(Text 15 pages, 3 Figures)

The long-term exposure of rat cultured dorsal root ganglion cells to bradykinin induced the release of prostaglandin E2 by the activation of cyclooxygenase-2

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Abstract

The effects of long-term exposure of primary cultured rat dorsal root ganglion (DRG) cells to bradykinin (BK), compared to short-term exposure, were investigated to establish whether BK could induce prostaglandin E2 (PGE2) release from DRG cells. Short-term exposure (30 min) resulted in a small but significant amount of PGE2 release which was mainly inhibited by a selective COX-1 inhibitor, SC-560 but only partially by a selective COX-2 inhibitor, NS-398, and did not induce COX-2 protein as determined by Western blotting. In contrast, long-term exposure (3 hr) induced a large amount of PGE2 release, which was completely abolished by indomethacin or NS-398. The level of COX-2 mRNA began to be detected by ribonuclease protection assay after 30 min of 100 nM BK exposure, maintained maximal expression for 1 h, and subsequently declined to the basal level. The level of COX-2 protein was expressed to follow the time course of COX-2 mRNA induction by BK in a delayed but similar kinetic manner. The expression of COX-2 induced by BK in DRG cells was inhibited by a BK B2 receptor antagonist, HOE140, but not a B1 receptor antagonist, Lys-des-Arg^{9}, (Leu^{8})-BK. Thus BK has been shown to induce COX-2 protein by B2 receptor, which may cause prostanoid generation in rat DRG cells, which may play an important role in the pathogenesis of inflammatory pain and hyperalgesia around the primary sensory neurons.

Bradykinin (BK) acts as an endogenous algesic substance on sensory neurons with the activation and sensitization of nociceptors to cause acute pain and hyperalgesia associated with inflammation, nerve injury, or rheumatinoid diseases [10].

By short-term exposure in DRG cells, BK could stimulate B2 receptor constitutively expressed in a subpopulation of rat primary sensory neurons [27], sensitize transient receptor potential vanilloid receptor 1 (TRPV1) by release from phosphatidylinositol 4,5-bisphosphate-mediated inhibition [9], and activate protein kinase C, which phosphorylates TRPV1, resulting in the sensitization of TRPV1 [21]. We have observed that BK sensitizes TRPV1 to facilitate capsaicin-induced $^{45}Ca^{2+}$ uptake by the activation of phospholilpase C in primary cultured rat dorsal root ganglion (DRG) cells [30].

On the other hand, sensitization by BK might involve long-term changes in gene expression. BK stimulates the production and release of a number of endogenous chemicals, for example neuropeptides such as substance P and calcitonin gene-related peptide, cytokines such as interleukin-1 β and TNF- α, 5-HT, ATP, histamine and prostanoids which can mediate neurogenic inflammation and inflammation-induced hyperalgesia [10]. There are some reports that BK induces prostaglandin E2 (PGE2) release from some cell types. For example, Jenkins *et al*. [13] demonstrated that BK induced PGE_2 release from cultured rat trigeminal ganglion neurons. Furthermore, Pang *et al*. [23] reported that BK-stimulated PGE_2 release and BK-induced activation of cyclooxygenase (COX)-2 were inhibited by cycloheximide in human airway smooth muscle cells. Two isoforms of COX, COX-1, and COX-2 have been identified, the former constitutively expressed in many cell types and possibly activated by short-term treatment with BK and the latter induced at the site of inflammation [31]. Recent works have reported that BK induced prostanoid production owing to the induction of COX-2 isoform in cultured fibroblasts [29], pulmonary artery smooth muscle cells [5], and human airway epithelial cells [7]. Thus COX-2 induction by BK in DRG cells may be involved in the excitation and sensitization of sensory neurons contributing

inflammation-induced hyperalgesic sensations, which has not been demonstrated.

Thus, we investigated PGE2 release by exposing primary cultured rat DRG cells to BK as a function of the treatment time associated with the change of COX activity. We found that long-term exposure to BK induced the transient expression of COX-2 mRNA and protein via B2 receptor, which was responsible for the production and release of PGE2 in DRG cells.

