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Sensitization of vanilloid receptor 1 induced by bradykinin via the activation of second messenger signaling cascades in rat primary afferent neurons

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Abstract

Vanilloid receptor 1 was recently reported to play an important role in hyperalgesia, but the mechanisms by which this receptor is activated by endogenous inflammatory mediators, such as bradykinin and nerve growth factor, are not yet fully understood. Here, we investigated whether bradykinin, which is a pain-producing inflammatory mediator, sensitizes vanilloid receptor 1 by inducing the activation of cyclooxygenases, phospholipase C and phospholipase A_2 in rat dorsal root ganglion cells. We demonstrated this using $^{45}Ca^{2+}$ uptake and inositol phosphates accumulation assays, bradykinin activates phospholipase C and cyclooxygenase-1 through the bradykinin B_2 receptor. The bradykinin B_2 receptor then sensitizes vanilloid receptor 1 activity by facilitating non-selective Ca^{2+} channel activity, increasing the intracellular Ca^{2+} concentration from the extracellular pool. These methods would be useful for screening new drugs for activity at vanilloid receptor 1. These data suggest that endogenous substances produced by several enzymes may be capable of producing a synergistic response involving the vanilloid receptor 1.

Keywords: Bradykinin; Cyclooxygenase; Phospholipase A_2; Phospholipase C; Vanilloid receptor 1

1. Introduction

Vanilloid receptor 1, a cloned capsaicin receptor, is a member of the transient receptor potential family of non-selective Ca^{2+} channels. Over 80% of dorsal root ganglion cells express vanilloid receptor 1 (Helliwell et al., 1998), which can be activated by capsaicin, noxious heat, extracellular protons, and a structurally heterogeneous group of compounds named vanilloids, encompassing also the endogenous ligands *N*-arachidonoyldopamine and *N*-oleoyldopamine (Caterina et al., 1997; Huang et al., 2002; Shin et al., 2002; Chu et al., 2003; Toth et al., 2003). Moreover, vanilloid receptor 1 knockout mice have deficits in thermal inflammation-induced hyperalgesia (Caterina et al., 2000; Davis et al., 2000). Thus the vanilloid receptor 1 is now considered to be a molecular detector that perceives various pain stimuli.

Capsaicin, a pungent chemical present in hot peppers, produces immediate pain or hyperalgesia, and an influx of Ca^{2+} in a dose-dependent manner in cultured sensory neurons (Wood et al., 1988). Recently, capsaicin was reported to bind to the intracellular domain of vanilloid receptor 1, opening a non-selective Ca^{2+} channel expressed predominately in sensory neurons (Caterina et al., 1997; Jung et al., 1999; Davis et al., 2000). Therefore, it is an excellent pharmacological tool for stimulating this receptor directly.

Bradykinin is a potent pro-inflammatory mediator which can stimulate a pain response in various animal models and in humans (Kameyama et al., 1987; Manning et al., 1991;

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Hargreaves et al., 1993; Pyne and Pyne, 1993; Vasko et al., 1994). A line of electrophysiological studies has shown that bradykinin sensitizes the vanilloid receptor 1 by means of endogenous substances produced by the activation of bradykinin B_2 receptors in dorsal root ganglion cells (Chuang et al., 2001; Shin et al., 2002) and by activating protein kinase C (Premkumar and Ahern, 2000; Sugiura et al., 2002).

Concomitantly released endogenous substances in dorsal root ganglion cells following stimulation of bradykinin B_2 receptors are likely to produce a synergistic response at the vanilloid receptor 1 stimulated by capsaicin to modulate the sensation of pain. Hence we examined the effect of several enzyme inhibitors and bradykinin B_2 antagonists which block the cascade of second messenger pathways stimulated by bradykinin and capsaicin, by monitoring $^{45}Ca^{2+}$ uptake and by measuring inositol phosphate (Ins(X)*P*) accumulation in rat primary dorsal root ganglion cells.

