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Involvement of endogenous nitric oxide in the mechanism of bradykinin-induced peripheral hyperalgesia

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1 When N^G-nitro-L-arginine methyl ester (L-NAME, 0.1–10 nmol) or N^G-monomethyl-L-arginine (L-NMMA, 10 nmol–1 μmol) was intradermally administered with bradykinin (BK, 3 nmol) into the instep of rat hind-paws, a dose-related suppression of BK-induced hyperalgesia, assessed by the paw-pressure test, was produced.

2 L-Arginine (1 μmol) but not D-arginine (1 μmol) reversed the suppressive effects of L-NAME (10 nmol) and L-NMMA (1 μmol) on BK-induced hyperalgesia.

3 Concomitant intradermal administration of BK (3 nmol) with haemoglobin (1 nmol) significantly suppressed BK-induced hyperalgesia in the paw-pressure test. The BK-induced hyperalgesia was abolished by concomitant intradermal administration of either a guanylate cyclase inhibitor, methylene blue (10 nmol), or LY83583 (1 nmol). In addition, KT5823 (1 nmol) or $\mathbf{R_p}$-8-bromoguanosine-3′:5′-cyclic monophosphothioate ($\mathbf{R_p}$-8-Br-cGMPS; 1 nmol), an inhibitor of cyclic GMP-dependent protein kinase, also significantly suppressed BK-induced hyperalgesia.

4 The carrageenin-induced hyperalgesia was significantly attenuated by L-NAME in a dose-dependent manner.

5 L-Arginine (1 μmol), sodium nitroprusside (1 μmol), dibutyryl cyclic GMP (1 μmol) or 8-bromo cyclic GMP (1 μmol) all failed to produce any significant relieving effect on the nociceptive threshold of rodent hind-paws. Concomitant administrations of each agent with a sub-threshold dose (0.1 nmol) of BK induced significant hyperalgesia.

6 $\mathbf{R_p}$-adenosine 3′:5′-cyclic monophosphothioate ($\mathbf{R_p}$-cAMPS; 1 nmol), an inhibitor of cyclic AMP-dependent protein kinase, significantly suppressed BK-induced mechanical hyperalgesia. Concomitant administration of forskolin (1 nmol) with 8-bromo cyclic GMP (100 nmol) induced significant hyperalgesia.

7 In the superfusion experiment of a blister base on the instep of rodent hind-paws, intradermally administered BK (3 nmol) significantly increased the outflow of both cyclic GMP and cyclic AMP from the blister base. Concomitant administrations of L-NAME (10 nmol) with BK significantly reduced the BK-induced outflow of cyclic GMP without affecting the cyclic AMP content.

8 These results suggest that the NO–cyclic GMP pathway is involved in the mechanism of BK-induced hyperalgesia, and an activation of both cyclic GMP-and cyclic AMP-second messenger system plays an important role in the production of peripherally induced mechanical hyperalgesia.

Keywords: Bradykinin; nitric oxide (NO); hyperalgesia; cyclic GMP; cyclic AMP; guanylate cyclase; cyclic GMP-dependent protein kinase; carrageenin

Introduction

Bradykinin (BK) has been shown to be a chemical mediator of inflammatory pain (Steranka *et al.*, 1988; Dray & Perkins, 1993). BK not only causes excitation and sensitization of primary afferent nociceptors but also elicits vasodilatation and enhances vascular permeability (Dray & Perkins, 1993). These multifaceted effects of BK contribute to the induction of inflammation in tissues.

BK-induced vasodilatation has been demonstrated to be mediated via endothelium-derived relaxing factor (EDRF), which was recently identified as nitric oxide (NO) (Palmer *et al.*, 1987). NO is synthesized from L-arginine by an enzyme called nitric oxide synthase (NOS) in the vascular endothelial cells (Palmer *et al.*, 1988). Stimulation of BK receptors on the endothelial cells activates NOS, and subsequently NO diffuses from the endothelial cells to smooth muscle cells. NO causes relaxation of the smooth muscle cells through activation of soluble guanylate cyclase and enhancement of intracellular cyclic GMP (Moncada *et al.*, 1991).

