INVITED REVIEW ARTICLE

Nagoya J. Med. Sci. **60.** 69 ~ 87, 1997

PERIPHERAL MECHANISM OF HYPERALGESIA — SENSITIZATION OF NOCICEPTORS —

KAZUE MIZUMURA

Department of Neural Regulation, Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-01

ABSTRACT

The peripheral mechanism of hyperalgesia is considered to be the result of nociceptor sensitization. As possible agents causing nociceptor sensitization, bradykinin, histamine, prostaglandin (PG) s, protons and nerve growth factor are evaluated with respect to their release into the injured tissue, their sensitizing potencies. Whether blocking these agents suppresses sensitization was also evaluated. In addition, the intracellular mechanisms by which bradykinin, histamine and PGs cause sensitization are reviewed.

Key Words: hyperalgesia, sensitization, nociceptor, inflammatory mediators, protons, nerve growth factor

INTRODUCTION

The main objective of this review is to consider the mechanism of sensitization of peripheral nociceptors, including the intracellular mechanism, as hyperalgesia in the periphery is considered to be due to sensitization of nociceptors. Before going into details, however, it would be better to offer a brief overview on hyperalgesia and related altered pain conditions, with reference to the terminology.

Changed pain states related to inflammation and neuropathic pain are characterized by alterations of pain perception that include an enhanced sensitivity to normally noxious stimuli (hyperalgesia) and an abnormal pain sensitivity to normally non-painful stimuli (allodynia, Fig. 1). This definition was given by IASP in 1994.[1] However, the word "hyperalgesia" is still used in different ways. When the skin is injured, the areas of hypersensitivity are found not only in the injured site but also in a much larger area extending well beyond the site of injury and into undamaged skin. In the first area, low intensity mechanical stimuli and warmth evoke pain (thus allodynia) and noxious stimuli causes more severe pain (hyperalgesia). Within the second area, low intensity mechanical stimuli causes pain (thus allodynia), but warmth does not (absence of thermal allodynia). Although different from the IASP definition of 1994, these regions have been called areas of *primary* and *secondary hyperalgesia*, respectively, based on the classical descriptions of Lewis[2] and Hardy et al.[3]

Of these two kinds of hyperalgesia, primary hyperalgesia can be explained by nociceptor sensitization: it has long been known that an injury induces a process of nociceptor sensitization (increased excitability and lowered threshold of nociceptors). Mild burn (or repetitive heat stimulation) has been used in experiments to study this phenomenon because the magnitude of

Correspondence to: Dr. Kazue Mizumura at the above address.

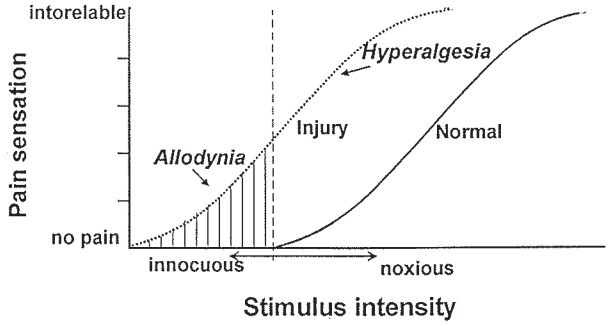

Fig. 1 Relationship between stimulus intensity and pain sensation in normal and injured conditions.

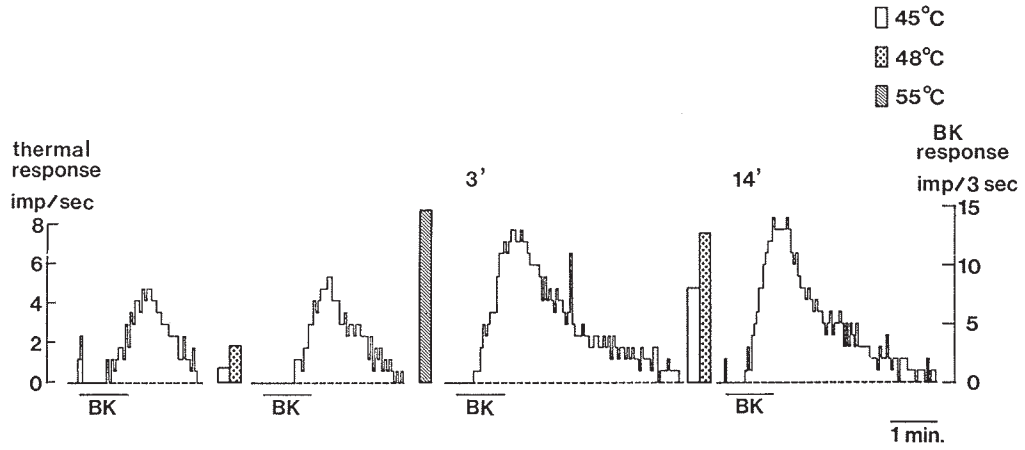

Fig. 2 Heat-induced sensitization of a polymodal receptor.
The bradykinin response (peri-stimulus time histogram) and the heat response (bar graph) of a testicular polymodal receptor unit are shown along the order of testing. BK: bradykinin 0.1 μM. Heat stimulation was applied by replacing bathing Krebs solution (34°C) with a preheated Krebs solution (45, 48 or 55°C). Note that after 55°C stimulation, both the heat and bradykinin responses were facilitated.

injury can be well controlled. One example of heat-induced sensitization of the nociceptor is shown in Fig. 2. It was demonstrated that after mild burn (strong heat stimulation at 55°C), the heat threshold decreased and the response to the same heat stimuli increased. These changes in nociceptor responses satisfactorily explain changes in pain sensation. Such changes in nociceptors are now considered to be induced by inflammatory mediators, tissue acidosis, raised temperature in the injured tissue and certain processes induced by increased production of nerve growth factor (NGF). This will be discussed later in more detail.

Secondary hyperalgesia, in contrast, is considered to be mediated by alterations in the central processing of sensory input and to be induced by arrival in the central nervous system of the afferent volleys that the injury evokes in peripheral nociceptors. Evidence for changes in the central nervous system is:

1. If the conduction of C-fiber activity is blocked during injury, allodynia and hyperalgesia in the area of secondary hyperalgesia does not develop upon recovery from the block, whereas primary hyperalgesia is seen.[4)]
2. Intraneural microstimulation of A-fibers at a place proximal to the secondary hyperalgesic area induces allodynia when the projection area is located inside the area of secondary hyperalgesia.[5)]

The alteration responsible for allodynia in the area of secondary hyperalgesia is also different from that of primary hyperalgesia. It is not due to the lowered mechanical threshold of nociceptors, because allodynia disappeared after the block of A-fibers which convey volleys from low threshold mechanoreceptors.[6)] Thus, allodynia is caused by a change in modality of the sensation evoked by low threshold mechanoreceptors, from touch to pain.

Central changes induced by nociceptive input have been intensively studied, and readers are asked to refer to the reviews for details.[7-10)]

This review will focus on the mechanism of peripheral sensitization of nociceptors by inflammatory mediators, because this is the area of the author's work. In addition, the effects of pH, NGF and recruitment of sleeping fibers will be briefly reviewed.

SENSITIZATION OF NOCICEPTORS BY INFLAMMATORY MEDIATORS AND OTHER CHANGES

To determine whether a substance mediates sensitization of nociceptors and causes pain and hyperalgesia, the following points must be clarified: 1) It must be known that a substance is produced in the injured tissue and its concentration. 2) The ability of a substance to excite and sensitize nociceptors at reasonable concentrations (that is, near concentrations measured in the injured tissue) must be demonstrated. 3) Inhibition of the action of a substance or substances should alleviate pain and/or hyperalgesia. In the following sections, these points will be considered separately.

1) Inflammatory mediators and other changes in inflamed tissues

In damaged and inflamed tissues, potassium and inflammatory mediators such as bradykinin, histamine, serotonin and prostaglandins (PGs) are released from damaged cells, blood plasma and inflammatory cells.[11-16)] In carageenin-inflammatory pouch fluid, 0.4 ng/ml (about 0.4 nM) of bradykinin has been detected.[14)] With regard to the histamine concentration in inflamed tissues, there is a large difference between reports: a concentration slightly less than 10 μM has been reported in pouch fluid inflamed with carrageenin,[16)] whereas in vivo microdialysis revealed that probe insertion induced release of about 40 nM of histamine.[17)] Close apposition of nerve fibers and mast cells containing histamine[18,19)] suggest a higher concentration near nerve terminals. The concentration of PGE_2 in inflamed tissue is reported to be on the order of 0.01 μM in exudate and 0.1 μM in abscess,[11,13,20,21)] and a 10-fold increase of PGE_2 above the control value was detected after induction of carrageenin inflammation in the temporomandibular joint by microdialysis combined with ELISA for PGE_2.[15)] Any absolute value was not given in the report.

A pronounced decrease in tissue pH up to 5.4 and 6.91 is reported in exudate of the abscess from painful inflammation[22)] and joints experimentally inflamed with urate crystals.[23)] Such pronounced decrease in pH in the inflamed tissue has recently been confirmed using pH-sensitive needle electrodes.[24)]

When applied exogenously, these inflammatory mediators not only induce pain but also induce hyperalgesia to heat and/or mechanical stimulation in humans.[24-30)] A good correlation

between decrease in pH and pain sensation has also been observed.[24] Therefore, these mediators and tissue acidosis are candidates for nociceptor sensitizers.

Cytokines also appear in inflamed tissues. They do not directory sensitize nociceptors, but rather do so through release of PG and other substances, or through other mechanisms such as induction of receptors for inflammatory mediators.

2) Bradykinin-induced excitation and facilitatory effect on the heat response through B2 receptor subtype

The testicular polymodal receptor activities described in the following sections were recorded using canine testis-spermatic nerve preparations in vitro.[31] These studies were done in collaboration with researchers at the Research Institute of Environmental Medicine, Nagoya University.