2. Materials and methods

2.1. Isolation and culture of dorsal root ganglion cells

Young adult Wistar rats (6–9 weeks) were housed in cages under a 12-h light/dark cycle with free access to food and water. All procedures used in the experiments were approved by the Animal Care and Use Committee of Hiroshima University School of Medicine, Hiroshima, Japan. Dorsal root ganglia were removed from young adult Wistar rats and were dissociated into single isolated neurons by enzyme treatment with 0.125% collagenase for 90 min (twice), followed by 0.25% trypsin for 30 min at 37 °C and by trituration with fire-polished Pasteur pipettes of decreasing tipdiameter (Inoue et al., 1999). The cells were plated on polyethyleneimine and laminin-coated dishes and incubated in Dulbecco's modified Eagle's medium containing 10% heat-inactivated horse serum, 1% penicillin/streptomycin and 200 mM glutamine. The cultures were incubated at 37 °C in a water-saturated atmosphere with 5% CO_2 for 5 days before the experiment. On the fifth day of culture, neurons exhibited globular cell bodies and extended axonal processes. Various non-neuronal cells, such as Schwann cells, fibroblasts and satellite cells, were also present as background.

2.2. Measurement of $^{45}Ca^{2+}$ uptake into dorsal root ganglion cells

Dorsal root ganglion cells were pre-incubated for 10 min in Krebs–HEPES buffer (NaCl 110, KCl 4.5, $CaCl_2$ 1.3, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, D-glucose 11.7, HEPES 5 (mM)) with or without test drugs. After pre-incubation, the buffer was discarded from each dish and $^{45}Ca^{2+}$-labeled buffer (final concentration 0.375 μCi/ml in Krebs–HEPES buffer without 4.5 mM $CaCl_2$) with or without test drugs was added to each dish and incubated for 10 min at 37 °C. After being washed with cold Krebs–HEPES buffer (Ca^{2+} full), cells were dissolved with 1 N NaOH at room temperature for 12 h. The cell lysate from each sample was counted with a liquid scintillation counter (Winter et al., 1988; Wood et al., 1988).

2.3. Measurement of the accumulation of Ins(X)P in dorsal root ganglion cells

Dorsal root ganglion cells were washed twice with Dulbecco's modified Eagle's medium (serum free) and cells were labeled with [^{3}H] myo-inositol (3 μCi/ml) for 20 h. After labeling, cells were washed twice with Krebs–HEPES buffer and pre-incubated in Krebs–HEPES containing 10 mM LiCl buffer with or without test drugs for 15 min. The various concentrations of bradykinin were added for 30 min to stimulate the phosphoinositide (PI) response. The reactions were terminated by addition of ice-cold 50% trichloroacetic acid (5% of final concentration) and the samples were cooled on ice for 60 min. The samples were neutralized with 200 mM KOH plus 50 mM Tris solution and centrifuged at 10,000 rpm for 5 min. The collected supernatant was applied to 1 ml Dowex-X8 columns (100–200 mesh, chloride form, Bio-Rad), and washed with 2 ml of 50 mM Tris solution, followed by 20 ml distilled water. Finally the Ins(X)*P* contained in these columns was extracted with 2 ml of 1N HCl and this was counted with a liquid scintillation counter (Berridge et al., 1983).

2.4. Statistics

All the experiments were repeated three or more times with similar results. The results in the text and in the figures are presented as percentages of the control and *n* indicates the number of cells tested. The control was not treated with any test drug. Statistical analyses were performed using Student's unpaired *t*-test. The differences between two groups were considered to be statistically significant when $P<0.05$.