Recently, such a NO-cyclic GMP pathway in peripheral tissues has been reported to be involved in the formation of inflammatory oedema induced by BK (Teixeira *et al.*, 1993), substance P (Hughes *et al.*, 1990), phospholipase A_2 (Cirino *et al.*, 1991) or carrageenin (Ialenti *et al.*, 1992). Involvement of NO in the pathophysiology of arthritis has also been suggested (Ialenti *et al.*, 1993; Stefanovic-Racic *et al.*, 1993; 1994). Hyperalgesia is also one of the major inflammatory symptoms observed (Treede *et al.*, 1992). Recent reports have demonstrated that the mechanisms of thermal hyperalgesia in the spinal cord involves activation of the NO-cyclic GMP pathway mediated through the N-methyl-D-aspartate (NMDA) receptor (Malmberg & Yaksh, 1993; Meller & Gebhart, 1993; Meller *et al.*, 1994). Furthermore, peripherally or centrally administered NO synthase inhibitors have been shown to produce an antinociceptive effect in mice (Moore *et al.*, 1991; 1993; Morgan *et al.*, 1992; Babbedge *et al.*, 1993a,b). In contrast to this finding, Duarte *et al.* (1990) have shown that

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acetylcholine-induced antinociception is mediated via the NO-cyclic GMP pathway. Together these findings suggest that NO is involved in the regulation of pain transmission.

Studies on the peripheral role(s) of the NO-cyclic GMP pathway involved in the mechanism of hyperalgesia have been limited. Recently, BK has been reported to activate the NO-cyclic GMP pathway in rat cultured sensory neurones (McGehee *et al.*, 1992; Bauer *et al.*, 1993). The role(s) of the NO-cyclic GMP pathway in BK-induced excitation and sensitization of primary afferent neurones is, however, still unclear. In the present study, we asked whether the NO-cyclic GMP pathway was involved in BK-induced peripheral hyperalgesia. In addition, clarification of the peripheral role(s) of the NO-cyclic GMP pathway in the mechanism of hyperalgesia was attempted.

Methods

Animals

Male Sprague-Dawley rats weighing 150–200 g were used in this study. Animal chow and water were provided *ad libitum*.

Measurement of nociceptive thresholds

The paw-pressure test was performed according to the method of Randall & Selitto (1957) with an analgesy-meter for the rat paw (MK-300, Muromachi Kikai Co. Ltd., Japan). Increasing pressure (g) was continuously applied to the instep of the hind-paw at a gradual weight-loading rate of 48 g s^{-1}. The pressure required to elicit a struggle response was used as the nociceptive threshold of the hind-paw tested. On the day before the experiment, nociceptive thresholds of the right hind-paw of each rat were measured for 4 times at 1 h intervals. Only animals with stable and reproducible thresholds were selected. On the day of the experiment, the baseline threshold of the right hind-paw was determined twice before drug injection. The mean of the values was defined as the control threshold.

Test agents were injected intradermally (i.d.) into the instep of the right hind-paw, the same site at which the control nociceptive threshold was previously measured.

For BK-induced hyperalgesia, agents were administered concomitantly with BK (final volume: 25 μl) in the instep of right hind-paws. Rats were tested at post-administration 5 min.

For carrageenin-induced hyperalgesia, 1% carrageenin in a volume of 50 μl saline was administered into the instep of the right hind-paw. N^G-nitro-L-arginine methyl ester (L-NAME) was injected into the same site in a volume of 25 μl, either 2 h after or 15 min before carrageenin injection. The nociceptive threshold was measured at 30 min intervals after the carrageenin injection.

The results are expressed as percentages of the control threshold.