Male Wistar rats weighing 200-300 g were housed in cages under a 12 h light/dark cycle and given access to food and water ad libitum. All procedures used in these studies were approved by the Animal Care and Use Committee of Hiroshima University, Hiroshima, Japan. The rat DRG were digested with 0.125% collagenase and 0.25% trypsin and mechanically dissociated by trituration. The cells were plated in 35 mm-diameter tissue culture dishes coated with polyethyleneimine and laminin and maintained at 37℃ under an atmosphere of 5% CO2 in Dulbecco's modified Eagle's medium supplemented with 10% horse serum, 2 mM glutamine, 50 mg/ml penicillin-streptomycin, and 50 ng/ml 2.5S mouse salivary gland nerve growth factor. The medium was changed every 3 days. The cultured rat DRG cells were treated with drugs tested on day 5 of culture. On the day, neurons exhibited globular cell bodies and slender axonal processes. Many non-neuronal cells such as Schwann cells and fibroblasts were present in the background.

PGE2 content in the culture medium was determined by enzyme immunoassay (Amersham Biosciences Co., Piscataway, NJ, USA) according to the manufacturer's protocol.

Total RNA in culture cells was prepared according to the method of Chomzynski and Sacchi [8]. The cDNAs encoding rat COX-2 (pCX2) and β-actin were prepared by reverse transcription PCR as described previously [12]. Ribonuclease protection assay was performed as described previously [12]. Briefly the radiolabeled antisense RNA probe for COX-2 and β–actin was added to 10 μg total RNA in hybridization buffer and incubated at 45℃ for 12 h. After the mixture was digested with 40 μg/ml RNase A at 30℃ for 1h and treated with proteinase K, the resulting hybridized

RNA was analyzed by electrophoresis on a 4% polyacrylamide/7 M urea gel. The intensity of each band on the gel was analysed using a model BAS2000 bioimaging analyzer (Fuji Film, Tokyo, Japan).

To determine the expression of COX-2 protein, DRG cells treated with BK (Peptide Institute Inc., Osaka, Japan) were lysed in 100 μL of lysis buffer containing 20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 10% glycerol, 20 μg/mL aprotinin, 20 μg/mL leupeptin, and 1 mM PMSF, and the lysate was centrifuged at 20,000 g for 10min at 4℃. The supernatant (30 μg protein/track) was analyzed by electrophoresis on a 10% SDS-polyacrylamide gel and electroblotted onto nitrocellulose membranes. The blot was blocked with 5% fat-free dried milk in 150 mM NaCl, 20 mM Tris/HCl pH7.4 and 0.02% Tween 20 (TBST) and incubated with primary monoclonal anti-human COX-2 antibody (1:2000 in TBST; Cayman Chemical, AnnArbor, MI, USA) at room temperature for 1 h. After washing with TBST, the blot was incubated with horseradish peroxidase-conjugated second anti-rabbit immunoglobulin G antibody (1:5000 in TBST; Amersham Biosciences Co., Piscataway, NJ, USA) for 1 h at room temperature. Immunoreactivity was detected using an enhanced chemiluminescence (ECL) detection system (Bio-Rad Lab, Inc., Hercules, CA, USA) and exposure to X-ray film (Fuji Film Tokyo Japan), and was quantified using software (Fuji Film, Tokyo, Japan).

Data are expressed as the mean ± SE values. The PGE2 amounts released and the relative levels of band intensity of the electrophoresis were evaluated by one-way ANOVA with pairwise comparison by the Bonferroni method. Differences were considered significant when the p value was <0.05.

As shown in Fig. 1a, short-term exposure of the DRG to BK at 1 μM for 30 min induced small but significant amounts of PGE2 release, while long-term exposure of BK at 1 μM for 3 h facilitated more than 8x PGE2 release compared with non-treated cells. Short-term exposure to

bradykinin-induced PGE2 release was partially inhibited by 10 nM SC-560, a selective COX-1 inhibitor or 1 μM NS-398, a selective COX-2 inhibitor respectively, and completely by 1 μg/mL indomethacin, a non-selective COX-1 and COX-2 inhibitor. Long-term exposure to bradykinin-induced PGE2 was partially inhibited by 10 nM SC-560, while it was completely inhibited by 1 μM NS-398 or 1 μg/mL indomethacin. Furthermore, the PGE2 release by 1 μM BK for 3 h was completely inhibited by HOE140, a BK B2 receptor selective antagonist but not by Lys-(Des-Arg^9, Leu^8)-BK, a BK B1 receptor antagonist (7.630±0.383 ng/mL PGE2 release by 1 μM BK, 6.380±0.077 ng/mL in the presence of 1 μM Lys-(Des-Arg^9, Leu^8)-BK and 0.551± 0.039 ng/mL in the presence of 10 μM HOE140). IL-1β did not release PGE2 during 30 min incubation, but induced PGE2 release during 3 h incubation. Simultaneously, the expression of COX-2 protein in DRG cells receiving long- and short-term exposure was measured by Western blotting as shown in Fig. 1b. The induction of COX-2 protein was detected after 3 hr exposure, not 30 min. COX-2 protein expression by long-term exposure to BK was not inhibited by COX inhibitors tested.