Bradykinin induces excitation in testicular polymodal receptors ≥ 10 nM at 34°C (Fig. 3). This threshold concentration of bradykinin for the excitation of the testicular polymodal receptor is the lowest among inflammatory mediators the author has studied so far (see below), and also the lowest to excite nociceptors in many tissues (2.6 μg/0.3 ml in cat muscle,[32] 0.26 μg/0.3 ml in cat knee joint afferents,[33] 10 nM - 10 μM in rat skin nerve preparation[34]). This excitatory effect is characterized by a long latency (about 15 s (for A-delta fibers) to 22 s (for C-fibers) at 0.1 μM (see ref. No.31), and decrease in the response magnitude on repetitive application at the relatively short interval of ca. 10 min (a process of tachyphylaxis).[31] A clear concentration-dependency of the response magnitude was observed.[31,35]

The bradykinin response is also dependent on the temperature of the stimulus solution: the response at 36°C starts earlier, reaches a higher discharge frequency and ends faster than at 30°C;[36] a different pattern of augmentation from that induced by PGs where the sensitized bradykinin response starts earlier but ends later.[37] One of the factors producing this facilitation pattern induced by temperature increase may be a change in the activity of the bradykinin degrading enzyme.[36] The threshold concentration decreases when the temperature of the stimulus solution is raised, namely 50 nM at 30°C and 9 nM at 36°C. This temperature dependency of the bradykinin response may partly explain the well-known phenomena that warming the inflamed skin aggravates pain whereas cooling alleviates it.

In addition to exciting polymodal receptors, bradykinin sensitizes the response to heat (45–48°C) of testicular polymodal receptors (Fig. 3).[35] This sensitization was observed from about 0.1 nM, a concentration 100 times lower than that necessary to excite the polymodal receptors. That means that induction of excitation is not required to facilitate the receptor. This sensitizing effect endured for a short period of time, diminishing in 10 min.[35] It should be noted that this sensitizing effect was observed during inhibition of PG production by acetylsalicylic acid (ASA).[35]

A similar sensitizing effect of bradykinin was noted in the heat response of cutaneous C-nociceptors of cats[38] and rats (a decrease in the threshold temperature by 5°C),[39] and on the mechanical response of cat joint receptors,[40] but at as high as 10 μg (injection volume unknown) or 10 μM, and 0.26 μg/0.3 ml (about 0.7 μM), respectively. The difference in the concentration necessary to induce sensitization might be based on the different application routes or accessibility in various tissues. In contrast, human pain rating was increased by intradermal injection of bradykinin ≥ 0.1 nM, but hyperalgesia to heat was observed with only bradykinin ≥ 10 nM.[28] In this human experiment, precedent pain sensation induced by bradykinin might have influenced the subsequent pain rating to heat.

In carageenin-inflammatory pouch fluid, 0.4 ng/ml (about 0.4 nM) of bradykinin was detected;[14] this concentration is higher than the threshold concentration to sensitize the heat response of testicular polymodal receptors.

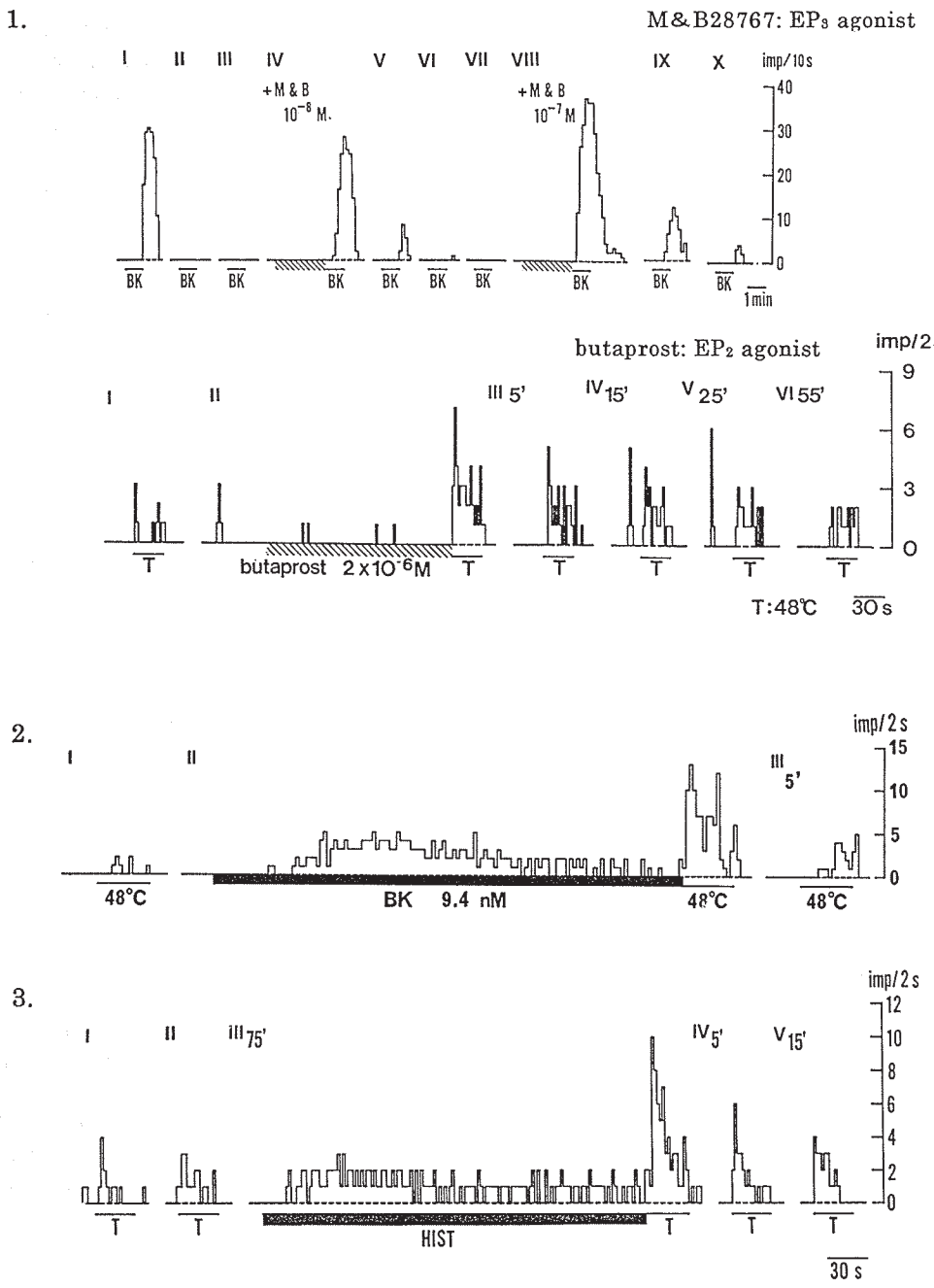

Fig. 3 Excitation and sensitization of the polymodal receptors induced by inflammatory mediators.
Peri-stimulus time histograms are shown in the order of testing. 1: Effects of prostaglandin EP agonists on the bradykinin (upper row) and heat (lower row) responses. From the second bradykinin application, no discharge was induced by bradykinin (tachyphylaxis). With the addition of an EP3 agonist (M&B28767) discharges reappeared. An EP2 agonist, butaprost, facilitated the heat response. Note that neither agonist induced any discharge by itself. 2: Effects of bradykinin. 3: Effects of histamine. Both bradykinin and histamine excited the receptor and sensitized the following heat response.

For the bradykinin receptor two subtypes (B1 and B2 receptors) were distinguished by pharmacological methods[41] and later confirmed by molecular cloning of cDNA.[42-44] In testicular polymodal receptors, Des-Arg^9-BK (a B1 receptor agonist) did not induce excitation, and des-Arg^9-[Leu^8]-BK (a B1 receptor antagonist) failed to block BK effects. In contrast [Thi^{5,8}, D-Phe^7]-BK (a B2 receptor antagonist) shifted the BK response curve to the right, and another B2 antagonist, D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (NPC349) suppressed the bradykinin-induced facilitation of the heat response. Therefore, the receptor subtype involved in excitation and sensitization of the heat response of testicular polymodal receptors by bradykinin (BK) is B2.[35,45]

The induction of B1 receptors after a long incubation in vitro,[46] in UV-induced inflammation[47] and NGF-induced hyperalgesic states,[48] (described later in this review) has been reported, but excitation by Des-Arg^9-BK was not observed in preparations incubated in vitro for longer than 5 hrs in a study of the author's lab. A recent report has shown that B1 receptors are expressed not on sensory receptor terminals but on cells other than neurons.[49] This might be the reason why we did not observe any B1 effect.

Our observations on mediation through the B2 receptor for bradykinin effects on testicular afferents are consistent with the reports on humans that pain induced by applying bradykinin to a blister base is mediated through the B2 receptor.[50]

3) Histamine-induced excitation and facilitatory effects on the heat response through H1 receptor subtype

In contrast to bradykinin, histamine induced excitation in only a portion of testicular polymodal receptors even when the concentration was increased to 1000 μM.[51] A sample recording is shown in Fig. 3. In the entire recorded population of units, the proportion of units that showed excitation ($>$ 10 impulses/1 min application of histamine) increased roughly with an increase in concentration, namely 7% at 1 μM, 26% at 10 μM, 79% at 100 μM, and 61% at 1000 μM.