Superfusion of blister base

Blister induction and superfusion over the blister base were performed according to the procedure of Khalil & Helme (1992) with slight modification. Briefly, the experiments were carried out under anaesthesia with sodium pentobarbitone and the body temperature of rats was maintained at 37°C by a isothermal pad (Braintree Scientific, U.S.A.). A blister was induced in the instep of the right hind-paw by applying a suction pressure of −20 kPa for 30 min before detaching the epidermis. The surface of the blister base, where the dermis was exposed, was then perfused with Krebs-bicarbonate solution at 0.1 ml min^{-1} by using a perfusion chamber and peristaltic pumps. Krebs-bicarbonate solution had the following composition: (in mM) NaCl 118, KCl 4.7, KH_2PO_4 1.19, $MgSO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.54, D-glucose 11, and was bubbled with 95% O_2 and 5% CO_2. To inhibit the phosphodiesterase activity, all experiments were carried out in the presence of 1 mM 3-isobutyl-1-methylxanthine (IBMX). After an initial equilibration period (40 min), the superfusates were collected at 10 min intervals and promptly frozen on dry ice. Immediately after the second fraction was collected, 25 μl of either physiological saline or drug solution was administered i.d. into the perfusion site. Both cyclic GMP and cyclic AMP levels in the superfusate samples were measured by radioimmunoassay (Amersham ^{125}I assay system, England).

Statistical analysis

Results were expressed as the mean plus or minus standard error mean (mean ± s.e.mean). Statistical differences between the groups were verified by either one- or two-way ANOVA followed by Dunnett's t test, Bonferroni modified t test or Student's unpaired t test.

Drugs

Bradykinin and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin were obtained from Peptide Institute (Japan). N^G-nitro-L-arginine methyl ester (L-NAME), D-arginine, haemoglobin, 8-bromo cyclic GMP, dibutyryl cyclic GMP and carrageenin lambda were purchased from Sigma Chemical Co. (U.S.A.). N^G-monomethyl-L-arginine (L-NMMA), LY83583 (6-(phenylamino)-5,8-quinolinedione) and $\mathbf{R}_p$-adenosine 3′,5′-cyclic monophosphothioate ($\mathbf{R}_p$-cAMPS) were from Research Biochemicals (U.S.A.). KT5823 ((8**R**, 9**S**, 11**S**)-(-)-2-N-methyl-9-methoxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1H,8H,11H-2,7b,11a-triazadibenzo[a,g]cycloocta-[cde]trinden-1-one) was from Biomol Research Laboratories (U.S.A.). Methylene blue, sodium nitroprusside and 3-isobutyl-1-methylxanthine (IBMX) were from Nacalai Tesque (Japan). Forskolin and L-arginine were from Wako (Japan) and Kyowa Hakko Kogyo (Japan), respectively. $\mathbf{R}_p$-8-bromoguanosine-3′:5′-cyclic monophosphothioate ($\mathbf{R}_p$-8-Br-cGMPS) was from BIOLOG Life Science Institute (Germany). LY83583 and KT5823 were dissolved in dimethyl sulphoxide (DMSO) and diluted to the desired concentration in physiological saline. All other drugs were dissolved with physiological saline.

Results

Effects of NO synthase inhibitors on BK-induced hyperalgesia

Administration (i.d.) of BK (0.3–3 nmol/paw) reduced the nociceptive threshold of the rat hind-paw in a dose-dependent manner. Because the ceiling effect was observed at doses exceeding 3 nmol, a dose of 3 nmol was employed to investigate the effects of NO synthase inhibitors. BK (3 nmol, i.d.) significantly reduced the nociceptive threshold by 27.4 ± 2.1% ($n=6$) of the basal value. BK-induced hyperalgesia peaked within 5 min and persisted for 30 min. This effect of BK on the nociceptive threshold was completely abolished by concomitant administration of D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (10 nmol, i.d.), a B_2 receptor antagonist (0.0 ± 2.8% in the nociceptive threshold; $n=6$, $P<0.01$).

NO synthase inhibitors, L-NAME (0.1–10 nmol) and L-NMMA (10 nmol–1 μmol), dose-dependently attenuated the BK-induced hyperalgesia and almost completely abolished the hyperalgesic effect of BK at their highest doses (Figure 1). The inhibitory effect of L-NAME on BK-induced hyperalgesia was about 100 fold more potent than that of L-NMMA. When given alone, neither L-NAME (10 nmol) nor L-NMMA (1 μmol) produced a significant effect on the nociceptive threshold (data not shown). L-Arginine (1 μmol), but not D-arginine (1 μmol), reversed the suppressive effects of L-NMMA (1 μmol) on the BK-induced hyperalgesia

(Figure 2). When L-NAME (10 nmol) was used, the hyperalgesia was similarly reversed by concomitant administration of L-arginine (1 μmol) (data not shown).