2.5. Materials

Bradykinin was obtained from Peptide Institute (Osaka, Japan). Dulbecco's modified Eagle's medium was from Nissui Pharmaceutical (Tokyo, Japan). Horse serum and penicillin/streptomycin were from GibcoBRL (Gaithersburg, MD, USA). Mouse laminin was from Upstate Biotechnology (Lake Placid, NY, USA). Nerve growth factor (2.5 S) was from Promega (Madison, WI, USA). $^{45}Ca^{2+}$ (2.4 mCi/ml) and [^{3}H] myo-inositol (18 Ci/mmol) were from Amersham Pharmacia Biotech (Amersham, UK). Arachidonyl trifluoromethyl ketone ($AACOCF_3$) was from Biomol (Plymouth Meeting, PA, USA). NS-398 (*N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanosulfonamide)

and SC-560 (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole) were from Cayman Chemical (Ann Arbor, MI, USA). Trypsin (2.5%) was from Invitrogen (Canada). Collagenase (Type I), capsazepine, indomethacin and polyethyleneimine, HOE 140 (D-Arg(Hyp^3, Thi^5, D-Tic^7, Oic^8) bradykinin), U73122 (1-[6-((17b-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione), and U73343 (1-[6-([17-3-methoxyestra-1,3,5(10)-trien-17-yl]amino)hexyl]-2,5-dione) were from Sigma (St. Louis, MO, USA).

3. Results

3.1. Effects of capsaicin and bradykinin on $^{45}Ca^{2+}$ uptake into dorsal root ganglion cells

As shown in Fig. 1, a 10-min treatment with capsaicin increased $^{45}Ca^{2+}$ uptake into dorsal root ganglion cells in a dose-dependent manner from 50 to 1000 nM, whereas bradykinin (50 nM) by itself did not significantly increase $^{45}Ca^{2+}$ uptake (113±7% of the control (100±7%)) into these cells. Based on these results, we selected 200 nM capsaicin and 50 nM bradykinin to examine their cooperative action on the vanilloid receptor 1 by measuring capsaicin-stimulated $^{45}Ca^{2+}$ uptake.

3.2. Effects of bradykinin on capsaicin-induced $^{45}Ca^{2+}$ uptake into dorsal root ganglion cells

Bradykinin (50 nM) enhanced significantly capsaicin-induced $^{45}Ca^{2+}$ uptake (177±6% of the control (100±4%)) compared with that of capsaicin stimulation alone (136±4% of the control). HOE 140, a specific bradykinin B_2 receptor antagonist, inhibited only the enhancement of $^{45}Ca^{2+}$ uptake induced by bradykinin (139±4% of the control), but did not influence capsaicin-induced $^{45}Ca^{2+}$ uptake. However, capsazepine, a vanilloid receptor 1 antagonist, depressed completely the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake elicited by bradykinin (90±5% of the control), as shown in Fig. 2. These results suggested that the bradykinin-induced enhancement of $^{45}Ca^{2+}$ uptake might be mediated through the activation of the bradykinin B_2 receptor, and that $^{45}Ca^{2+}$ uptake could involve the non-selective Ca^{2+} channel in the vanilloid receptor 1 in dorsal root ganglion cells.