A histamine (100 and 1000 μM) response $>$ 0.5 impulses/s was observed only in units with conduction velocity (CV) of 10 m/s or slower, but not in those with CV faster than 10 m/s,[51] although a small response was observed also in the latter group. Histamine-induced discharges were significantly greater in units with CV $\leq$ 10 m/s at all concentrations $\geq$ 10 μM, thus units were tentatively divided into slow (CV $\leq$ 10 m/s) and fast (CV $>$ 10 m/s) CV groups. In the slow-CV group, significant excitation by histamine (1 min application) was observed at $\geq$ 10 μM whereas a 10 $\times$ higher concentration was necessary for the fast-CV group (in Table 1 of ref. No. 51). These threshold concentrations required to excite the testicular polymodal receptors are much higher than for bradykinin (see previous section). It is interesting to note that the difference in sensitivity to histamine is related to conduction velocity, but not related to myelination.

Histamine excitation was characterized by a long latency (18 s at 10 μM and 9 s at 100 μM for the slow-CV group) and long duration after washing.[51] It also showed prominent tachyphylaxis on repetitive application at an interval of ca. 10 min: the second response to 1000 μM histamine was reduced to 34% of the first one in the slow-CV group.

Histamine can also sensitize the heat response of the testicular polymodal receptors,[51,52] as shown in the sample recording in Fig. 3. It should be noted that this sensitizing effect was observed irrespective of preceding histamine-induced excitation. Significant facilitation of the heat response was observed $\geq$ 10 μM in the slow-CV group, and $\geq$ 100 μM in the fast-CV group. The magnitude of sensitization tended to increase with higher histamine concentrations. It has been reported that histamine stimulates release of PG,[53] but the sensitizing effect of histamine was also observed under inhibition of cyclooxygenase by ASA, suggesting that PG production is not the sole mechanism for this effect.

The threshold concentration of histamine necessary to sensitize the heat response is thus much higher (10,000 ×) than that for bradykinin, and 10 × higher than that for PGE_2 (see below). A concentration slightly less than 10 μM has been reported in pouch fluid inflamed with carrageenin,[16] and in vivo microdialysis revealed that probe insertion induced release of about 40 nM histamine.[17] Close apposition of nerve fibers and mast cells containing histamine[18,19] suggests the occurrence of a locally high concentration of histamine. Thus, sensitization of the heat response in testicular afferents may occur naturally under inflammatory or tissue damaging conditions.

The histamine (100 μM)-induced excitation of the slow-CV group was significantly suppressed by a H1 receptor antagonist (D-chlorphenilamine maleate) but not by other antagonists (famotidine for H2 and thioperamide maleate for H3 receptor).[51] The facilitatory effect of histamine on the heat response was also suppressed by the H1 receptor antagonist in both slow- and fast-CV groups.[51] These results strongly suggest that both excitation and facilitation of the heat response induced by histamine are mediated through the H1 receptor.

Involvement of the H1 receptor in histamine-induced excitation of cutaneous nociceptors was previously noted by Lynn[54] and the present observations suggest that visceral nociceptors share a common histamine receptor subtype with cutaneous afferents mediating itch.[55] Expression of H1 receptor mRNA in primary sensory neurons has been demonstrated.[56]

4) Prostaglandins − excitation and facilitatory effects on the heat and bradykinin responses

Prostaglandin (PG) E_2 up to 0.1 μM never induced excitation in testicular polymodal receptors,[37] and up to 10 μM induced only a quite small increase in discharge rate, if any, when applied in naive preparations. However, clear excitation was sometimes induced by PGE_2 at 0.1 μM when applied shortly after bradykinin application. This excitation is considered to be a facilitated response to residual bradykinin.[37]

A weak excitatory effect of PGI_2 was observed in about one half of the multi-fiber recordings at 0.1 μM where PGE_2 never induced excitation. Thus, PGI_2 is apparently more potent than PGE_2 in its excitatory effect.[57]

Excitation by PGs also was lacking in cutaneous[34] and muscular afferents,[58] and the absence of a pain-inducing action by PGE_2 by itself is suggested by behavioral and reflex studies.[59] In contrast to these results, clear excitatory effects were reported in cat knee joint afferents with PGE_2[60] and rats with PGI_2,[61] but this might be a consequence of both the surgical procedures and the higher concentration used in these experiments.

A sensitizing effect of PGE_2 on the bradykinin response was observed from 0.01 μM, a concentration at which PGE_2 by itself never induced excitation in naive preparations, with both cumulative and simultaneous application methods.[37,57] The magnitude of sensitization increased by application of a higher concentration of PGE_2. A similar sensitizing effect was observed with PGI_2,[57] but it was not confirmed by the use of the more selective IP receptor agonist, cicaprost.

The sensitizing effects of PGE_2 on the bradykinin response are reported for cutaneous afferents,[62] muscular group IV afferents[58] and joint afferents.[60] It is puzzling that a significant sensitizing effect of PGE_2 on the bradykinin response of nociceptors is not observed in rat skin-nerve preparations in vitro,[34] although hyperalgesic effects of PGE_2 in the skin have been reported in humans.[63,64]

A sensitizing effect on the heat responses was observed only from 100 × higher concentration than needed to sensitize the bradykinin response.[65] It was confirmed that 1 μM PGI_2 also sensitized the heat responses of polymodal receptors. The magnitude of sensitization by 1 μM PGI_2 was larger than that by 1 μM PGE_2 (1.5 impulses/s with PGI_2 whereas 0.5 impulses/s with PGE_2).

A similar sensitizing effect of PGE_2 on the heat response was observed in cat cutaneous nociceptors;[66] the exact concentration needed is not clear because of intra-arterial infusion (0.5-5 μg/min).

The concentration of PGE_2 in inflamed tissue is reported to be on the order of 0.01 μM in exudate and 0.1 μM in abscess.[11,13,20,21] The reported values of PG are high enough to sensitize, at least, the bradykinin response.

For E series prostaglandins, three receptor subtypes are distinguished, EP1, EP2 and EP3, by pharmacological methods[67] and they are confirmed by cDNA cloning.[68-70] An EP4 subtype was later found.[71] For clarification of receptor subtypes involved, the sensitizing effects of agonists specific for the 3 receptor subtypes were studied. The bradykinin response was significantly facilitated by the EP3 receptor agonist M&B28767 ≥ 10 nM (Fig. 3), but neither by the EP1 receptor agonist 17-phenyl trinor PGE_2 nor the EP2 receptor agonist butaprost.[72,73] The concentration-response curve for PGE_2 was not shifted to the right by the EP1 antagonist, AH6809.[72] In contrast, the heat response was facilitated by all of these agonists, but butaprost, which was totally ineffective for the bradykinin response, was the most potent among these three (Fig. 3).[73,74] Although the effects of antagonists could not be studied because of a lack of specific receptor antagonists for EP2 and EP3, it was concluded from these results that sensitization of the bradykinin response is mediated by the EP3 receptor subtype whereas the heat response is sensitized through activation of the EP2 receptor subtype.

5) Effects of tissue acidosis on nociceptor activities

Proton action on afferents were mainly studied in rat skin-nerve preparation and cultured dorsal root ganglion neurons.

Thirty-eight % of polymodal receptors recorded from rat skin-nerve preparations in vitro were excited by lowering pH, with a threshold between 6.9 and 6.1, and the maximum response was observed at pH 5.2. No tachyphylaxis was observed. When low pH solution was applied with a mixture of inflammatory mediators (5-HT, PG, histamine and bradykinin), the proportion of polymodal receptors excited and the magnitude of pH response increased,[75] suggesting either a synergistic or sensitizing role of inflammatory mediators.

Mechanical sensitivity of polymodal receptors was lowered after a long application of low pH solution, and this sensitizing effect of pH was observed irrespective of whether a fiber was excited by protons,[76] a similar observation to those for histamine and PGs in testicular polymodal receptors.

Sensitization of mechanical response by low pH is worth noting because sensitization of cutaneous nociceptors has been somewhat puzzling: in human cutaneous polymodal receptors mechanical sensitivity cannot be sensitized by bradykinin although its heat sensitivity can be sensitized,[28] and sensitization of mechanical response of joint nociceptors by inflammatory mediators has been well documented.[40,60] Mechanical thresholds of the polymodal receptor in the rat skin-nerve preparations remained unchanged by applications of inflammatory mediators.[34] In addition, only a small subpopulation of specialized nociceptors, the high-threshold mechanoreceptive A-delta-fibers, in the rat have thus far been shown to lower their threshold to mechanical stimuli (with von Frey hair) in response to cutaneous injury.[77] Now, local acidosis is reported to effectively lower the mechanical thresholds of a majority of cutaneous nociceptors.

Proton has been considered to be an endogenous ligand of the receptor for capsaicin, which specifically excites thin fiber afferents, and induce conduction block or degeneration of thin fibers when applied in high concentrations.[78] Because capsaicin-sensitive populations of small DRG neurons,[79] which are thought to be cell bodies of thin-fiber afferents, and those of cutaneous polymodal receptors,[76] do not completely overlap with pH-sensitive populations, and

a capsaicin antagonist, capsazepine, blocks DRG neuron response to capsaicin but not to protons,[80] many researchers now throw doubt on this hypothesis.

6) Role of nerve growth factor (NGF) in sensitization of nociceptors

Nerve growth factor has been known as a survival factor for both sympathetic and sensory neurons during development. The number of neurons that need NGF for survival gets smaller as development proceeds. Despite this change in dependence on NGF for survival, many small diameter sensory neurons in the adult have high-affinity NGF receptors (trk-A receptors) and retrogradely transport NGF from their target tissues. This suggests that NGF has a physiological role in the adult that is distinct from that in development. It has been revealed that animals subjected to pre- or postnatal anti-NGF treatments appear to suffer from a deceased sensitivity to noxious mechanical and heat stimuli. This leads to a hypothesis that NGF play roles in pain modulation.

Recently, increased production of NGF has been reported in actual and experimental inflammatory conditions.[81-83] Skin keratinocytes appear to be major sources of NGF in vivo.