Effects of NO-cyclic GMP pathway inhibitors on BK-induced hyperalgesia

The effects of various inhibitors of the NO-cyclic GMP pathway on BK-induced hyperalgesia are summarized in Figure 3. The BK-induced hyperalgesia was abolished by the NO inactivating haemoglobin (1 nmol). The inhibitors of soluble guanylate cyclase, methylene blue (10 nmol) and LY83583 (1 nmol), also significantly suppressed the hyperalgesic effect induced by BK (3 nmol). KT5823 (1 nmol) or $\mathbf{R}_p$-8-Br-cGMPS (1 nmol), inhibitors of cyclic GMP-dependent protein kinase, significantly attenuated BK-induced hyperalgesia. When administered alone, these inhibitors did not display any significant effect on the nociceptive threshold.

Effects of L-NAME on carrageenin-induced hyperalgesia

Carrageenin (1%), administered i.d. into the instep of the rat hind-paw in a volume of 50 μl, produced long-lasting hyperalgesia. Administration of L-NAME (10 and 30 nmol) 2 h after carrageenin-injection elicited a significant inhibitory effect on the carrageenin-induced hyperalgesia ($F(2,143)=44.574$, $P<0.001$; Figure 4). In a similar manner, pretreatment with L-NAME (10 and 100 nmol) significantly

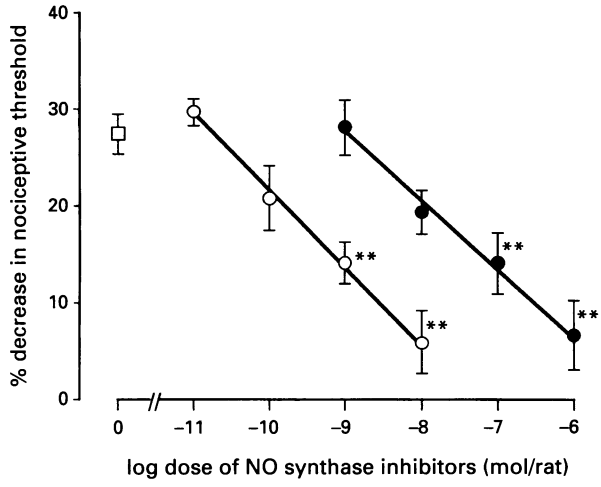

Figure 1 Dose-dependent suppressive effects of nitric oxide synthase inhibitors on bradykinin (BK)-induced hyperalgesia assessed by the paw-pressure test in rats. BK was injected intradermally into the rat hind-paw at a dose of 3 nmol (□). Either N^G-nitro-L-arginine methyl ester (L-NAME, ○) or N^G-monomethyl-L-arginine (L-NMMA, ●) was administered concomitantly with BK (3 nmol). Decreases (%) in the nociceptive threshold at 5 min after i.d. administration of drugs are indicated. Each point is the mean ± s.e.mean of six observations. $^{**}P<0.01$ when compared with the BK-injected group.

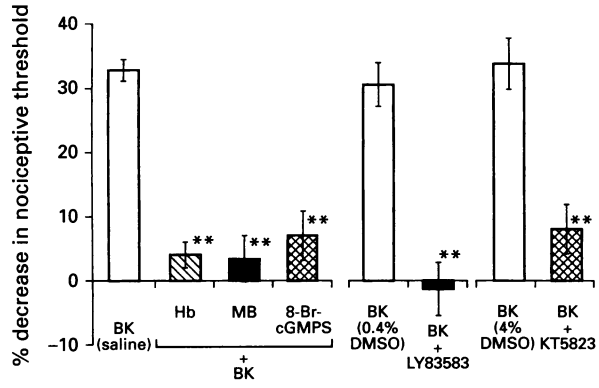

Figure 3 Effects of inhibitors of nitric oxide (NO)-cyclic GMP pathway on bradykinin (BK)-induced hyperalgesia. BK (3 nmol) was co-administered with haemoglobin (Hb, 1 nmol), methylene blue (MB, 10 nmol), LY83583 (1 nmol), KT5823 (1 nmol) or $\mathbf{R}_p$-8-bromoguanosine-3′:5′-monophosphothioate (Rp-8-Br-cGMPS, 1 nmol). Hb, MB and $\mathbf{R}_p$-8-Br-cGMPS were dissolved in physiological saline. LY83583 and KT5823 were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO for LY83583 and KT5823 were 0.4 and 4%, respectively. BK was dissolved in the respective vehicles. Data indicate percentage decreases in the nociceptive threshold at 5 min after i.d. administration of drugs. Results show the mean with s.e.mean of six observations. $^{**}P<0.01$ in comparison with each control group.