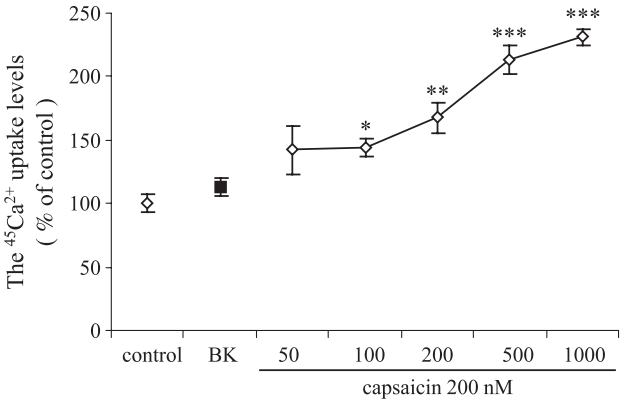

Fig. 1. Effects of bradykinin and capsaicin on $^{45}Ca^{2+}$ uptake into rat cultured dorsal root ganglion cells. Dorsal root ganglion cells were cultured for 5 days with nerve growth factor and then the cells were stimulated with bradykinin (BK, 50 nM) or various concentrations of capsaicin for 10 min at 37 °C in $^{45}Ca^{2+}$-labeled buffer (final concentration 0.375 μCi/ml in Krebs–HEPES buffer without 4.5 mM $CaCl_2$), respectively. After extensive washing in Ca^{2+}-containing buffer, the amount of $^{45}Ca^{2+}$ accumulated in the cells in each well was counted with a liquid scintillation counter. Data are expressed as means±S.E. (bars) from three separate experiments. ** or *** denoted $P<0.01$ or 0.001, compared with the control, respectively.

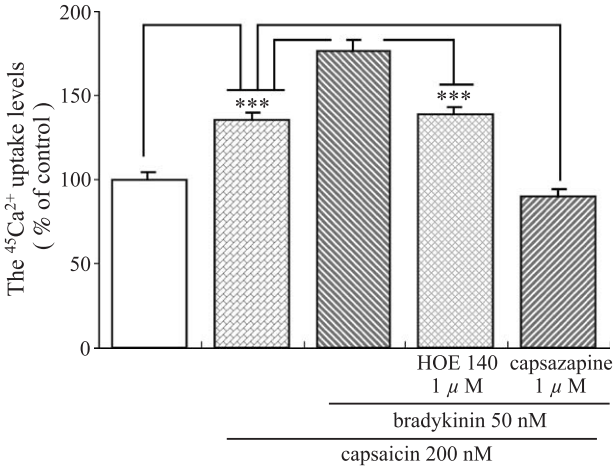

Fig. 2. Effects of HOE 140 and capsazepine on the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin in rat cultured dorsal root ganglion cells. Dorsal root ganglion cells were stimulated with 50 nM bradykinin in addition to 200 nM capsaicin, or capsaicin plus 1 μM HOE140 or 10 μM capsazepine for 10 min at 37 °C in $^{45}Ca^{2+}$-labeled buffer (final concentration 0.375 μCi/ml in Krebs–HEPES buffer without 4.5 mM $CaCl_2$). After extensive washing in Ca^{2+}-containing buffer, the amount of $^{45}Ca^{2+}$ accumulated in the cells in each well was determined. Data are expressed as means±S.E. (bars) from four to five separate experiments. ** or *** denoted $P<0.01$ or 0.001, respectively.

3.3. Effects of cyclooxygenase and phospholipase C inhibitors on the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin

To study whether the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin was associated with the activation of cyclooxygenase, we used SC-560 (a highly selective cyclooxygenase-1 inhibitor), indomethacin (a non-selective cyclooxygenase-1, -2 inhibitor), and NS-398 (a highly selective cyclooxygenase-2 inhibitor). Both cyclooxygenase-1 inhibitors returned $^{45}Ca^{2+}$ uptake levels to those induced by capsaicin without having an effect on $^{45}Ca^{2+}$ uptake by themselves (data not shown), but NS-398 did not have an effect on the enhancement of $^{45}Ca^{2+}$ uptake induced by bradykinin (Fig. 3). These results suggest that the bradykinin enhancement of $^{45}Ca^{2+}$ uptake induced by capsaicin may be mediated through activation of cyclo-

