended on PKG activity (343). It was shown that the effect of the NO donor (that was preserved in cell-free patches) was mediated by *S*-nitrosylation of cysteine residues in the SUR1 subunit of the K^+_{ATP} channels. Whether this mechanism operates in small, presumably nociceptive DRG neurons, is unclear. In agreement with the proposed peripheral antinociceptive role of NO, abundant evidence has been provided that activation of the L-arginine-NO-cGMP-PKG-K^+_{ATP} channel pathway plays a significant role in the peripheral antinociceptive action of various opioid and nonopioid analgesics (for a review, see Refs. 128, 610).

A local administration of chemically diverse NO donors reversibly blocked action potential propagation in both demyelinated, and less effectively, normal axons of the rat (598). In rat peripheral nerves, an NO donor reversibly eliminated the action potential conduction in both myelinated and unmyelinated fibers, whereas an analog of cGMP was ineffective (655). Interestingly, this effect of the NO donor depended on the presence of the endoneurium. Furthermore, NO donors were reported to block fast TTX-S as well as both slow and persistent TTX-R currents in DRG neurons independently of the cGMP-PKG pathway by modifying SH groups of the channel proteins, e.g., through *S*-nitrosylation (605). No effect of the NO donors was revealed on inactivation of voltage-gated Na^+ channels, suggesting that a real block of channel conductance may be involved. In axotomized cultured DRG neurons, endogenous NO produced by upregulation of NOS was shown to block fast TTX-S and slow TTX-R Na^+ currents (604). It can be concluded that both activation of K^+_{ATP} channels (via the GC-cGMP-PKG pathway) and blockade of voltage-gated Na^+ channels (independently of cGMP) may contribute to antinociceptive effects of NO in the periphery.

D. Opposing/Inconsistent Effects of Peripheral Nitric Oxide on Nociception Revealed in the Same Experimental Model (Table 5)

According to section VI, *B* and *C*, it appears that in the periphery the NO-cGMP pathway can play either a pronociceptive or antinociceptive role or may be without effect, depending on the model and the experimental conditions.

1. *Opposing effects of nitric oxide revealed by variation of experimental protocols in the same study*

There are examples that even in the same study, both pronociceptive and antinociceptive effects of NO could be revealed in the same model depending on the experimental arrangement. The tissue level of NO may be one important factor determining the effect of NO as suggested by a study in mice in which a low dose of the NO precursor L-arginine applied intraplantarly enhanced the second phase of formalin-induced nociception, whereas a higher dose had an inhibitory effect and an intermediate dose was without effect (341, 372). Both actions of L-arginine were diminished by coapplied NOS inhibitor. The authors could not exclude the possibility that the pronociceptive action of NO was due to its proinflammatory activity.

An opposite concentration-dependent dual action of NO became evident when the effects of locally applied NO donors were investigated on tactile allodynia induced by surgical incision in rats: lower concentrations of an NO donor reduced allodynia in a GC-dependent fashion, whereas higher concentrations intensified allodynia, independently of GC (584). Similarly, a low dose of the systemically applied NO donor sodium nitroprusside decreased the severity of inflammation in CFA-induced arthritis of rats along with a reduction of mechanical hyperalgesia while higher doses aggravated both inflammation and hyperalgesia raising the possibility that changes in hyperalgesia were secondary to alterations of inflammation (237).

Another theory, put forward to explain the dual role of NO in peripheral nociception, assumes that the tissue environment can determine the nature of NO action. The mechanical hyperalgesia induced by intradermal injection of bradykinin in the rat hindpaw was diminished by local inhibiton of NOS, GC, or PKG, whereas the same response induced by subcutaneous injection of bradykinin was potentiated by local pretreatment with a GC inhibitor (124, 525). Intracutaneous injection of an NO donor facilitated whereas its subcutaneous administration inhibited PGE_2-induced mechanical hyperalgesia in the rat measured with the electronic von Frey method (751). Evidence was provided that production of cGMP was involved in both the hyperalgesic and antihyperalgesic actions of NO donors. Furthermore, intracutaneous injection of an NO precursor, NO donor, or cGMP analog produced a hyperalgesic action, whereas upon subcutaneous administration these agents failed to alter the mechanonociceptive threshold. In contrast, a cAMP analog induced hyperalgesia upon both intracutaneous and subcutaneous administration showing the marked difference in the action of the two cyclic nucleotides.