Fig. 2a shows the typical autoradiography of the ribonuclease protection assay for COX-2 mRNA in DRG cells incubated with 100 nM BK for 1 h. COX-2 mRNA was detected as a band of 577 bases, and β-actin mRNA was detected as an internal standard at a position corresponding to 310 bases. Fig. 2b shows the quantitative levels of COX-2 mRNA normalized to constant levels of β-actin mRNA in DRG cells treated with 100 nM BK for the indicated periods. The time-dependent and transient induction of COX-2 mRNA by BK was first observed 30 min after the addition of BK, then peaked at 1 h at about 5-fold the control (0 time); thereafter, COX-2 mRNA levels declined, reaching the control level after 3 h. In contrast, the level of COX-1 mRNA expression was constitutive and unchanged when the cultured DRG cells were incubated with 1 μM BK (data not shown). The BK-induced COX-2 mRNA expression after incubation for 1 h was inhibited by HOE140 but not by Lys-(Des-Arg^9, Leu^8)-BK (Fig. 2a , c).

Fig. 3a represents the relative protein expression of COX-2 by Western blotting in DRG cells treated with 1 μM BK for 1, 3, 6 and 12 h as a function of the time course. The expression of COX-2 protein was significant as early as 3 h after BK stimulation continued at 6 h, and declined at 12 h almost to the control level. The significant expression of COX-2 protein compared with the unstimulated control by 1 μM BK for 6 h was inhibited by HOE140 but not by Lys-(Des-Arg^9, Leu^8)-bradykinin (Fig. 3b).

We showed that the long-term, not short-term, exposure of primary cultured rat DRG cells to BK is responsible for PGE2 production by COX-2 activation. To our knowledge, this study is the first to demonstrate directly that BK induces COX-2 protein in rat DRG cells and that this induction is involved in BK-induced PGE2 generation. In human airway epithelial cells, BK has been shown to act through B2 receptor and activate the Ras/Raf-1/ERK pathway, which increases IκB kinase activity and finally induces NF-κB activation [7]. We have demonstrated that the effects of BK in this study are mediated by B2 receptor and preliminary observed that COX-2 protein production by BK was inhibited by U0126, a inhibitor of ERK (data not shown). Thus, possible similar mechanisms to airway epithelial cells might underlie our DRG cells leading to COX-2 expression by long-term exposure to BK, and are now under investigation.

The early release of PGE2 by BK exposure for 30 min was significantly inhibited by COX-1 inhibitor, suggesting that constitutive COX-1 is responsible for PGE2 production in short-term treatment. In our DRG cells, COX-1 mRNA was constitutively expressed [12] whether the cells were treated with BK or not. While NS-398 caused the small but significant inhibition of the early PGE2 release, maybe because the functional amount of COX-2 protein produced by 30 min exposure was too small to be detected by Western blotting analysis. Indeed, COX-2 mRNA was already expressed significantly at 30 min treatment (Fig. 2).

The change in COX-2 protein accumulation follows very closely the kinetic of COX-2 mRNA

expression, which was rapid and transient. This indicates that DRG cells have a high rate system of COX-2 turnover. Previous studies in our laboratory [12] have shown that interleukin-1β, a proinflammatory cytokine, induced the rapid appearance of COX-2 mRNA followed by a declining phase, and the steady-state expression persisted until 24 h. In WISH cells, the rapid and transient expression of COX-2 was induced by epidermal growth factor [24], while the steady–state expression of COX-2 mRNA and increasing accumulation of COX-2 protein for 24 h were induced by IL-1β and TNF [1]. Thus, the persistence of COX-2 expression is greatly influenced by the stimulus employed.