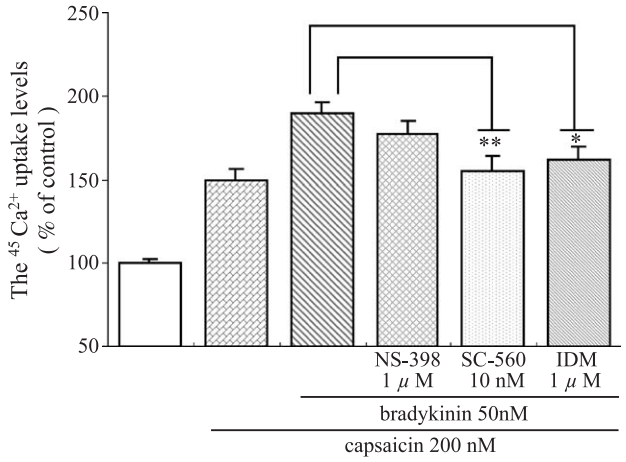

Fig. 3. Effects of cyclooxygenase inhibitors (NS-398, SC-560 and indomethacin) on the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin in rat cultured dorsal root ganglion cells. Dorsal root ganglion cells were stimulated with 50 nM bradykinin in addition to 200 nM capsaicin, or capsaicin plus cyclooxygenase inhibitors (1 μM of NS-398, 10 nM of SC-560 or 1 μM of indomethacin (IDM)) for 10 min at 37 °C in $^{45}Ca^{2+}$-labeled buffer (final concentration 0.375 μCi/ml in Krebs–HEPES buffer without 4.5 mM $CaCl_2$). After extensive washing in Ca^{2+}-containing buffer, the amount of $^{45}Ca^{2+}$ accumulated in the cells in each well was determined. Data are expressed as means±S.E. (bars) from six to seven separate experiments. ** or *** denoted $P<0.01$ or 0.001, respectively.

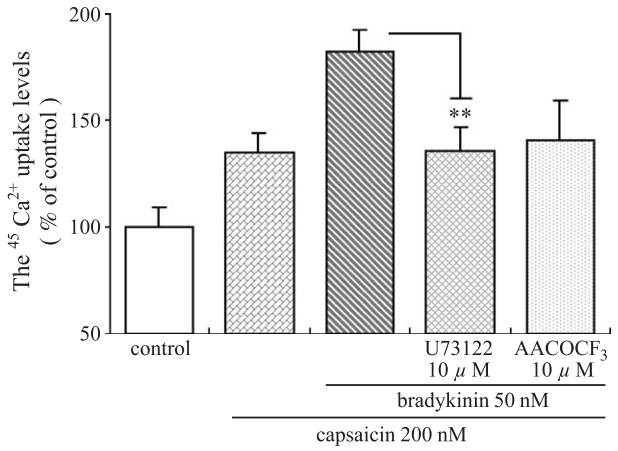

Fig. 4. Effects of U73122 and $AACOCF_3$ on the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin in rat cultured dorsal root ganglion cells. Dorsal root ganglion cells were stimulated with 50 nM bradykinin in addition to 200 nM capsaicin, or capsaicin plus 10 μM of U73122 or 10 μM of $AACOCF_3$ for 10 min at 37 °C in $^{45}Ca^{2+}$-labeled buffer (final concentration 0.375 μCi/ml in Krebs–HEPES buffer without 4.5 mM $CaCl_2$). After extensive washing in Ca^{2+}-containing buffer, the amount of $^{45}Ca^{2+}$ accumulated in the cells in each well was determined. Data are expressed as means±S.E.(bars) from four to ten separate experiments. ** denoted $P<0.01$.

oxygenase-1, which is the key enzyme in prostaglandin biosynthesis.

Two selective inhibitors of phospholipase C and cytosolic phospholipase A_2 were used to investigate whether phospholipase C or/and cytosolic phospholipase A_2 is involved in sensitizing the non-selective Ca^{2+} channels of the vanilloid receptor 1 stimulated by capsaicin and bradykinin. As shown in Fig. 4, both U73122 (a selective inhibitor of phospholipase C) and $AACOCF_3$ (a selective inhibitor of cytosolic phospholipase A_2) tended to decrease the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin (182±10%) to levels induced by capsaicin stimulation alone (135±9%). Pretreatment of dorsal root ganglion cells with U73122 resulted in a significant reduction of the capsaicin-induced $^{45}Ca^{2+}$ uptake enhanced by bradykinin (136±11%), but the effect of $AACOCF_3$ varied and did not reach significance (141±18 %). U73343 (10 μM), the inactive analogue of U73122, did not have any effect on $^{45}Ca^{2+}$ uptake (Data not shown). These results suggest the activation of phospholipase C by bradykinin in dorsal root ganglion cells is involved in the sensitization of the vanilloid receptor 1.