Adult NGF treatment induced mechanical hyperalgesia within 24 hours and heat hyperalgesia within one hour. Because the mechanical threshold of peripheral nociceptors did not change, mechanical hyperalgesia may be caused by central changes. In contrast, heat hyperalgesia is considered to be of peripheral origin.[84,85] Since NGF stimulates mast cells to induce degranulation,[85] substances released from mast cells are considered to be responsible for sensitization. Later experiments have shown that heat hyperalgesia, but not mechanical hyperalgesia, induced by NGF is blocked by B1 antagonist, but not by B2 antagonist.[48] Because bradykinin response is mediated through the B2 receptor in nociceptors in normal tissues,[45,50] B1 receptors are considered to be produced de novo after NGF treatment. In the work from the author's lab, NGF is seen to increase the percentage of DRG neurons responding to bradykinin, but this response is mediated through B2 receptors.[86] In other experiments, no appearance of B1 receptor on afferent neurons and afferent fibers was confirmed.[49] From these observations, it is concluded that B1 receptors appear on cells other than afferents, and mediators released from these cells may sensitize the nociceptor terminals.

7) Does suppression of action of inflammatory mediators alleviate nociceptor sensitization in injury?

In model burns (heat stimulation at 55°C for 30 s), neither B2 nor B1 antagonist suppressed on-going activities appearing after this strong heat stimulation (unpublished observation from the author's lab). Rather, they induced or facilitated on-going activities. The B1 agonist des-Arg^9-BK sometimes induced excitation after 55°C stimulation. Furthermore, the sensitized heat response was not suppressed by these antagonists. For the moment, the involvement of bradykinin in heat-induced sensitization requires further study.

In kininogen deficient humans, pain and hyperalgesia are also induced after burn injury,[87] suggesting that bradykinin is not essential in inducing pain and hyperalgesia after a burn. Similarly, King et al.[88] failed to block heat-induced sensitization of the heat response of polymodal nociceptors of rabbits by a bradykinin-degrading enzyme (carboxypeptidase B).

In other inflammatory conditions such as those induced by ultra-violet irradiation, Freund's complete adjuvant, or NGF, sensitized heat response was suppressed by a B1 antagonist des-Arg^9-[Leu^8]-BK but not by a B2 antagonist HOE140.[47,48,89]

Involvement of PGs in inflammatory pain has long been established, as seen in the facts that analgesic action of non-steroidal anti-inflammatory drugs is explained mainly based on their cyclooxygenase inhibiting action. Their involvement in heat-induced nociceptor sensitization was

not clear, thus it was evaluated using acetylsalicylic acid (ASA).[90] The heat and bradykinin responses after 55°C stimulation in the presence of ASA were greater than before 55°C stimulation, but smaller than in its absence. In addition, the duration of the sensitized state for the heat and bradykinin responses was also shorter in the presence of ASA. The incidence of spontaneous discharges after 55°C stimulation was significantly lower in the presence of ASA than in its absence. These results lead to the conclusion that PGs play some role in the heat-induced sensitization of both the heat and bradykinin responses. Whether receptor subtypes for PGs change in inflammatory condition as in the case of bradykinin, is not known because of the lack of type-specific antagonists.

It must be additionally mentioned that in inflamed tissue, a new type of prostaglandin synthesizing enzyme, cyclooxygenase-2 (COX-2), is induced.[91] This enzyme is a little different from the constitutive type of cyclooxygenase, COX-1, suggesting the possibility of blocking excessive PG production in inflamed tissue without interfering with PG production necessary for maintaining normal cell function.[92-95]

Histamine might also be involved in the heat-induced sensitization of nociceptors, because suppression of the heat-induced sensitization could be obtained only by the application of a combination of agents that would inhibit the production of PGs, would degrade bradykinin, and would antagonize the effects of serotonin and histamine.[88] The involvement of histamine in sensitization in other inflammation models has not been evaluated yet.

Anti-NGF might also alleviate nociceptor sensitization, but a need for a large amount of antibody to NGF has prevented this kind of experiment. An antagonist against NGF receptor (trk A) would facilitate the experiment.

RECRUITMENT OF "SILENT (SLEEPING)" NOCICEPTORS IN INFLAMED TISSUES

It is long known there are many C-afferent fibers in cutaneous nerves. In human peroneal nerves, for example, only 12% are sympathetic units, while 45% are C mechano-heat receptors, 13% are C-mechanoreceptors, 6% are C-heat nociceptors, and the remaining 24% are mechano-insensitive-heat insensitive units (CMiHi). One third of these CMiHi units were excited by topically applied mustard oil. After application of mustard oil/capsaicin, C-mechanoreceptors, C-heat nociceptors or CMiHi units were sensitized to heating and/or to mechanical stimuli.[96] These CMiHi units are named "silent" or "sleeping" nociceptors. In the cat knee joint, induction of inflammation by kaolin and carrageenin also excites previously mechanically insensitive C-fibers and enhances their responses to mechanical manipulation of the corresponding tissue.[97] Recruitment of these "sleeping (silent) nociceptors" in inflammatory conditions will increase the barrage of afferent impulses arriving at the spinal cord, thus facilitating the responses of nociceptive neurons in the spinal cord as well as in the upper level nociceptive pathways. However, the existence of "silent nociceptors" has not been confirmed in all afferent systems (negative examples: canine testicular afferents (unpublished observation from our lab), rat gall bladder afferents (personal communication)). It is not known what awakens these "sleeping" nociceptors.

INTRACELLULAR MECHANISM OF SENSITIZATION

1) Increasing intracellular cAMP facilitates the heat response but suppresses the bradykinin response

Among the receptors of inflammatory mediators mentioned above, the PGE receptor EP2

subtype and the IP receptor for PGI_2 are known to stimulate adenylyl cyclase and to increase intracellular cyclic AMP, whereas some isoforms of the EP3 subtype are reported to suppress cAMP production.[67] As described above, PGE_2, through the EP2 receptor, and PGI_2 facilitate the heat response, whereas PGE_2, through activation of the EP3 receptor, facilitates the bradykinin response. Thus, it can be expected that the heat response and the bradykinin response are modified differently by activation of adenylyl cyclase.

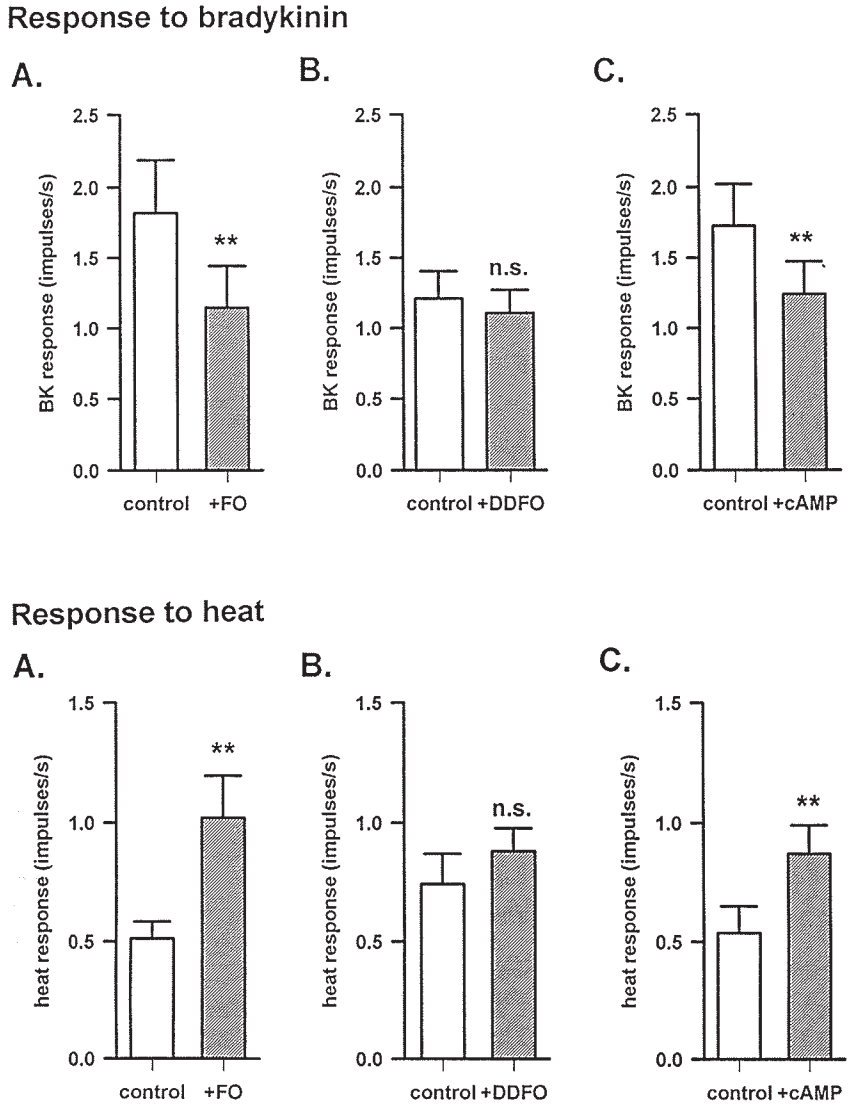

Fig. 4 Increasing cAMP facilitates the heat response but suppresses the bradykinin response of testicular polymodal receptors.
Upper row: response to bradykinin; lower row: response to heat. FO: forskolin 10 μM, DDFO: 1,9 dideoxy-forskolin (inactive analog of forskolin) 10 μM, cAMP: mixture of dibutyryl cAMP (20-100 μM) and 3-isobutyl-1-methyl xanthine (20-100 μM). **: $p < 0.01$ compared with the control response (paired t test). n.s.: not significant.