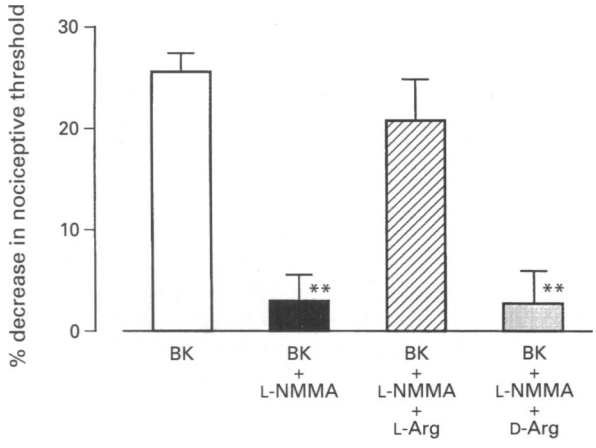

Figure 2 The effect of N^G-monomethyl-L-arginine (L-NMMA) on bradykinin (BK)-induced hyperalgesia was reversed by L-arginine (L-Arg), but not D-arginine (D-Arg). BK (3 nmol) was concomitantly administered intradermally (i.d.) with either L-NMMA (1 μmol), L-Arg (1 μmol) or D-Arg (1 μmol). Data indicate percentage decreases in the nociceptive threshold at 5 min after i.d. administration of drugs. Results show the mean with s.e.mean of six observations. $^{**}P<0.01$ when compared with the BK-injected group.

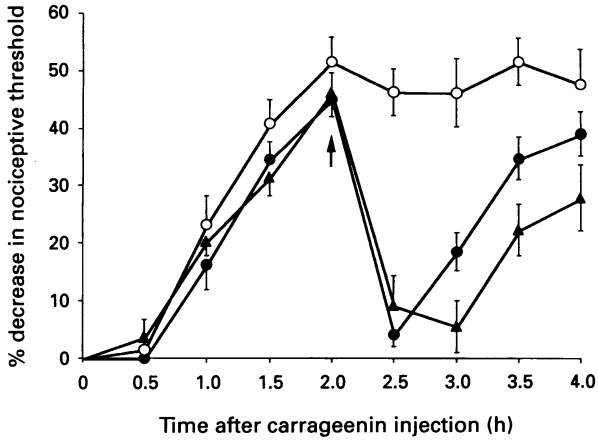

Figure 4 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) on carrageenin-induced hyperalgesia in rats: 1% carrageenin in a volume of 50 μl was administered into the instep of the rat hind-paws. Either L-NAME (●; 10 nmol, $n=6$ or ▲; 30 nmol, $n=6$) or saline (○; $n=6$) in a volume of 25 μl was injected intradermally 2 h (arrow in figure) after carrageenin injection. Data indicate percentage decreases in the nociceptive threshold. L-NAME (10 and 30 nmol) significantly inhibited decreases of the nociceptive threshold ($F(2,143)=44.574$; $P<0.001$, two-way ANOVA).

suppressed the hyperalgesic effect in a dose-dependent manner ($F(2,107)=13.905$, $P<0.001$; data not shown).

Effects of NO-cyclic GMP pathway activators on BK-induced hyperalgesia

When administered alone, L-arginine (1 μmol), sodium nitroprusside (1 μmol) or cyclic GMP analogues (dibutyryl cyclic GMP, 1 μmol; 8-bromo cyclic GMP, 1 μmol) produced no significant effect on the nociceptive threshold of the rat hindpaw (Figure 5). Concomitant administration of the respective agent with a sub-threshold dose (0.1 nmol) of BK induced significant hyperalgesia (Figure 5).