3.4. Effects of cyclooxygenase inhibitors on accumulation of Ins(X)P stimulated by bradykinin

As bradykinin, acting via the bradykinin B_2 receptor, can also increase the hydrolysis of PIP_2 to activate phospholipase C, the extent of Ins(X)*P* accumulation is considered to be direct evidence of phospholipase C activation. We examined the effects of a bradykinin B_2 antagonist and inhibitors, which showed inhibitory effects on $^{45}Ca^{2+}$ uptake, on Ins(X)*P* accumulation in dorsal root ganglion cells labeled with [^{3}H] myo-inositol and stimulated by bradykinin. As shown in Fig. 5, bradykinin increased the amount of Ins(X)*P* accumulated in a dose-dependent manner. The increase in Ins(X)*P* accumulation induced by bradykinin was inhibited completely by pretreatment with HOE 140. Finally, we examined whether the increase in

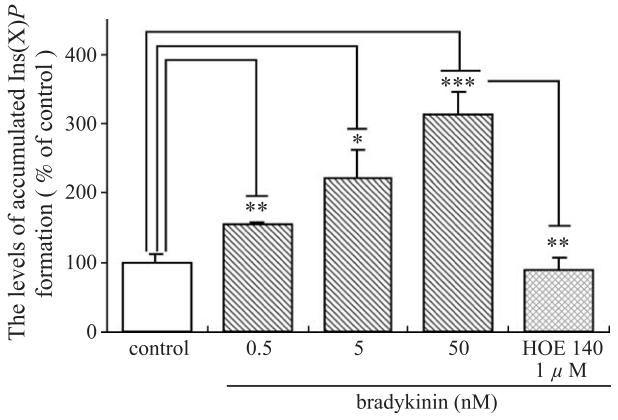

Fig. 5. Effects of bradykinin on the accumulation of Ins(X)*P* in rat cultured dorsal root ganglion cells. Dorsal root ganglion cells were grown for 5 days with nerve growth factor and then the cells were labeled for 20 h in Dulbecco's modified Eagle's medium containing 3 μCi/ml of [^{3}H] myo-inositol. After labeling, the cells were pre-incubated for 15 min in Krebs–HEPES buffer containing 10 mM LiCl with or without HOE 140 before treatment with bradykinin (the time of treatment was 30 min). Data are expressed as means±S.E.(bars) from three to nine separate experiments. ** or *** denoted $P<0.01$ or 0.001, respectively.

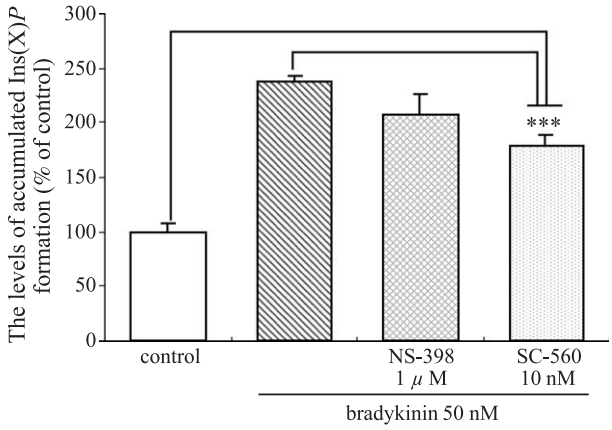

Fig. 6. Effects of bradykinin with or without indomethacin, NS-398 or SC-560 on the accumulation of Ins(X)*P* in rat cultured dorsal root ganglion cells. Dorsal root ganglion cells were grown for 5 days with nerve growth factor and then the cells were labeled for 20 h in Dulbecco's modified Eagle's medium containing 3 μCi/ml of [^{3}H] myo-inositol. After labeling, the cells were pre-incubated for 15 min in Krebs–HEPES buffer containing 10 mM LiCl and with or without the inhibitors of cyclooxygenases before treatment with 50 nM of bradykinin (the time of treatment was 30 min). Data are expressed as means±S.E. (bars) from five to seven separate experiments. *** denoted $P<0.001$.