Stimulation of adenylyl cyclase by forskolin (5-10 μM) seldom induced excitation in polymodal receptors. A mixture of dibutyryl cAMP (a membrane permeable analog of cAMP, abbrev. dBcAMP, 20-100 μM) and isobutyl-methyl xanthine (inhibitor of phosphodiesterase, abbrev. IBMX, 20-100 μM) did not induce excitation.[98]

Forskolin reversibly facilitated the heat response.[99] Similarly, a mixture of dBcAMP and IBMX facilitated the heat response, but the inactive analog of forskolin, 1,9 dideoxy-forskolin (DDFO, 10 μM) did not (Fig. 4).[98]

In three units, prostaglandin-induced sensitization of the heat response was studied in the presence and absence of an adenylyl cyclase inhibitor, 2,5-dideoxyadenosine (DDA, 200 μM for 10 min). The magnitude of sensitization was smaller in the presence of DDA in all three units, suggesting that PG-induced sensitization of the heat response is mediated by activation of adenylyl cyclase.

In contrast, the bradykinin response was suppressed by forskolin[100] or by a mixture of dBcAMP and IBMX[98] (Fig. 4). This difference from the heat response was not based on the units used for the bradykinin response and for the heat response, because both effects were observed in the same single fiber. In close agreement with our results, Kress et al. also reported that cAMP analog facilitates the heat response of rat cutaneous nociceptors, but not the bradykinin response using rat skin-nerve preparation in vitro.[101]

In contrast to our results and those obtained in the rat skin-nerve preparations, however, several studies have shown the facilitatory effects of cAMP on bradykinin responses.[102,103] The common factor in these studies is that the neurons used were in early development stages, and in culture.[103] In cultured DRG neurons, sensitivity to bradykinin is known to be quite variable.[104-106] The intracellular machinery that intervenes between the bradykinin receptor and ion channels may possibly be different between adult neurons in vivo and those in developing neurons, or neurons maintained in culture. Alternatively, the effects of cAMP might vary depending on cellular activities which are yet unknown.

In order to explain the opposite effects of cAMP on the bradykinin and heat responses, we might consider sensitizing mechanisms for heat and bradykinin responses that could be differentially influenced by cAMP. As noted above, the bradykinin response of testicular polymodal receptors is mediated through the B2 receptor which activates phospholipase C to increase intracellular diacylglycerol (DAG).[107] Bradykinin may by this route activate protein kinase C (PKC), an enzyme thought to be involved in excitatory responses.[108,109] Some reports have demonstrated inhibitory interactions between A kinase and C kinase.[110-112] If such interactions also exist in the polymodal receptor terminals, then elevating cAMP leading to A-kinase activation might in turn inhibit bradykinin responses that depend on C-kinase activity.

On the other hand, the opposite effects of cAMP on these two responses fits well with our observation that the PGE receptor subtype involved in the sensitization of the heat response is the EP2 subtype which stimulates adenylyl cyclase and that involved in the bradykinin response is the EP3 subtype which suppresses adenylyl cyclase. Suppression of the bradykinin response by an increase in cAMP is apparently contradictory to the long-standing hypothesis that the hyperalgesic effect of PGE_2 is mediated by an increase in cAMP,[113,114] but that hypothesis is based on experiments using mechanical stimulation. The EP receptor subtype involved in the sensitization of the mechanical response remains unknown. This response may be mediated through the same subtype as the heat response.

2) Effects of phorbol esters which activate protein kinase C (PKC)

There is considerable evidence that activation of phospholipase C is involved in the responses to bradykinin through B2 receptors[107] and to histamine through H1 receptors,[115] and that

activation of phospholipase C results in increased intracellular concentrations of inositol trisphosphate and DAG. The latter is known to activate PKC,[116] which is found in high concentrations in the nervous system. In neuroblastoma-glioma hybrid cells (NG-108 cells) activation of PKC by DAG has been shown to mimic the inward current induced by bradykinin.[108] It has also been suggested that activation of PKC is involved in the effect of bradykinin on primary afferents.[117] We therefore studied effects of phorbol esters, which stimulate PKC, on polymodal receptor activities.

Phorbol 12,13-dibutyrate (PDBu), a phorbol ester, induced excitation in polymodal receptors after a long latency (longer than 5 min at 1 μM) and lasted long after rinsing out the PDBu.[118] The magnitude of excitation was, however, much smaller than those induced by histamine or bradykinin, although the discharge rate significantly increased at ≥ 0.1 μM.

PDBu ≥ 0.01 μM also facilitated the heat response. Facilitation by PDBu at 0.01 μM disappeared in 5 min, but facilitation induced by PDBu > 0.1 μM lasted longer than 25 min.[118] The magnitude of facilitation by PDBu at 1 μM was comparable to that induced by bradykinin or histamine. A similar facilitatory effect was also induced by an another phorbol ester, phorbol 12-myristate 13-acetate (PMA). This facilitatory effect was not induced by an inactive analog of phorbol ester, 4-α phorbol 12,13-didecanoate (0.1 μM). Since this facilitatory effect of PDBu at 0.1 μM was not observed in the presence of staurosporine (1 μM, 13 min),[118] a protein kinase inhibitor, it was confirmed that this facilitatory effect was induced through activation of PKC.

The study clearly showed that the concentration of PDBu needed to induce a significant augmenting effect on the heat response of polymodal receptors was ≥ 0.01 μM, whereas a ten times higher concentration (≥0.1 μM) was necessary to excite them. Higher concentrations of

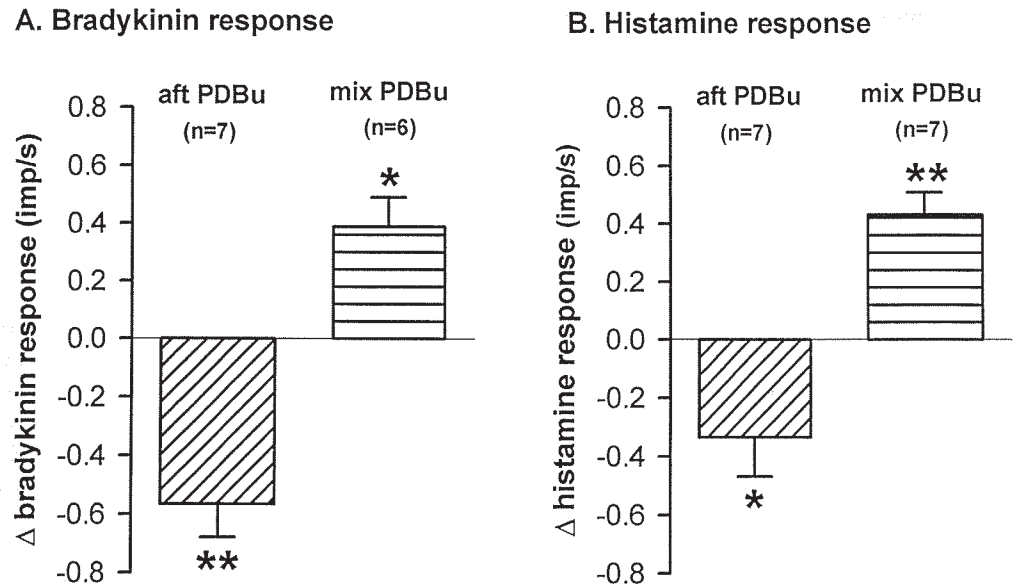

Fig. 5 Phorbol ester has dual effects on the bradykinin as well as histamine responses, depending on application period.
Ordinate: change induced by phorbol ester in the bradykinin (A) or histamine (B) response. aft PDBu: PDBu 0.1 μM was applied for 5 min prior to bradykinin application; mix PDBu: PDBu 0.1 μM was applied simultaneously with bradykinin. *: $p<0.05$; **: $p<0.01$ compared with the control bradykinin (or histamine) response (paired t test).

bradykinin and PGE_2 were also required to excite polymodal receptors than to facilitate their heat responses.[35,65]

The effects of phorbol esters, both excitatory and facilitatory, were observed at a higher concentration (1 μM) in other sensory nerve preparations.[119-121] Possible reasons for this concentration difference may be due to differences in the species used and in the routes by which PDBu was applied.

When PDBu was applied previous to bradykinin application, as used for the heat response, the bradykinin response was suppressed[122] (Fig. 5). When PDBu was applied simultaneously with bradykinin, clear facilitation of the bradykinin response was observed[122] (Fig. 5). These results suggest that two mechanisms, facilitated and inhibited by PKC, are involved in the bradykinin response of polymodal receptors. An inactive analog of phorbol ester, 4-α phorbol 12,13-didecanoate (0.1 μM), induced no significant effect on the bradykinin response, confirming that both facilitatory and suppressive effects of PDBu are mediated through activation of PKC.

In order to demonstrate whether PKC activation is implicated in the bradykinin response in testicular afferents as in cultured cells, the effects of bradykinin were studied in the presence of staurosporine. For this study, more than a 1-hour interval elapsed between bradykinin applications to prevent tachyphylaxis. Bradykinin-induced excitation was suppressed by staurosporine, and sensitization of the heat response was also suppressed.

Quite similar to the bradykinin response, the response to histamine was either facilitated or suppressed by PDBu, depending on the application period (Fig. 5, unpublished observation from author's lab). An absence of the effects of 4-α phorbol 12,13-didecanoate (0.1 μM) on the histamine response confirmed involvement of activation of PKC in PDBu effects.

These similarities in the effects of phorbol ester suggest that some intracellular processes are common between the bradykinin and histamine actions on the nociceptors.

Application of phorbol ester for longer than several hours is known to desensitize PKC,[123,124] and desensitization of phospholipase C in 5 min by phorbol ester through PKC activation[125] has also been reported. These mechanisms may be implicated in the suppression of the bradykinin response by PDBu pretreatment.