Effects of activation of cyclic AMP second messenger system on peripheral mechanical hyperalgesia

R_p-cAMPS (1 nmol), an inhibitor of cyclic AMP-dependent protein kinase, significantly suppressed BK (3 nmol)-induced mechanical hyperalgesia (Figure 6). Concomitant i.d. administration of forskolin (1 nmol) with 8-bromo cyclic GMP (100 nmol) induced significant hyperalgesia (Figure 6).

Effects of BK and L-NAME on efflux of cyclic GMP and cyclic AMP from the blister base

After an initial equilibration period (40 min), a constant efflux of both cyclic nucleotides from the blister base was observed. Cyclic GMP and cyclic AMP contents of the fraction just before drug administration were 245.1 ± 28.5 and 640.5 ± 68.3 fmol 10 min^{-1} ($n=15$), respectively. Administration (i.d.) of saline slightly increased the efflux of both cyclic nucleotides, although only the increase of the cyclic GMP content was significant. Similar treatment with BK (3 nmol, i.d.) enhanced the efflux of both cyclic GMP and cyclic AMP from the blister base markedly. These increases of both cyclic nucleotides, significantly greater than that induced by saline (Table 1), were observed only in the first fraction after BK injection. Concomitant administration of L-NAME (10 nmol) and BK significantly inhibited the BK-induced efflux of cyclic GMP, but not that of cyclic AMP.

Discussion

In the present study, the NO-cyclic GMP pathway was demonstrated to play a role in the mechanism of peripheral mechanical hyperalgesia. The present experiments demonstrated that BK activated the peripheral NO-cyclic GMP pathway via the B_2 receptor. NO synthase inhibitors reduced the BK-induced hyperalgesia without eliciting any effect on the nociceptive threshold. This finding suggested that activation of NO synthase was involved in BK-induced sensitization of primary sensory nerve endings, but not in their excitation.

The inhibitory effect of L-NAME on BK-induced hyperalgesia was 100 times more potent than that of L-NMMA, and L-arginine reversed both inhibitory effects. According to Babbedge *et al.* (1993a), the antinociceptive effect of L-NAME is several orders more potent than that of L-NMMA. However, the inhibitory effects of both inhibitors on oedema formation have been found to be equipotent by Hughes *et al.* (1990) and Ialenti *et al.* (1992). Therefore, the relative inhibitory potency of NO synthase inhibitors on BK-induced hyperalgesia is consistent with regard to nociception rather than oedema formation.

Haemoglobin, which inactivates extracellular NO, abolished BK-induced hyperalgesia, suggesting that NO produced by BK exerts its action by diffusing from the generator cells to target cells. However, it is very difficult to identify the NO-generator and target cells on the basis of our present findings. In the intradermal tissues, BK receptors are distributed on various

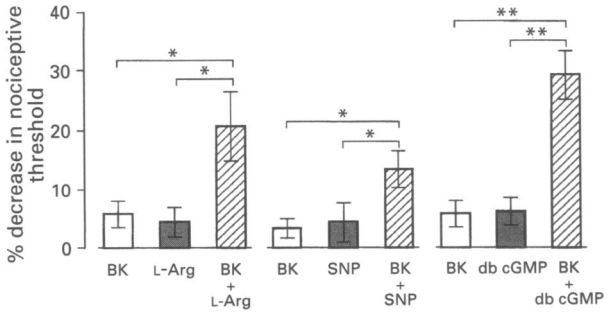

Figure 5 Effects of activators of NO-cyclic GMP pathway on the bradykinin (BK)-induced hyperalgesia. BK (0.1 nmol), L-arginine (L-Arg, 1 μmol), sodium nitroprusside (SNP, 1 μmol) or dibutyryl cyclic GMP (db cGMP, 1 μmol) was injected intradermally (i.d.) into the hindpaw of rats. Data indicate percentage decreases in the nociceptive threshold 5 min after i.d. administration of drugs. Results show the mean with s.e.mean of six observations. $*P<0.05$; $**P<0.001$ in comparison with each control group