Ins(X)*P* accumulation elicited by bradykinin was inhibited by cyclooxygenase inhibitors. The effects of SC-560 and NS-398 on Ins(X)*P* accumulation stimulated by bradykinin are shown in Fig. 6. Bradykinin (50 nM) increased significantly the amount of Ins(X)*P* accumulated (237±6%) compared with the control (100±8%). The increased Ins(X)*P* accumulation stimulated by bradykinin was significantly inhibited by SC-560 (178±11% of the control (100±8%)), but not by NS-398 (208±18% of the control (100±8%)).

4. Discussion

The vanilloid receptor 1 is a member of the transient receptor potential (TRP) family of non-selective Ca^{2+} channels that can be directly activated by capsaicin (Oh et al., 1996; Jung et al., 1999) and endogenous capsaicin-like substances. Indeed, Hwang et al. (2000) showed, in electrophysiological experiments, that several products of lipoxygenases directly enhance capsaicin-induced non-selective Ca^{2+} channel activity in isolated membrane patches of sensory neurons. Shin et al. (2002) also demonstrated that bradykinin activates non-selective Ca^{2+} channels in cultured sensory neurons via lipoxygenase products. Recently, Ferreira et al. (2004) demonstrated that intraplantar administration of bradykinin in mice produces severe nociception mediated by non-selective Ca^{2+} channel stimulated by phospholipase C pathway activation and lipoxygenase products. Therefore, bradykinin is likely to produce inflammatory pain by activating not only phospholipase C but also lipoxygenases as well as cyclooxygenases. We have shown in this study that the measurement of vanilloid receptor 1 activity, monitored by $^{45}Ca^{2+}$ uptake and the amount of Ins(X)*P* accumulated, provides new insight into the mechanism of sensitization of vanilloid receptor 1 by bradykinin in rat dorsal root ganglion cells.

As capsaicin binds to vanilloid receptor 1 and induces an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) through Ca^{2+} release from the ryanodine-sensitive Ca^{2+} store in addition to Ca^{2+} entry through capsaicin-activated non-selective Ca^{2+} channels in the plasma membrane (Eun et al., 2001), we exposed cultured rat dorsal root ganglion cells to a Ca^{2+}-free Krebs–HEPES solution containing $^{45}Ca^{2+}$ to evaluate non-selective Ca^{2+} channel activity. In our study, we demonstrated that capsaicin increased $^{45}Ca^{2+}$ uptake into dorsal root ganglion cells in a dose-dependent manner, while capsazepine, a vanilloid receptor 1 antagonist, completely inhibited $^{45}Ca^{2+}$ uptake induced by capsaicin. These results suggest that an increase in capsaicin-induced $^{45}Ca^{2+}$ uptake reflects the activity of non-selective Ca^{2+} channels in the vanilloid receptor 1.