Other factors may also lead to opposing NO effects. The mechanical hyperalgesia in zymosan-induced arthritis in rats was reduced by local (intraarticular) pretreatment with nonselective or iNOS-selective inhibitors before zymosan injection; however, these drugs were without effect if they were given after development of arthritis (609). As NO donors given locally after development of arthritis inhibited mechanical hyperalgesia without affecting edema, pointing to an antinociceptive effect of NO, the authors ascribed the former effect to inhibition of synovial inflammation by reduced formation of the proinflammatory NO. NO donors produced either an increase or decrease in mechanosensitivity of rat dural nociceptors, with the sensitized neurons having higher baseline mechanical thresholds than the desensitized ones, suggesting that neurons with lower sensitivity are more likely to be sensitized (426). In this study, an involvement of cGMP in the inhibitory action of NO was shown.

2. *Experimental paradigms yielding inconsistent results regarding nitric oxide*

In the rat formalin test, neuronal activity recorded from dorsal horn neurons during the second phase was reduced by intraplantar pretreatment with a NOS inhibitor (260), but formalin-induced behavioral nociception was not affected by local inhibition of NOS or GC similarly to locally applied NO donors (405, 541, 730). In the acetic acid-induced writhing assay in mice, locally applied sildenafil exhibited an inhibitory effect that was enhanced by L-arginine and reduced by NOS or GC inhibitor, but NOS or GC inhibition alone failed to alter the response (312, 561).

In rats with streptozotocin-induced diabetes, decreases in the number of NOS-expressing neurons, in nNOS expression and cGMP content were revealed in DRGs together with mechanical hyperalgesia that all were completely prevented by insulin treatment (360, 628). In subsequent studies, however, no significant change in nNOS mRNA levels was observed in DRG neurons; moreover, increased NOS activity was revealed as late as 12 mo after diabetes induction (446, 806). This could not be explained by consistent or substantial increases in eNOS, nNOS, or iNOS synthesis, suggesting that an increase in NOS efficiency could have been involved. No significant change in nNOS expression was observed during the 12 mo follow-up period, which is in contrast to the nNOS upregulation seen following peripheral axotomy (see above). In 2- but not 12-mo diabetic animals, an increase in eNOS expression was observed in peripheral nerves and DRGs in the perineurium and DRG capsule, respectively. The expression of iNOS was decreased at both time points in peripheral nerves, but it was unchanged in DRGs.

E. Concluding Remarks and Open Questions

It is rather challenging to reconcile the great amount of apparently contradictory data supporting either a pronociceptive or an antinociceptive effect of NO in the periphery. Regarding the former action, one must remember that the known proinflammatory effects of NO may indirectly enhance nociception/hyperalgesia by aggravating inflammation in not only inflammatory paradigms but also in most neuropathic models involving some kind of inflammation. Several factors including concentration, tissue environment, and stage of inflammatory hyperalgesia may determine whether NO induces a pronociceptive or antinociceptive effect. It is worth mentioning that such a dual role for NO has also been proposed for the central processing of pain (for review, see Ref. 128).

Concerning the pronociceptive actions of NO, exogenous NO can evoke overt pain in humans and, in addition, hyperalgesia in animals. NO is produced in tissues inflamed by CFA, kaolin, heat injury, plantar incision, or zymosan and is involved in the evoked nocifensive behavior and/or hyperalgesia. At least a part of these pronociceptive actions appears to be independent of the proinflammatory effects of the radical. Regarding neuropathic pain, firm evidence for an involvement of peripheral NO as a pronociceptive agent in neuropathic hyperalgesia is only available for the CCI model in which an early and transient role for eNOS-derived NO in hyperalgesia was suggested that is followed by a later and sustained contribution of NO produced by nNOS and iNOS. In this model, NO appears to exert its hyperalgesic effects by activation of the GC-cGMP-PKG pathway. In other paradigms based on peripheral nerve injury, NOS upregulation was revealed, but no consonant functional data are available. In diabetic neuropathy, conflicting data have been reported regarding NOS expression. Therefore, further studies are needed in both mechanical and metabolic forms of nerve injury to clarify the role of NO. Regarding the molecular mechanism(s) of the peripheral pronociceptive actions of NO, more studies are needed, especially using models that reflect the activity of the peripheral terminals of nociceptive sensory neurons (e.g., electrophysiological recordings). Of the mechanisms revealed so far, most support exists for activation of TRPV1, TRPA1, and ASIC channels by NO in a cGMP-independent fashion probably through *S*-nitrosylation of cysteine residues of the ion channel proteins.