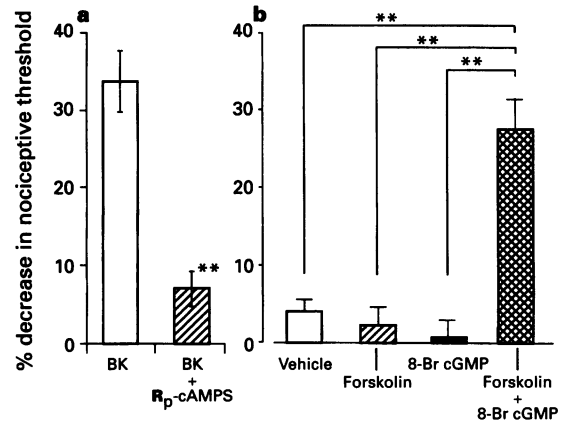

Figure 6 Effect of a cyclic AMP-dependent protein kinase inhibitor on bradykinin (BK)-induced hyperalgesia (a) and effects of forskolin and 8-bromo cyclic GMP (8-Br cGMP) on the nociceptive threshold (b). (a) BK (3 nmol) was co-administered with R_p-adenosine-3′:5′-cyclic monophosphothioate (R_p-cAMPS, 1 nmol). BK and R_p-cAMPS were dissolved in saline. (b) Vehicle (1% ethanol), forskolin (1 nmol) and 8-Br cGMP (100 nmol) were injected intradermally (i.d.) into the hind-paw of rats. Forskolin was dissolved in ethanol and diluted to the final concentration of 1% ethanol. Data indicate percentage decreases in the nociceptive threshold at 5 min after i.d. administration of drugs. Results show the mean with s.e.mean of six observations. $**P<0.01$ in comparison with each control group.

Table 1 Effects of bradykinin (BK) and N^G-nitro-L-arginine methyl ester (L-NAME) on efflux of cyclic nucleotides from the blister base

Treatment	*Cyclic GMP* (% increase)	*Cyclic AMP* (% increase)
Saline	26.9 ± 6.4	11.6 ± 7.0
BK (3 nmol)	165.8 ± 24.4**	107.7 ± 16.6**
BK (3 nmol) + L-NAME (10 nmol)	72.5 ± 7.7††	61.3 ± 18.8

Results are the mean ± s.e.mean of five experiments. Data are expressed as a percentage increase of the efflux of cyclic nucleotide content from the fraction collected just before drug injection. $**P<0.01$ and $††P<0.01$ when compared with the saline- and BK-injected groups, respectively.

types of cells, such as nociceptive neurones and endothelial cells (Dray & Perkins, 1993). One or more cell types may be the generator cell of NO, which is involved in the generation of BK-induced hyperalgesia. However, the target cell of NO is likely to be a primary sensory neurone, because hyperalgesia is a phenomenon that involves the sensitization of sensory nerve endings. Recently, the presence of NO synthase in primary sensory neurones of dorsal root ganglia has been shown (Aimi *et al.*, 1991; Terenghi *et al.*, 1993; Zhang *et al.*, 1993). In primary cultures of the dorsal root ganglion, BK activates soluble guanylate cyclase via the release of NO (McGehee *et al.*, 1992; Bauer *et al.*, 1993). The fact that inhibitors of guanylate cyclase, methylene blue and LY83583 reduced the BK-induced hyperalgesia demonstrates that NO released by BK similarly activates soluble guanylate cyclase. These findings suggest that the i.d. administered BK probably acts on the NO generator cells, such as primary sensory neurones or endothelial cells, to induce an increase in the intracellular cyclic GMP content in primary sensory neurones via the release of NO. BK induces increases in the cyclic GMP content in primary sensory neurones, and this eventually results in selective desensitization to the action of BK (Burgess *et al.*, 1989; McGehee *et al.*, 1992; Rueff *et al.*, 1994). Therefore, activation of the NO -cyclic GMP pathway by BK might induce both sensitization to noxious mechanical stimuli and desensitization to further BK action.