Two subtypes of BK receptors, B1 and B2, have been cloned from the rat genome [18, 20], and their mediated responses can be distinguished pharmacologically by the effective potencies of BK receptor ligands. The inhibitory effects of selective antagonists on COX-2 mRNA and protein production and PGE2 release suggests that the actions of BK-induced COX-2 expression in DRG cells are mediated by B2 receptors. B2 receptors are constitutively expressed in a subpopulation of rat DRG neurons [27] and are upregulated in response to nerve injury [25]; however, there are contradictory reports about the existence of B1 receptors in rat DRG neurons. Some detected B1 receptor mRNA by RT-PCR in normal DRG [15, 17], and another indicated the lack of B1 receptor expression in both normal conditions and after nerve injury [6]. In our results, the selective B2-receptor antagonist HOE140 completely abolished the effects of BK, suggesting no contribution of B1 receptors in BK actions in our cultured rat DRG cells.

BK induces sensory neuron excitation and sensitization of nociceptors, which probably underlies the phenomenon of peripheral hyperalgesia during inflammation. BK is not only a powerful inflammatory mediator by itself [10] but can directly activate cPLA2 to release arachidonic acid [11] and induce COX-2 expression. Consequently, BK induces exaggerated prostanoid production around sensory neurons. Prostanoids have long been known to sensitize sensory neurons to facilitate the release of neuropeptides such as substance P, neurokinin A and

calcitonin gene related peptide [19, 28], which mediate neurogenic inflammation and contribute to nociceptor sensitization in an autocrine manner. We observed that BK induced the facilitation of capsaicin-evoked substance P release by long-term treatment for 3 h in our rat cultured DRG cells [22].

The rat cultured DRG cells we used were fed with 50 ng/mL NGF. NGF has several important effects on the morphological and functional properties of sensory neurons. Although the cultured DRG cells from a fetus or newborn rats cannot survive without NGF, adult rat cultured DRG cells can survive without NGF. However, the phenotype of adult rat cultured DRG cells changes with NGF, which enhances the sensitivity of DRG neurons to capsaicin and noxious heat [4], and increases the expression of substance P, TrkA receptor, TRPV1, and P2X3 ATP receptors [2, 3, 16, 26]. These events may be reflected in the facilitation of processing nociceptive signals. This preparation might therefore reflect the pathophysiological conditions of inflammation-induced hyperalgesia. In the case of B2 receptors, NGF has been reported to increase B2 receptor expression in mouse DRG [14]; however, we observed no effects of NGF on B2 receptor mRNA levels in our DRG cells (data not shown) and the question of whether our DRG cells reflect pathophysiological conditions remains unclear and is under study.

In conclusion, our studies have demonstrated that rat DRG cells release PGE2 in response to BK stimulation by two different mechanisms. Although both are mediated by B2 receptors, one is due to COX-1 activity in short-term treatment with BK and the other is mainly due to the induction of COX-2 in long-term treatment. These results are consistent with the hypothesis that COX-1 is a constitutive isoform, while COX-2 is an inducible isoform responsible for the generation of prostanoids under inflammatory conditions. Since peripheral inflammation induces the local production of kinin and proinflammatory cytokines, and since IL-1β [12] and BK have been shown to induce COX-2 protein, which may cause prostanoid generation, this may play an important role in the pathogenesis of inflammatory pain and hyperalgesia around primary afferent neurons.

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References

[1] T. Albert, H.C. Su, P.D. Zimmerman, J.D. Iams, D.A. Kniss, Interleukin-1 beta regulates the inducible cyclooxygenase in amnion-derived WISH cells, Prostaglandins 48 (1994) 401-416.

[2]F. Amaya, G. Shimosato, M. Nagano, M. Ueda, S. Hashiomoto, Y. Tanaka, H. Suzuki, M. Tanaka, NGF and GDNF differentially regulate TRPV1 expression that contributes to development of inflammatory thermal hyperalgesia, Eur. J. Neurosci. 20 (2004) 2303-2310.

[3]D.L. Bennett, S. Averill, D.O. Clary, J.V. Priestley, S.B. McMahon, Postnatal changes in the expression of the trkA high-affinity NGF receptor in primary sensory neurons, Eur. J. Neurosci. 8 (1996) 2204-2208.

[4]J.K. Bonnington and P.A. McNaughton, Signalling pathways involved in the sensitization of mouse nociceptive neurons by nerve growth factor, J. Physiol. 551 (2003) 433-446.