The antinociceptive actions of NO have mostly been established in studies on inflammatory mediators and models (PGE_2, bradykinin, cytokines, carrageenan, and oral surgery). The only neuropathic model studied so far is experimental diabetes. The antihyperalgesic actions of NO appear to be predominantly mediated by the cGMP-PKG-K^+_{ATP} channel signaling pathway. Further support for this inhibitory mechanism has been provided by studies on a great number of opioid and nonopioid analgesics as well as numerous natural products.

VII. GENERAL CONCLUSIONS

Extensive evidence indicates that bradykinin fulfills the criteria for being a typical peripheral mediator involved in both inflammatory and neuropathic pain: exogenously applied bradykinin causes nociceptor activation and hyperalgesia to heat, mechanical, or chemical stimuli; both B_1 and B_2 receptors are upregulated in established animal models of acute, subacute, or chronic inflammation and also nerve injury. In these models, either B_1 or B_2 receptor antagonists exert antihyperalgesic effects that are confirmed in bradykinin receptor knockout animals. Similarly, prostanoids, LOX products, and NO have been shown to contribute to both inflammatory and neuropathic pain in the periphery, i.e., at the level of nociceptors or their axons in the nerve. In addition, they are involved in several nociceptor-activating and sensitizing actions of bradykinin, and they, especially prostanoids, may sensitize nociceptors to bradykinin. Thereby positive feedback loops are established between bradykinin and the secondary mediators that are presumably involved in peripheral amplification of pain and hyperalgesia. These mutual interactions as well as the multiplicity and redundancy of the inflammatory mediators discourage searching for an ideal target of antinociception. Concerning the peripheral pronociceptive actions of bradykinin, prostanoids, LOX products, PAF and NO, one must remember that all these mediators have proinflammatory actions as well, meaning that their pronociceptive effects may include a component that is secondary to aggravation of inflammation. Another confounding factor is that all these mediators can contribute to pain and hyperalgesia by actions in the central nervous system. It makes difficult to interpret results, in terms of peripheral nociception, obtained with knockout animals or systemically applied receptor antagonists, channel blockers, and enzyme inhibitors possibly passing the blood-brain barrier.

FIGURE 4 summarizes intracellular signaling mechanisms and membrane targets of bradykinin and/or prostanoids (for the other mediators only insufficient data are available) that are likely to mediate their pronociceptive effects not only in somata but also peripheral terminals of sensory neurons. These include facilitation of TRPV1, TRPA1, HCN, $Na_v1.9$, and Ca^{2+}-activated Cl^- channels as well as