Vasodilatation and plasma extravasation induced by BK are mediated by NO (Palmer *et al.*, 1987; 1988; Khalil & Helme, 1992; Teixeira *et al.*, 1993). In addition, carrageenin-induced oedema and plasma extravasation are reduced by i.d. administration of NO synthase inhibitors (Ialenti *et al.*, 1992). In the present study, we further showed that NO was involved in the production of carrageenin-induced mechanical hyperalgesia. These findings indicate that peripherally produced NO may play an important role in the production of inflammatory responses such as hyperalgesia, vasodilatation and plasma extravasation. I.d. administration of NO synthase inhibitors reduces skin blood flow (Hughes *et al.*, 1990; Lawrence & Brain, 1992; Kajekar *et al.*, 1995). Reduction of skin blood flow possibly influences oedema formation and the delivery of chemical mediators. Therefore, L-NAME-induced reduction of skin blood flow may also contribute to the suppression of carrageenin-induced hyperalgesia.

We have demonstrated the role of the NO-cyclic GMP pathway in peripherally induced mechanical hyperalgesia. Furthermore, involvement of the NO-cyclic GMP pathway in the maintenance of hyperalgesia is also suggested, because L-NAME, administered 2 h after carrageenin-injection, significantly inhibited the carrageenin-induced hyperalgesia. Further studies to clarify the role(s) of the NO-cyclic GMP pathway in chronic hyperalgesia are warranted.

Cyclic GMP-dependent protein kinase, ion channel and phosphodiesterases are the many effector targets for cyclic GMP (Schmidt *et al.*, 1993). Cyclic GMP-dependent protein kinase inhibitors, such as KT5823 and $\mathbf{R}_p$-8-Br-cGMPS, elicit potent inhibitory effects on BK-induced hyperalgesia. To our knowledge, this is the first work that demonstrates the involvement of the NO-cyclic GMP pathway and cyclic GMP-dependent protein kinase in the mechanism of mechanical hyperalgesia of primary afferents.

When administered alone, activators of the NO-cyclic GMP pathway, such as L-arginine, sodium nitroprusside and membrane permeable cyclic GMP analogs, did not display any significant hyperalgesic effect. However, concomitant administration of these agents with a sub-threshold dose of BK triggered significant hyperalgesia. These results manifest that activation of the NO-cyclic GMP pathway alone is not enough, although this event is essential to trigger peripherally induced mechanical hyperalgesia. Therefore, apart from activation of the NO-cyclic GMP pathways, BK or the BK-activated second messenger system(s) may be required to complement the manifestation of peripherally induced mechanical hyperalgesia. The cyclic AMP second messenger system, inclusive of the cyclic AMP-dependent protein kinase, contributes to the induction of primary afferent mechanical hyperalgesia (Taiwo *et al.*, 1989; Taiwo & Levine, 1991). Suppression of BK-induced hyperalgesia by $\mathbf{R}_p$-cAMPS confirms the involvement of cyclic AMP-dependent protein kinase in BK-induced mechanical hyperalgesia. Furthermore, concomitant administration of forskolin with 8-bromo cyclic GMP induced significant hyperalgesia. These findings suggest that BK activates both cyclic GMP- and cyclic AMP-second messenger system to induce the mechanical hyperalgesia.

In order to examine whether BK stimulated both cyclic GMP- and cyclic AMP-second messenger systems in the intradermal tissue, we performed a superfusion experiment on the blister base. Efflux of cyclic GMP and cyclic AMP has been shown to reflect their respective intracellular contents (Burton *et al.*, 1990; Heuze-Joubert *et al.*, 1992; Rosenberg, 1992). Efflux of cyclic GMP from the blister base was significantly stimulated by i.d. injection of BK at a dose that would induce peripheral hyperalgesia. Concomitant administration of L-NAME with BK significantly suppressed the BK-induced increase of cyclic GMP efflux. These data further indicated that i.d. administered BK did increase cyclic GMP content through activation of NOS. The involvement of the cyclic AMP second messenger system in peripheral hyperalgesia (Taiwo *et al.*, 1989; Pitchford & Levine, 1991; Taiwo & Levine, 1991) is also confirmed by the BK-induced efflux of cyclic AMP in our experiment.

In summary, the results of this investigation indicate that both the NO-cyclic GMP pathway and cyclic AMP second messenger system are involved in the BK-induced peripheral mechanical hyperalgesia.

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