CONCLUSIONS

In this review, the sensitizing effects of bradykinin, histamine, PGs and protons were demonstrated, and possible involvement of these substances in inflammation-induced hyperalgesia was discussed. It was also noted that the receptor subtypes for bradykinin and prostaglandin synthesizing enzyme, COX, differ in inflamed conditions, indicating the possibility of pain being differentially modulated between normal and inflamed tissues. In this review, the interaction of inflammatory mediators was not discussed. Such interaction may occur, because many substances are released into the tissue at the same time, and some of them share a common intracellular pathway as described in the chapter of the intracellular mechanism. This issue remains open for future study.

REFERENCES

1) Merskey, H. and Bogduk, N.: Classification of Chronic Pain, (1994) IASP press, Seattle.
2) Lewis, T.: Pain (1942), MacMillan, New York.
3) Hardy, D., Wolff, H.G. and Goodell, H.: Experimental evidence on the nature of cutaneous hyperalgesia. *J. Clin. Invest.*, 29, 115-40 (1950).

4) Lamotte, R.H., Shain, C.N., Simone, D.A. and Tsai, E.F.P.: Neurogenic Hyperalgesia — Psychophysical Studies of Underlying Mechanisms. *J. Neurophysiol.*, 66, 190-211 (1991).
5) Torebjörk, H.E., Lundberg, L.E.R. and Lamotte, R.H.: Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. *J. Physiol. (Lond.)*, 448, 765-80 (1992).
6) Cervero, F. and Laird, J.M.A.: Mechanisms of allodynia: Interactions between sensitive mechanoreceptors and nociceptors. *Neuroreport*, 7, 526-8 (1996).
7) Schaible, H.-G.: On the role of tachykinins and calcitonin gene-related peptide in the spinal mechanisms of nociception and in the induction and maintenance of inflammation-evoked hyperexcitability in spinal cord neurons (with special reference to nociception in joints). In The Polymodal Receptor — A Gateway to Pathological Pain, edited by Kumazawa, T., Kruger, L., and Mizumura, K., pp.423-41 (1996), Elsevier, Amsterdam.
8) Yoshimura, M.: Slow synaptic transmission in the spinal dorsal horn. In The Polymodal Receptor — A Gateway to Pathological Pain, edited by Kumazawa, T., Kruger, L., and Mizumura, K., pp.443-62 (1996), Elsevier, Amsterdam.
9) Randic, M.: Plasticity of excitatory synaptic transmission in the spinal cord dorsal horn. In The Polymodal Receptor — A Gateway to Pathological Pain, edited by Kumazawa, T., Kruger, L., and Mizumura, K., pp.463-506 (1996), Elsevier, Amsterdam.
10) Woolf, C.J. and Doubell, T.P.: The pathophysiology of chronic pain — increased sensitivity to low threshold A beta-fibre inputs. *Current Opinion in Neurobiology.*, 4, 525-34 (1994).
11) Barbieri, E.J., Orzechowski, R.F. and Rossi, G.V.: Measurement of prostaglandin E_2 in an inflammatory exudate: effects of nonsteroidal anti-inflammatory agents. *J. Pharmacol. Exp. Ther.*, 201, 769-77 (1977).
12) Fazekas, I.G., Kosa, F., Viragos-Kis, E. and Basch, A.: Free and total histamine content of the skin in experimental burn injuries. *Z. Rechtmed.*, 72, 203 (1973) [Abstract].
13) Greaves, M.W., Songergaard, J. and McDonald-Gibson, W.: Recovery of prostaglandins in human cutaneous inflammation. *Br. Med. J.*, 2, 258-60 (1971).
14) Kumakura, S., Kamo, I. and Tsurufuji, S.: Role of bradykinin in the vascular permeability response induced by carrageenin in rats. *Br. J. Pharmacol.*, 93, 739-46 (1988).
15) Swift, J.O., Alton, T. and Hargreaves, K.M.: Prostaglandin E2 immunoreactivity is released during inflammation of the rabbit TMJ. *Society for Neuroscience Abstracts*, 18, 690 (1992) [Abstract].
16) Willis, A.L.: Release of histamine, kinin and prostaglandins during carrageenin-induced inflammation in the rat. In Prostaglandins, Peptides and Amines, edited by Monterazza, P. and Horton, E.W., pp.31-8 (1969), Academic, London.
17) Anderson, C., Andersson, T. and Andersson, R.G.G.: In vivo microdialysis estimation of histamine in human skin. *Skin Pharmacol.*, 5, 177-83 (1992).
18) Keith, I.M., Jin, J. and Saban, R.: Nerve mast cell interaction in normal guinea pig urinary bladder. *J. Comp. Neurol.*, 363, 28-36 (1995).
19) Kruger, L., Silverman, J.D., Mantyh, P.W., Sternini, C. and Brecha, N.C.: Peripheral patterns of calcitonin gene-related peptide general somatic sensory innervation: cutaneous and deep terminations. *J. Comp. Neurol.*, 280, 291-302 (1989).
20) Higgs, G.A., Moncada, S., Salmon, J.A. and Seager, K.: The source of thromboxane and prostaglandins in experimental inflammation. *Br. J. Pharmacol.*, 79, 863-8 (1983).
21) Ohuchi, K., Sato, H. and Tsurufuji, S.: The content of prostaglandin E and prostaglandin F_{2a} in the exudate of carrageenin granuloma of rats. *Biochim. Biophys. Acta*, 424, 439-48 (1976).
22) Häbler, C.: Über den K- und Ca-Gehalt von Eiter und Exsudaten und seine Beziehungen zum Entündungsschmerz. *Klin. Wochenschr.*, 8, 1569-72 (1929).
23) McCarty, D.J., Phelps, P. and Pyenson, J.: Crystal-induced inflammation in canine joints-I. An experimental model with quantification of the host response. *J. Exp. Med.*, 124, 99-114 (1966).
24) Issberner, U., Reeh, P.W., Kreysel, H.W. and Steen, A.E.: Correlation between proton concentration and pain in leg ulcers. *Abstracts: 8th World Congress on Pain*, 123 (1996) [Abstract].
25) Jensen, K., Tuxen, C., Pedersen-Bjergaard, U., Jansen, I., Edvinsson, L. and Olesen, J.: Pain, wheal and flare in human forearm skin induced by bradykinin and 5-hydroxytryptamine. *Peptides*, 11, 1133-8 (1990).
26) Ferreira, S.H., Nakamura, M. and Castro, M.S.A.: The hyperalgesic effects of prostacyclin and prostaglandin E_2. *Prostaglandins*, 16, 31-7 (1978).
27) Keele, C.A. and Armstrong, D.: Substances Producing Pain and Itch, (1964) Arnold, London.
28) Manning, D.C., Raja, S.N., Meyer, R.A. and Campbell, J.N.: Pain and hyperalgesia after intradermal injection of bradykinin in humans. *Clin. Pharmacol. Ther.*, 50, 721-9 (1992).
29) Hardy, J.D., Wolff, H.G. and Goodell, H.: The nature of cutaneous hyperalgesia. In Pain Sensations and Reactions, edited by Hardy, J.D., Wolff, H.G., and Goodell, H., pp.173-215 (1952), Williams & Wilkins, Baltimore.

30) Rosenthal, S.R. and Minard, D.: Experiments on histamine as the chemical mediator for cutaneous pain. *J. Exp. Med.*, 70, 415-25 (1939).
31) Kumazawa, T., Mizumura, K. and Sato, J.: Response properties of polymodal receptors studied using in vitro testis superior spermatic nerve preparations of dogs. *J. Neurophysiol.*, 57, 702-11 (1987).
32) Fock, S. and Mense, S.: Excitatory effects of 5-hydroxytryptamine, histamine and potassium ions on muscular group IV afferent units: a comparison with bradykinin. *Brain Res.*, 105, 459-69 (1976).
33) Messlinger, K., Pawlak, M., Schepelmann, K. and Schmidt, R.F.: Responsiveness of slowly conducting articular afferents to bradykinin: effects of an experimental arthritis. *Pain*, 59, 335-43 (1994).
34) Lang, E., Novak, A., Reeh, P.W. and Handwerker, H.O.: Chemosensitivity of fine afferents from rat skin in vitro. *J. Neurophysiol.*, 63, 887-901 (1990).
35) Kumazawa, T., Mizumura, K., Minagawa, M. and Tsujii, Y.: Sensitizing effects of bradykinin on the heat responses of the visceral nociceptor. *J. Neurophysiol.*, 66, 1819-24 (1991).
36) Kumazawa, T., Mizumura, K. and Sato, J.: Thermally potentiated responses to algesic substances of visceral nociceptors. *Pain*, 28, 255-64 (1987).
37) Mizumura, K., Sato, J. and Kumazawa, T.: Effects of prostaglandins and other putative chemical intermediaries on the activity of canine testicular polymodal receptors studied in vitro. *Pflügers Arch.*, 408, 565-72 (1987).
38) Handwerker, H.O.: Pharmacological modulation of the discharge of nociceptive C-fibres. In Sensory Functions of the Skin in Primates with Special Reference to Man (Wenner-Green Center International Symposium, vol. 27), edited by Zotterman, Y. pp.427-39 (1976), Pergamon, Oxford.
39) Koltzenburg, M., Kress, M. and Reeh, P.W.: The nociceptor sensitization by bradykinin does not depend on sympathetic neurons. *Neuroscience*, 46, 465-73 (1992).
40) Neugebauer, V., Schaible, H.-G. and Schmidt, R.F.: Sensitization of articular afferents to mechanical stimuli by bradykinin. *Pflügers Arch.*, 415, 330-5 (1989).
41) Regoli, D., Barabe, J. and Park, W.K.: Receptors for bradykinin in rabbit aortae. *Can. J. Physiol. Pharmacol.*, 55, 855-67 (1977).
42) Hess, J.F., Borkowski, J.A., Young, G.S., Strader, C.D. and Ransom, R.W.: Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem. Biophys. Res. Commun.*, 184, 260-8 (1992).
43) Yokoyama, S., Kimura, Y., Taketo, M., Black, J.A., Ransom, B.R. and Higashida, H.: B-2 bradykinin receptors in NG108-15 cells – cDNA cloning and functional expression. *Biochem. Biophys. Res. Commun.*, 200, 634-41 (1994).
44) Menke, J.G., Borkowski, J.A., Bierilo, K.K., Macneil, T., Derrick, A.W., Schneck, K.A., Ransom, R.W., Strader, C.D., Linemeyer, D.L. and Hess, J.F.: Expression cloning of a human B-1 bradykinin receptor. *J. Biol. Chem.*, 269, 21583-6 (1994).
45) Mizumura, K., Minagawa, M., Tsujii, Y. and Kumazawa, T.: The effects of bradykinin agonists and antagonists on visceral polymodal receptor activities. *Pain*, 40, 221-7 (1990).
46) Regoli, D., Marceau, F. and Barabe, J.: *De novo* formation of vascular receptors for bradykinin. *Can. J. Physiol. Pharmacol.*, 56, 674-7 (1978).
47) Perkins, M.N. and Kelly, D.: Induction of bradykinin-B_1 receptors in vivo in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat. *Br. J. Pharmacol.*, 110, 1441-4 (1993).
48) Rueff, A., Dawson, A.J.L.R. and Mendell, L.M.: Characteristics of nerve growth factor induced hyperalgesia in adult rats: dependence on enhanced bradykinin-1 receptor activity but not neurokinin-1 receptor activation. *Pain*, 66, 359-72 (1996).
49) Davis, C.L., Naeem, S., Phagoo, S.B., Campbell, E.A., Urban, L. and Burgess, G.M.: B-1 bradykinin receptors and sensory neurones. *Br. J. Pharmacol.*, 118, 1469-76 (1996).
50) Whalley, E.T., Clegg, S., Stewart, J.M. and Vavrek, R.J.: The effect of kinin agonists and antagonists on the pain response of the human blister base. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 336, 652-5 (1987).
51) Koda, H., Minagawa, M., Leng Si-Hong, Mizumura, K. and Kumazawa, T.: H1 receptor mediated excitation and facilitation of the heat response by histamine in canine visceral polymodal receptors studied in vitro. *J. Neurophysiol.*, 76, 1396-404 (1996).
52) Mizumura, K., Minagawa, M., Koda, H. and Kumazawa, T.: Histamine-induced sensitization of the heat response of canine visceral polymodal receptors. *Neurosci. Lett.*, 168, 93-6 (1994).
53) Juan, H. and Sametz, W.: Histamine-induced release of arachidonic acid and of prostaglandins in the peripheral vascular bed. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 314, 183-90 (1980).
54) Lynn, B.: Structure, function and control: Afferent nerve endings in the skin. In Handbook of Experimental Pharmacology, edited by Greaves, M.W. and Schuster, S., pp.175-92. (1989), Springer-Verlag, Berlin.