[5] D.A. Bradbury, R. Newton, T.-M. Zhu, J. Stocks, L. Corbett, E.D. Holland, L.H. Pang, A.J. Knox, Effect of bradykinin, TGF-β1, IL-1β, and hypoxia on COX-2 expression in pulmonary artery smooth muscle cells, Am. J. Physiol. Lung Cell Mol. Physiol. 283 (2002) L717-L725.

[6] M. Brand, A. Klusch, O. Kurzai, M. Valdeolmillos, R.F. Schmidt, M. Petersen, No evidence for bradykinin B1 receptors in rat dorsal root ganglion neurons, Neuroreport 12 (2001) 3165-3168.

[7] B.-C. Chen, C.-C. Yu, H.-C. Lei, M-S. Chang, M.-J. Hsu, C.-L. Huang, M.-C. Chen, J.-R. Sheu, T.-F. Chen, T.-L. Chen, H. Inoue, C.-H. Lin, Bradykinin B2 receptor mediates NF-κB activation and cyclooxygenase-2 expression via the Ras/Raf-1/ERK pathway in human airway epithelial cells, J. Immunol. 173 (2004) 5219-5228.

[8]P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1987 156-159.

[9] H. Chuang, E.D. Prescott, H. Kong, S. Shields, S.-E. Jordt, A.I. Basbaum, M.V. Chao, D. Jullius, Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition, Nature 411 (2001) 957-962.

[10] A. Dray, M. Perkins, Bradykinin and inflammatory pain, Trends Neurosci. 16 (1993) 99-104.

[11] S. Harwalkar, C.H. Chang, N.O. Dulin, J.G. Douglas, Role of phospholipase A2 isozymes in agonist-mediated signaling in proximal tubular epithelium, Hypertension. (1998)31,809-814.

[12] A. Inoue, K. Ikoma, N. Morioka, K. Kumagai, T. hashimoto, I. Hide, Y. Nakata, Interleukin-1 β induces substance P release from primary afferent neurons through the cyclooxygenase-2 system, J. Neurochem. 73 (1999) 2206-2213.

[13] D.W. Jenkins, L.A. Sellers, W. Feniuk, P.P. Humphrey, Characterization of bradykinin-induced prostaglandin E2 release from cultured rat trigeminal ganglion neurons, Eur. J. Pharmacol. 469 (2003) 29-36.

[14]Y-J. Lee, O. Zachrisson, D.A. Tonge, P.A. McNaughton, Upregulation of bradykinin B2 receptor expression by neurotrophic factors and nerve injury in mouse sensory neurons, Mol. Cell. Neurosci. 19 (2002) 186-200.

[15] D. Levy, D.W. Zochodne, Increased mRNA expression of the B1 and B2 bradykinin receptors and antinociceptive effects of their antagonists in an animal model of neuropathic pain, Pain 86 (2000) 265-271.

[16]R.M. Lidsay, A.J. Harmar, Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons, Nature 337 (1989) 362-364.

[17] Q.P. Ma, R. Hill, D. Sirinathsighj, Basal expression of bradykinin B1 receptor in peripheral sensory ganglia in the rat. Neuroreport 11 (2000) 4003-4005.

[18] A.E. McEaxhern, E.R. Shelton, S. Bhakta, R. Obernolte, C. Bach, P. Zuppan, J. Fujisaki, R.W.

Aldrich, K. Jarnagin, Expression cloning of a rat B2 bradykinin receptor, Proc. Natl. Acad. Sci. USA (1991), 7724-7728.

[19] P. Newbold, S.D. Brain, The modulation of inflammatory oedema by calcitonin gene-related peptide, Br. J. Pharmacol. 108 (1993) 705-710.

[20] A. Ni, K.X. Chai, L. Chao, J. Chao, Molecular cloning and expression of rat bradykinin B1 receptor, Biochim. Biophys. Acta 1442 (1998) 177-185.

[21] M. Numazaki, T. Tominaga, H. Toyooka, M. Tominaga, Direct phosphorylation of capsaicin receptor VR1 by protein kinase C ε and identification of two target serine residues, J. Biol. Chem. 277 (2002) 13375-13378.

[22] K. Oshita, A. Inoue, H.-B, Tand, Y. Nakata, M. Kawamoto, O. Yuge, CB1 cannabinoid receptor stimulation modulates transient receptor potential vanilloid receptor 1 activates in calcium influx and substance P release in cultured dorsal root ganglion cells, J. Pharmacol. Sci. 97 (2005) 377-385.