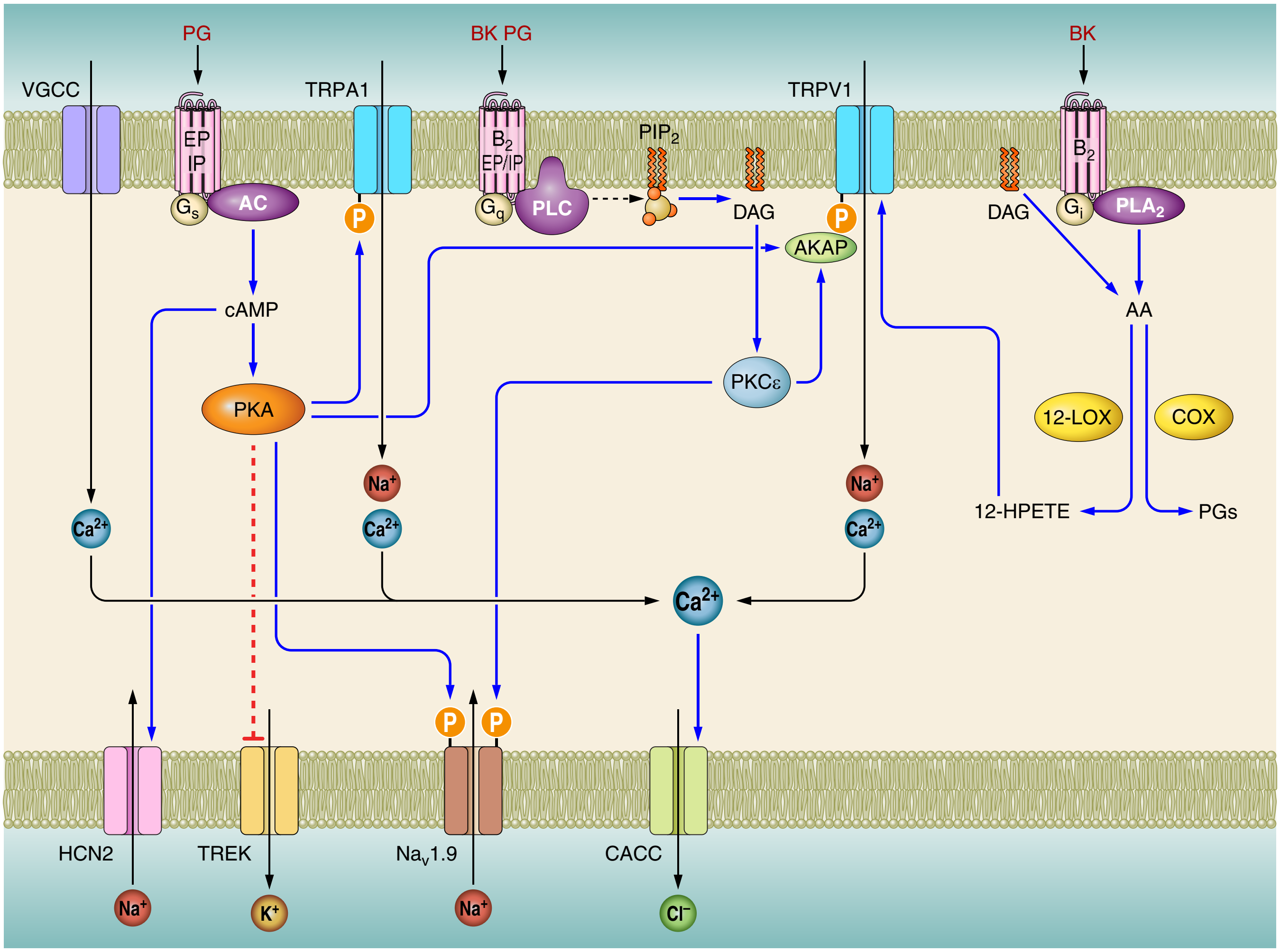


FIGURE 4. Putative signal transduction mechanisms of bradykinin and prostaglandins in peripheral endings of nociceptive sensory neurons based on studies employing single-fiber recording, neuropeptide release or behavioral tests reflecting the activity of peripheral nociceptors. Blue arrow: activation of a target or stimulation of synthesis of a substance; red line: inhibition of a target (dashed line indicates a likely inhibitory effect); dashed black arrow: cleavage of a substance. Not shown are the minor outward K^+ currents in case of TRPV1, TRPA1, and HCN2 channels. VGCC, voltage-gated Ca^{2+} channels; CACC, calcium-activated Cl^- channel; HCN2, hyperpolarization-activated cyclic nucleotide-gated channel; TREK, mechanosensitive K^+ channel. For other abbreviations, see text. AKAP is only shown when its involvement was directly revealed.

inhibition of TREK-1 channels. In addition to the membrane receptors and these target structures of inflammatory mediators, intracellular mechanisms of their sensitizing actions (e.g., TRPV1 and TRPA1 facilitation via AKAP, PKC, and PKA) may also represent promising targets for development of novel analgesic drugs (744). NO, unlike other mediators discussed in the present review, may exert peripheral antinociceptive effects as well, possibly under presensitized conditions and as a factor in analgesic drug actions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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