55) Douglas, W.W.: Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, edited by Goodman, A.G., Goodman, L.S., Rall, T.W., and Murad, F., 7th, pp.605-38 (1985), Macmillan, New York/Tronto/London.
56) Senba, E. and Kashiba, H.: Sensory afferent processing in multi-responsive DRG neurons. In The Polymodal Receptor — A Gateway to Pathological Pain, edited by Kumazawa, T., Kruger, L., and Mizumura, K., pp.387-410 (1996), Elsevier, Amsterdam.
57) Mizumura, K., Sato, J. and Kumazawa, T.: Comparison of the effects of prostaglandins E_2 and I_2 on testicular nociceptor activities studied in vitro. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 344, 368-76 (1991).
58) Mense, S.: Sensitization of group IV muscle receptors to bradykinin by 5-hydroxytryptamine and prostaglandin E_2. *Brain Res.*, 225, 95-105 (1981).
59) Raja, S.N., Meyer, R.A. and Campbell, J.N.: Peripheral mechanisms of somatic pain. *Anesthesiology*, 68, 571-90 (1996).
60) Schaible, H.-G. and Schmidt, R.F.: Excitation and sensitization of fine articular afferents from cat's knee joint by prostaglandin E_2. *J. Physiol. (Lond.)*, 403, 91-104 (1988).
61) Birrell, G.J., Mcqueen, D.S., Iggo, A., Coleman, R.A. and Grubb, B.D.: PGI_2-induced activation and sensitization of articular mechanonociceptors. *Neurosci. Lett.*, 124, 5-8 (1991).
62) Chahl, L.A. and Iggo, A.: The effects of bradykinin and prostaglandin E_1 on rat cutaneous afferent nerve activity. *Br. J. Pharmacol.*, 59, 343-7 (1977).
63) Ferreira, S.H.: Prostaglandins, aspirin-like durgs and analgesia. *Nature New Biology*, 240, 200-3 (1972).
64) Crunkhorn, P. and Willis, A.L.: Cutaneous reactions to intradermal prostaglandins. *Br. J. Pharmacol.*, 41, 49-56 (1971).
65) Mizumura, K., Minagawa, M., Tsujii, Y. and Kumazawa, T.: Prostaglandin E_2-induced sensitization of the heat response of canine visceral polymodal receptors in vitro. *Neurosci. Lett.*, 161, 117-9 (1993).
66) Handwerker, H.O.: Influence of prostaglandin E_2 on the discharge of cutaneous nociceptive C-fibres induced by radiant heat. *Pflügers Arch.*, 355, R116 (1975) [Abstract].
67) Coleman, R.A., Smith, W.L. and Narumiya, S.: International Union of Pharmacology classification of prostanoid receptors: Properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.*, 46, 205-29 (1994).
68) Sugimoto, Y., Namba, T., Honda, A., Hayashi, Y., Negishi, M., Ichikawa, A. and Narumiya, S.: Cloning and expression of a cDNA for mouse prostaglandin E receptor EP_3 subtype. *J. Biol. Chem.*, 267, 6463-6 (1992).
69) Regan, J.W., Bailey, T.J., Pepperl, D.J., Pierce, K.L., Bogardus, A.M., Donello, J.E., Fairbairn, C.E., Kedzie, K.M., Woodward, D.F. and Gil, D.W.: Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP_2 subtype. *Mol. Pharmacol.*, 46, 213-20 (1994).
70) Watabe, A., Sugimoto, Y., Honda, A., Irie, A., Namba, T., Negishi, M., Ito, S., Narumiya, S. and Ichikawa, A.: Cloning and expression of cDNA for a mouse EP1 subtype of prostaglandin E receptor. *J. Biol. Chem.*, 268, 20175-8 (1993).
71) Coleman, R.A., Grix, S.P., Louttit, J.B., Mallett, A. and Sheldrick, R.L.G.: A novel inhibitory prostanoid receptor in piglet saphenous vein. *Prostaglandins*, 47, 151-68 (1994).
72) Kumazawa, T., Mizumura, K. and Koda, H.: Involvement of EP_3 subtype of prostaglandin E receptors in PG E_2-induced enhancement of the bradykinin response of nociceptors. *Brain Res.*, 632, 321-4 (1993).
73) Kumazawa, T., Mizumura, K., Koda, H. and Fukusako, H.: EP receptor subtypes implicated in the PGE_2-induced sensitization of polymodal receptors in responses to bradykinin and heat. *J. Neurophysiol.*, 75, 2361-8 (1996).
74) Kumazawa, T., Mizumura, K. and Koda, H.: Possible involvement of the EP_2 receptor subtype in PGE_2-induced enhancement of the heat response of nociceptors. *Neurosci. Lett.*, 175, 71-3 (1994).
75) Steen, K.H., Steen, A.E. and Reeh, P.W.: A dominant role of acid pH in inflammatory excitation and sensitization of nociceptors in rat skin, in vitro. *J. Neurosci.*, 15, 3982-9 (1995).
76) Steen, K.H., Reeh, P.W., Anton, F. and Handwerker, H.O.: Protons selectively induce lasting excitation and sensitization to mechanical stimulation of nociceptors in rat skin, invitro. *J. Neurosci.*, 12, 86-95 (1992).
77) Reeh, P.W., Kocher, J.B.L. and Handwerker, H.O.: Sensitization of nociceptive cutaneous nerve fibers from the rat's tail by noxious mechanical stimulation. *Exp. Brain Res.*, 65, 505-12 (1987).
78) Szolcsányi, J.: Capsaicin-sensitive sensory nerve terminals with local and systemic efferent functions: facts and scopes of an unorthodox neuroregulatory mechanism. In The Polymodal Receptor — A Gateway to Pathological Pain, edited by Kumazawa, T., Kruger, L., and Mizumura, K., pp.343-59 (1996), Elsevier, Amsterdam.
79) Kasai, M., Mizumura, K. and Kumazawa, T.: Characteristics of capsaicin-sensitive neurones freshly dissociated from rat dorsal root ganglion. *Environ. Med.*, 39, 45-8 (1995).