[23] L. Pang, A.J. Knox, PGE2 release by bradykinin in human airway smooth muscle cells: involvement of cyclooxygenase-2 induction, Am. J. Physiol. 273 (1997) L1132-L1140.

[24] D.J. Perkins, D.A. Kniss, Rapid and transient induction of cyclo-oxygenase 2 by epidermal growth factor in human amnion-derived WISH cells, Biochem J. 321 (1997) 677-681.

[25] M. Petersen, A.S. Eckert, G. Segond von Banchet, B. Heppelmann, A. Klusch, K.D. Kniffki, Plasticity in the expression of bradykinin binding sites in sensory neurons after mechanical nerve injury, Neuroscience 83 (1998) 949-959.

[26]M.S. Ramer, E.J. Bradbury, S.B. McMahon, Nerve growth factor induces P2X(3) expression in sensory neurons. J. Neurochem. 77 (2001) 864-875.

[27] G. Segond von Banchet, M. Peterse, B. Heppelmann, Bradykinin receptors in cultured rat dorsal root ganglion cells: influence of length of time in culture, Neuroscience 75 (1996)

[28] M.D. Southall, M.R. Vasko, Prostaglandin receptor subtypes, EP3C and EP4, mediate the prostaglandin E2-induced cAMP production and sensitization of sensory neurons, J. Biol. Chem.

(2001) 276, 16083-16091.

[29] K. Tanaka, H. Kawasaki, K. Kurata, Y. Aikawa, Y. Tsukamoto, T. Inaba, T-614, a novel antirheumatic drug, inhibts both the activity and induction of cyclooxygenase-2 (COX-2) in cultured fibroblasts, Jpn. J. Pharmacol. 67 (1995) 305-314.

[30] H.-B. Tang, A. Inoue, K. Oshita, Y. Nakata, Sensitization of vanilloid receptor 1 induced by bradykinin via the activation of second messenger signaling cascades in rat primary afferent neurons, Eur. J. Pharmacol. 498 (2004) 37-43.

[31] J.R. Vane, Y.S. Bakhle, R.M. Botting, Cyclooxygenase 1 and 2, Annu. Rev. Pharnacol. Toxicol. 38 (1998) 97-120.

Legends

Fig. 1. Release of PGE2 and expression of COX-2 protein by exposing of rat DRG cells to BK for 30 min or 3 h. Rat DRG cells were incubated with 1 μM BK in the absence (BK) and presence of 10 nM SC-560 (BK+SC), 1 μM NS-398 (BK+NS) or 1 μg/mL indomethacin (BK+IDM) and 1 ng/mL IL-1β (IL-1β) for 30 min (left panel) or 3 h (right panel) at 37℃. a) PGE2 levels in the medium of non-treated DRG cells (C) and each treated DRG cell are shown. Data are the mean ± SEM (bars) values of four independent experiments. * $p<0.05$ compared with non-treated control (C) and + $p<0.05$ compared with BK-alone treatment (BK). b) Typical Western blottings of COX-2 protein or β-actin protein in non-treated DRG cells (C) and each treated DRG cell are shown. Numbers under the blots indicate the relative levels of COX-2 proteins expressed as the ratio to control (non-treated cells) of the band intensity ratio of COX-2 protein versus β-actin protein in treated DRG cells. These are the mean ± SEM values of four independent experiments. *$p<0.05$ compared with non-treated DRG cells (C).

Fig. 2. Expression of COX-2 mRNA by BK in rat DRG cells. a) A typical autoradiography of

COX-2 mRNA analysed by a ribonuclease protection assay. Each lane represents the protected bands of COX-2 mRNA (577b) and β-actin mRNA (310b) as an internal standard expressed in rat DRG cells nontreated and incubated with 100 nM BK for 1h in the absence (BK) or presence of BK receptor antagonists, a B2 antagonist, 10 μM HOE140 (HOE) or a B1 antagonist, 1 μM Lys-(Des-Arg^9, Leu^8)-BK (B1 ant), the labeled probes for COX-2 (Pcx2; 632bases) and for β-actin (Pact; 390bases). b) Relative hybridization levels for COX-2 mRNA are expressed as the band intensity ratio of the mRNA (intensity of COX-2 mRNA/intensity of β-actin mRNA) in rat DRG cells incubated with BK for 10 m, 30 m, 1 h, 3 h or 12 h. Data are the mean ± SEM (bars) values of three independent experiments. *$p<0.05$ compared with nontreated cells. c) Relative hybridization levels for COX-2 mRNA in rat DRG cells nontreated (C) and incubated with BK for 1 h in the absence (BK) or presence of BK receptor antagonists, a B2 antagonist, 10 μM HOE140 (HOE) or a B1 antagonist, 1 μM Lys-(Des-Arg^9, Leu^8)-BK (B1 ant) expressed as described in the legends for (b). Data are the mean ± SEM (bars) values of four independent experiments. *$p<0.05$ compared with nontreated cells. ++$p<0.05$ compared with BK-treated cells.