As shown in Fig. 1, bradykinin in concentrations as low as 50 nM alone had no effect on $^{45}Ca^{2+}$ uptake, but it enhanced significantly the $^{45}Ca^{2+}$ uptake induced by capsaicin. These results are in agreement with a previous report by Chuang et al. (2001), who suggested, on the basis electrophysiological experiments, that bradykinin might modulate vanilloid receptor 1 by binding to its own receptors and activating a second messenger signaling cascade which sensitizes vanilloid receptor 1 activity in dorsal root ganglion cells. Our data also demonstrate that the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin returned to the level of capsaicin-induced $^{45}Ca^{2+}$ uptake after incubation with HOE 140 (bradykinin B_2 antagonist), suggesting that the sensitization of vanilloid receptor 1 by bradykinin B_2 receptor primarily involves the activation of non-selective Ca^{2+} channels by capsaicin. Bradykinin probably stimulates the Gq protein-coupled bradykinin B2 receptor to activate phospholipase C, which catalyzes the hydrolysis of membrane PIP_2 to yield inositol triphosphate and diacylgycerol in dorsal root ganglion cells. Therefore there is another possibility that bradykinin could increase $[Ca^{2+}]_i$ via Ca^{2+} release from inositol triphosphate-sensitive Ca^{2+} stores and stimulate Ca^{2+} entry through non-selective Ca^{2+} channels. However the enhancement of capsaicin-induced $^{45}Ca^{2+}$ uptake by bradykinin was completely inhibited by the vanilloid receptor 1 antagonist capsazepine. These data may suggest that $^{45}Ca^{2+}$ entry through non-selective Ca^{2+} channels could be reflected by vanilloid receptor 1 activity in dorsal root ganglion cells.

Although we do not know the exact mechanisms underlying the bradykinin-induced sensitization of the vanilloid receptor 1 in our $^{45}Ca^{2+}$ uptake assay, we propose that phospholipase C and/or cyclooxygenase-1 are involved. Cytosolic phospholipase A_2 might be involved in this sensitization, as specific inhibitors of

phospholipase C and cyclooxygenase-1 inhibited $^{45}Ca^{2+}$ uptake significantly, and the cytosolic phospholipase A_2 inhibitor also probably inhibited $^{45}Ca^{2+}$ uptake but this did not reach significance level in rat dorsal root ganglion cells stimulated by bradykinin. These data are in part consistent with the results of the recent report by Ferreira et al. (2004), who showed that the nociceptive response induced by the peripheral administration of bradykinin in mice was decreased by pretreatment with inhibitors of phospholipase C, protein kinase C or phospholipase A_2.

Phospholipase C activated by stimulation of the bradykinin B_2 receptor catalyzes the hydrolysis of membrane PIP_2 to yield inositol triphosphate and diacylgycerol, and Mueller et al. (2002) have reported that it is able to increase agonist-induced levels of Ins(X)*P* and arachidonic acid. Shin et al. (2002) also demonstrated that bradykinin, acting at the bradykinin B_2 receptor, excites vanilloid receptor 1 activity via the production of 12-lipoxygenase metabolites of arachidonic acid. In view of these data, it seems likely that bradykinin can sensitize the non-selective Ca^{2+} channel activity of the vanilloid receptor 1 via an intracellular second messenger pathway involving activation of phospholipase C and/or cyclooxygenase-1 as well as lipoxygenase in dorsal root ganglion cells.

SC-560 partially inhibited the increase in Ins(X)*P* accumulation stimulated by bradykinin through the loss of phospholipase C-γ activity due to inhibition by the SC-560 modified cyclooxygenase-1. In contrast to SC-560, the cyclooxygenase-2 inhibitor NS 398 had no significant influence on the increase in Ins(X)*P* accumulation. As cyclooxygenase-2 seems to be an inducible enzyme (Pang and Knox, 1997), the time required for bradykinin stimulation (30 min) may not be long enough to induce cyclooxygenase-2 activity during the measurement of Ins(X)*P* accumulation.

In conclusion, this study demonstrates that the inflammatory mediator bradykinin can, through the activation of bradykinin B_2 receptor, induce the sensitization of vanilloid receptor 1 by facilitating non-selective Ca^{2+} channel activity in cultured dorsal root ganglion cells, as measured in $^{45}Ca^{2+}$ uptake and Ins(X)*P* accumulation assays. These methods would be useful for screening new drugs for activity at vanilloid receptor 1; for monitoring the effect of enzymes on second messenger pathways in order to elucidate the molecular mechanism of inflammatory pain induced by bradykinin in primary afferent neurons; and for developing new therapeutic strategies for the treatment of inflammatory pain.

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