80) Kasai, M., Kumazawa, T. and Mizumura, K.: Different receptors are involved in the responses to capsaicin and proton cultured neurons from adult rat dorsal root ganglia. *Neurosci. Lett.*, (In Press).
81) Donnerer, J. and Stein, C.: Evidence for an increase in the release of CGRP from sensory nerves during inflammation. *Ann. N. Y. Acad. Sci.*, 657, 505-6 (1992).
82) Donnerer, J., Schuligoi, R., Stein, C. and Amann, R.: Upregulation, Release and Axonal Transport of Substance-P and Calcitonin Gene-Related Peptide in Adjuvant Inflammation and Regulatory Function of Nerve Growth Factor. *Regul. Pept.*, 46, 150-4 (1993).
83) Constantinou, J., Reynolds, M.L., Woolf, C.J., Safieh-Garabedian, B. and Fitzgerald, M.: Nerve growth factor levels in developing rat skin: upregulation following skin wounding. *Neuroreport.*, 5, 2281-4 (1994).
84) Lewin, G.R., Ritter, A.M. and Mendell, L.M.: Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. *J. Neurosci.*, 13, 2136-48 (1993).
85) Lewin, G.R. and Mendell, L.M.: Nerve growth factor and nociception. *Trends Neurosci.*, 16, 353-9 (1993).
86) Kasai, M., Kumazawa, T. and Mizumura, K.: Nerve growth factor increases neurons responding to bradykinin cultured from adult rat dorsal root ganglia. *Neurosci. Res.*, (submitted).
87) Raja, S.N., Campbell, J.N., Meyer, R.A. and Colman, R.W.: Role of kinins in pain and hyperalgesia: psychophysical studies in a patient with kininogen deficiency. *Clin. Sci.*, 83, 337-41 (1992).
88) King, J.S., Gallant, P., Myerson, V. and Perl, E.R.: The effects of anti-inflammatory agents on the responses and the sensitization of unmyelinated (C) fiber polymodal nociceptors. In Sensory Functions of the Skin in Primates with Special Reference to Man (Wenner-Gren Center International Symposium, vol. 27), edited by Zotterman, Y. pp.441-61 (1976), Pergamon, Oxford.
89) Perkins, M.N., Campbell, E. and Dray, A.: Antinociceptive activity of the bradykinin B_1 and B_2 receptor antagonists, des-Arg^9,[Leu^8]-BK and HOE 140, in two models of persistent hyperalgesia in the rat. *Pain*, 53, 191-7 (1993).
90) Mizumura, K., Sato, J., Minagawa, M. and Kumazawa, T.: Incomplete suppressive effect of acetylsalicylic acid on the heat sensitization of canine testicular polymodal receptor activities. *J. Neurophysiol.*, 72, 2729-36 (1994).
91) Ristimaki, A., Garfinkel, S., Wessendorf, J., Maciag, T. and Hla, T.: Induction of cyclooxygenase-2 by interleukin-1 alpha. Evidence for post-transcriptional regulation. *J. Biol. Chem.*, 269, 11769-75 (1994).
92) Kennedy, B.P., Chan, C.C., Culp, S.A. and Cromlish, W.A.: Cloning and expression of rat prostaglandin endoperoxide synthase (cyclooxygenase)-2 cDNA. *Biochem. Biophys. Res. Commun.*, 197, 494-500 (1993).
93) Meade, E.A., Smith, W.L. and Dewitt, D.L.: Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.*, 268, 6610-4 (1993).
94) Futaki, N., Takahashi, S., Yokoyama, M., Arai, I., Higuchi, S. and Otomo, S.: NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity invitro. *Prostaglandins*, 47, 55-9 (1994).
95) Anderson, G.D., Hauser, S.D., Mcgarity, K.L., Bremer, M.E., Isakson, P.C. and Gregory, S.A.: Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. *J. Clin. Invest.*, 97, 2672-9 (1996).
96) Schmidt, R., Schmelz, M., Forster, C., Ringkamp, M., Torebjork, E. and Handwerker, H.: Novel classes of responsive and unresponsive C nociceptors in human skin. *J. Neurosci.*, 15, 333-41 (1995).
97) Schaible, H.-G. and Schmidt, R.F.: Time course of mechanosensitivity changes in articular afferents during a developing experimental arthritis. *J. Neurophysiol.*, 60, 2180-95 (1988).
98) Mizumura, K., Koda, H. and Kumazawa, T.: Opposite effects of increased intracellular cyclic AMP on the heat and bradykinin responses of canine visceral polymodal receptors in vitro. *Neurosci. Res.*, 25, 335-41 (1996).
99) Mizumura, K., Koda, H. and Kumazawa, T.: Augmenting effects of cyclic AMP on the heat response of canine testicular polymodal receptors. *Neurosci. Lett.*, 162, 75-7 (1993).
100) Mizumura, K., Minagawa, M., Koda, H. and Kumazawa, T.: Forskolin does not augment the bradykinin response of canine visceral polymodal receptors in vitro. *Neurosci. Lett.*, 166, 195-8 (1994).
101) Kress, M., Rodl, J. and Reeh, P.W.: Stable analogues of cyclic AMP but not cyclic GMP sensitize unmyelinated primary afferents in rat skin to heat stimulation but not to inflammatory mediators, in vitro. *Neuroscience*, 74, 609-17 (1996).
102) Dray, A., Patel, I.A., Perkins, M.N. and Rueff, A.: Bradykinin-induced activation of nociceptors: receptor and mechanistic studies on the neonatal rat spinal cord-tail preparation in vitro. *Br. J. Pharmacol.*, 107, 1129-34 (1992).

103) Cui, M. and Nicol, G.D.: Cyclic AMP mediates the prostaglandin E_2-induced potentiation of bradykinin excitation in rat sensory neurons. *Neuroscience*, 66, 459-66 (1995).
104) Lindsay, R.M. and Rang, H.P.: Effects of bradykinin on the membrane currents of rat sensory neurones in tissue culture. *J. Physiol. (Lond.)*, 390, 74p (1987).
105) Kitakoga, O. and Kuba, K.: Bradykinin-induced ion currents in cultured rat trigeminal ganglion cells. *Neurosci. Res.*, 16, 79-93 (1993).
106) Kano, M., Kawakami, T., Hikawa, N., Hori, H., Takenaka, T. and Gotoh, H.: Bradykinin-responsive cells of dorsal root ganglia in culture: Cell size, firing, cytosolic calcium, and substance P. *Cell Mol. Neurobiol.*, 14, 49-57 (1994).
107) Miller, R.J.: Bradykinin highlights the role of phospholipid metabolism in the control of nerve excitability. *Trends Neurosci.*, 10, 226-8 (1987).
108) Brown, D.A. and Higashida, H.: Inositol 1,4,5-trisphosphate and diacylglycerol mimic bradykinin effects on mouse neuroblastoma x rat glioma hybrid cells. *J. Physiol. (Lond.)*, 397, 185-207 (1988).
109) Rang, H.P., Bevan, S. and Dray, A.: Nociceptive peripheral neurons: cellular properties. In Textbook of Pain, edited by Wall, P.D. and Melzack, R., 3rd, pp.57-78 (1994), Churchill Livingstone, Edinburgh, London, Madrid, Melburne, New York, Tokyo.
110) Lazarowski, E.R. and Lapetina, E.G.: Activation of platelet phospholipase C by fluoride is inhibited by elevation of cyclic AMP. *Biochem. Biophys. Res. Commun.*, 158, 440-4 (1989).
111) McAtee, P. and Dawson, G.: Rapid dephosphorylation of protein kinase C substrates by protein kinase A activators results from inhibition of diacylglycerol release. *J. Biol. Chem.*, 264, 11193-9 (1989).
112) Campbell, M.D., Subramaniam, S., Kotlikoff, M.I., Williamson, J.R. and Fluharty, S.J.: Cyclic AMP inhibits inositol polyphosphate production and calcium mobilization in neuroblastoma x glioma NG108-15 cells. *Mol. Pharmacol.*, 38, 282-8 (1990).
113) Ferreira, S.H. and Nakamura, M.I.: Prostaglandin hyperalgesia, a cAMP/Ca^{2+} dependent process. *Prostaglandins*, 18, 179-89 (1979).
114) Taiwo, Y.O. and Levine, J.D.: Further confirmation of the role of adenyl cyclase and of cAMP-dependent protein kinase in primary afferent hyperalgesia. *Neuroscience*, 44, 131-5 (1991).
115) Hill, S.J.: Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.*, 42, 45-83 (1990).
116) Kaczmarek, L.K.: The role of protein kinase C in the regulation of ion channels and neurotransmitter release. *Trends Neurosci.*, 10, 30-4 (1987).
117) Dray, A., Bettaney, J., Forster, P. and Perkins, M.N.: Bradykinin-induced stimulation of afferent fibres is mediated through protein kinase C. *Neurosci. Lett.*, 91, 301-7 (1988).
118) Leng, S., Mizumura, K., Koda, H. and Kumazawa, T.: Excitation and sensitization of the heat response induced by a phorbol ester in canine visceral polymodal receptors studied in vitro. *Neurosci. Lett.*, 206, 13-6 (1996).
119) Kopp, U.C. and Smith, L.A.: A role for protein kinase C in bradykinin-mediated activation of renal pelvic sensory receptors. *Am. J. Physiol.*, 269, R331-8 (1995).
120) Rang, H.P. and Ritchie, J.M.: Depolarization of nonmyelinated fibers of the rat vagus nerve produced by activation of protein kinase C. *J. Neurosci.*, 8, 2606-17 (1988).
121) Schepelmann, K., Messlinger, K. and Schmidt, R.F.: The effects of phorbol ester on slowly conducting afferents of the cat's knee joint. *Exp. Brain Res.*, 92, 391-8 (1993).
122) Mizumura, K., Koda, H. and Kumazawa, T.: Different effects of protein kinase-C activation on the responses to bradykinin and to heat of visceral polymodal receptors studied in vitro. *Soc. Neurosci. Abstr.*, 21, 643 (1995) [Abstract].
123) Stabel, S., Rodriguez Pena, A., Young, S., Rozengurt, E. and Parker, P.J.: Quantitation of protein kinase C by immunoblot – expression in different cell lines and response to phorbol esters. *J. Cell Physiol.*, 130, 111-7 (1987).
124) Matthies, H.J.G., Palfrey, H.C., Hirning, L.D. and Miller, R.J.: Down regulation of protein kinase C in neuronal cells: effects on neurotransmitter release. *J. Neurosci.*, 7, 1198-206 (1987).
125) Smith, C.D., Uhing, R.J. and Snyderman, R.: Nucleotide regulatory protein-mediated activation of phospholipase C in human polymorphonuclear leukocytes is disrupted by phorbol esters. *J. Biol. Chem.*, 262, 6121-7 (1987).