Fig. 3. Expression of COX-2 protein by BK in rat DRG cells. a) Relative levels of COX-2 protein are expressed as the band intensity ratio to those of β-actin protein in rat DRG cells incubated with 1 μM BK for 1, 3, 6, or 12 h. Data are the mean ± SEM (bars) values of three independent experiments. *$p<0.05$ compared with nontreated cells. (lower panel) A typical autoradiography of COX-2 protein (71k) analysed by Western blotting. b) Relative levels of COX-2 protein in rat DRG cells nontreated (C) and incubated with 1 μM BK for 3 h in the absence (BK) or presence of BK receptor antagonists, a B2 antagonist, 10 μM HOE140 (HOE) or a B1 antagonist, 1 μM Lys-(Des-Arg^9, Leu^8)-BK (B1 ant). Data are the mean ± SEM (bars) values of four independent experiments. *$p<0.05$ compared with nontreated cells. +$p<0.05$ compared with BK-treated cells.

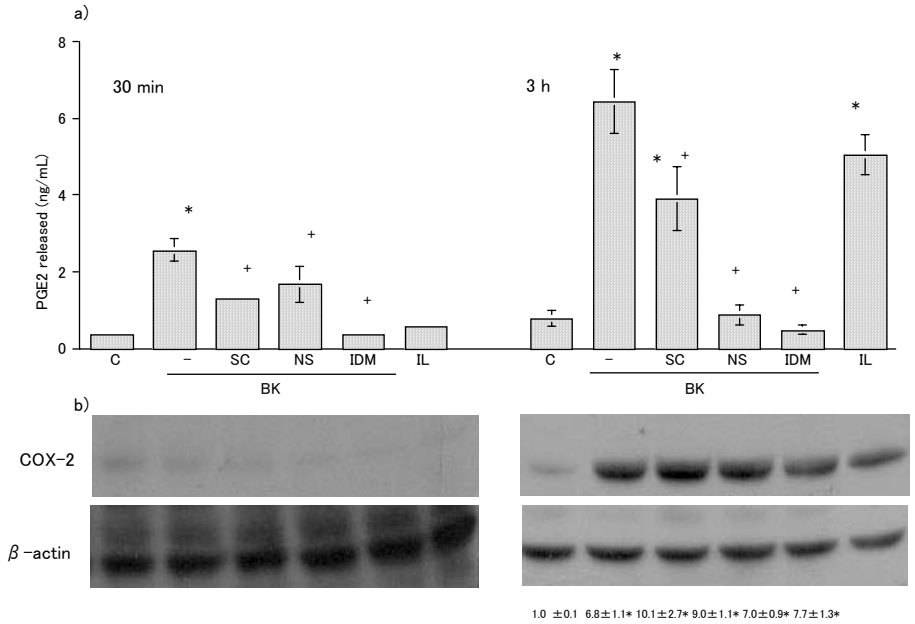

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Fig.1

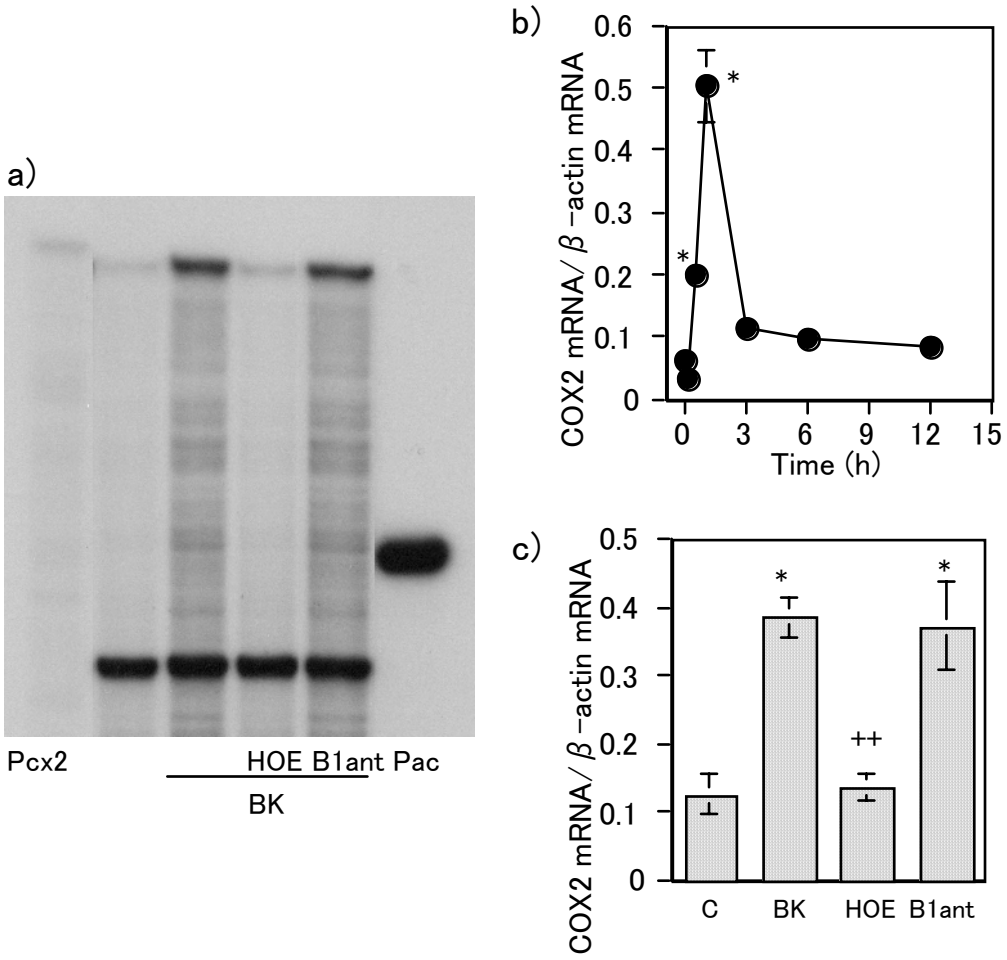

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Fig. 2

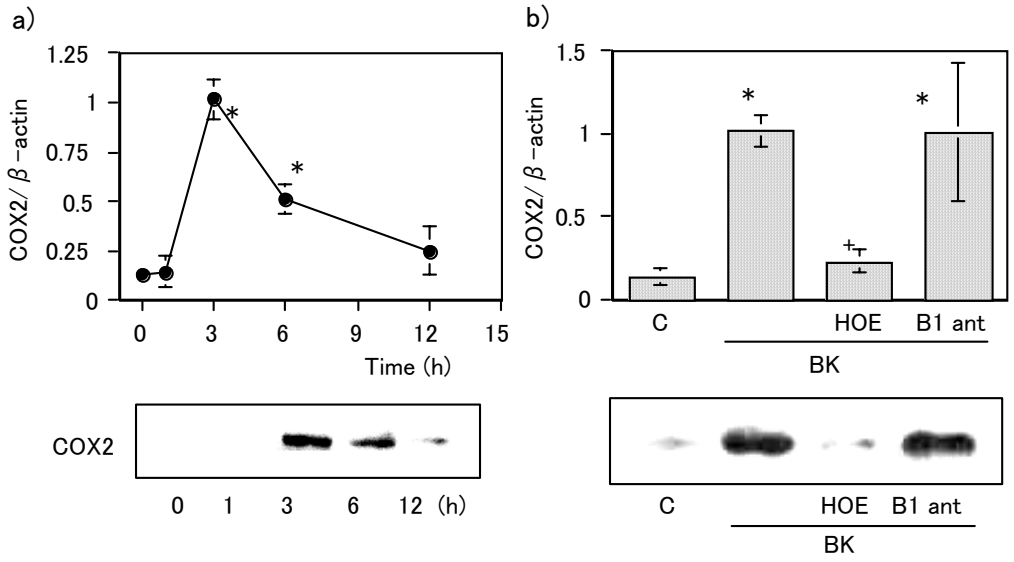

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Fig. 3