Direct activation of capsaicin receptors by products of lipoxygenases: Endogenous capsaicin-like substances

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Edited by Julius Axelrod, National Institutes of Health, Bethesda, MD, and approved March 17, 2000 (received for review July 16, 1999)

Capsaicin, a pungent ingredient of hot peppers, causes excitation of small sensory neurons, and thereby produces severe pain. A nonselective cation channel activated by capsaicin has been identified in sensory neurons and a cDNA encoding the channel has been cloned recently. However, an endogenous activator of the receptor has not yet been found. In this study, we show that several products of lipoxygenases directly activate the capsaicin-activated channel in isolated membrane patches of sensory neurons. Among them, 12- and 15-(*S*)-hydroperoxyeicosatetraenoic acids, 5- and 15-(*S*)-hydroxyeicosatetraenoic acids, and leukotriene B_4 possessed the highest potency. The eicosanoids also activated the cloned capsaicin receptor (VR1) expressed in HEK cells. Prostaglandins and unsaturated fatty acids failed to activate the channel. These results suggest a novel signaling mechanism underlying the pain sensory transduction.

Capsaicin has a unique effect on the pain sensory system and is a potential candidate for clinical use as an analgesic (1, 2). Capsaicin excites sensory neurons by binding to its receptor in the plasma membrane and activating ligand-gated, nonselective cation channels (3, 4). The capsaicin receptor (VR1) that was cloned recently (5) belongs to a family of transient receptor potential (TRP) channels responsible for light-sensitive current in *Drosophila* photoreceptors and is present exclusively in small sensory neurons (5). VR1 has been reported to be activated by noxious heat and acid (5, 6), suggesting that the capsaicin receptor may mediate both thermal and chemical pain. H^+ has been suggested to be an endogenous activator of the capsaicin receptor (6, 7); however, this is still controversial because H^+ failed to activate the capsaicin channel in cultured dorsal root ganglion neurons (4, 8).

Products of lipoxygenases (LOs) have been implicated in mediating inflammatory nociception because various LO products are produced during inflammation (9) and cause hyperalgesia when injected intradermally (10, 11). In addition, products of LOs often function as intracellular messengers in neurons. Among their actions, products of LOs act directly on K^+ channels in *Aplysia* sensory neurons (12, 13) and mammalian cardiac muscle cells (14). We recently showed that capsaicin binds to the intracellular side of the receptor channel, suggesting the presence of an endogenous intracellular activator (8). In addition, the hyperalgesic neural response induced by inflammation was blocked by capsazepine (15), a capsaicin receptor blocker (16), suggesting that an endogenous capsaicin-like substance is produced and causes hyperalgesia by opening capsaicin-activated channels. In this study, we tested the hypothesis that certain products of LOs directly open capsaicin-activated channels.

Materials and Methods

Primary Cultures and Single-Channel Recording. Primary cultures of sensory neurons isolated from dorsal root ganglions of neonatal rats were prepared, and the patch-clamp technique was used to record single-channel currents as described previously (4, 8). Pipette or control bath solution contained 130 mM NaCl, 2 mM $MgCl_2$, 5 mM EGTA, and 10 mM NaOH/Hepes (pH 7.2). For the K^+ substitution experiment, the control bath solution containing 140 mM Na^+ was changed to a solution containing 130 mM KCl, 2 mM $MgCl_2$, 5 mM EGTA, and 10 mM KOH/Hepes. All experiments were performed at room temperature. Borosilicate glass pipettes with tip resistance of 2 MΩ (Narishige, Tokyo, Japan) were pulled, polished, and coated with Sylgard (Dow-Corning). Junctional potentials were canceled. After gigaseals were formed with the glass pipettes, the inside-out patch configuration was used to study single-channel currents. Channel currents were recorded using a patch-clamp amplifier (Axopatch 200A; Axon Instruments, Foster City, CA). The output of the amplifier was filtered at 5 kHz with an 8-pole, low-pass Bessel filter. Data were digitized and stored on videotapes for later analysis. For chart recording, the output of amplifier was filtered at 500 Hz (Frequency Devices, Haverhill, MA) and fed into a thermal array chart recorder (TA-240; Gould, Cleveland, OH). The digitized data stored on videotapes were also imported to a personal computer (IBM pentium compatible) for computer analysis of single-channel currents.

Channel open probability (P_o) or amplitude of single-channel currents was obtained using the pCLAMP software (version 6.02; Axon Instruments). P_o of single channels was obtained from the ratio of the areas under the curves representing open events divided by the sum of the areas under the curves representing both open and closed events. The half-amplitude algorithm in FETCHAN (Axon Instruments) was used for detecting open events. Channel activity (NP_o) was calculated as a product of the number of functional channels (N) in the patch and P_o, NP_o, or P_o was determined only from patches that contained <6 functional capsaicin-activated channels.

Expression of VR1 in HEK 293 Cells. HEK 293 cells (CRL-1573; American Type Culture Collection) were cotransfected with pcDNA3-VR1 (5) and pHOOK-1 (Invitrogen). Forty-eight to 96 h after transfection, single-channel currents were recorded from the transfected cells isolated using Capture-Tec beads (Invitrogen).

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: TRP, transient receptor potential; LO, lipoxygenase; P_o, channel open probability; NP_o, channel activity; HPETE, hydroperoxyeicosatetraenoic acid; GASP, genetic algorithm similarity program; i_{cap}, capsaicin-activated single-channel currents; HETE, hydroxyeicosatetraenoic acid; LT, leukotriene; PG, prostaglandin; AA, arachidonic acid.

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Table 1. The fitness score, the number of pharmacophore point, and internal energy of 10 GASP alignments of 12-(*S*)-HPETE and capsaicin

GASP alignment	Fitness score	Pharmacophore point	Internal energy*
1	1,699	3	297
2	1,982	4	498
3	1,842	3	1,490
4	1,964	4	73
5	2,117	5	928
6	1,991	3	185
7	2,041	4	211
8	1,680	4	546
9	1,900	4	453
10	1,834	3	76

*Internal energy was calculated using a Lennard-Jones 6–12 potential (19, 20).

Structure Comparison. Low-energy conformations of capsaicin and 12-(*S*)-hydroperoxyeicosatetraenoic acid (12-(*S*)-HPETE) or other LO products were obtained using a SYBYL molecular modeling program (SYBYL; Tripos Associates, St. Louis, MO) (17). The parameters used for energy minimization were a conjugated gradient method, Tripos force field, Gasteiger-Hückel charges, and convergence criterion of 0.005 kcal/mol^{-1}Å^{-1}. After extracting low-energy conformations, structures of the two molecules were superimposed using a genetic algorithm similarity program (GASP; Tripos Associates) (18). The parameters used for the analysis are as follows: maxops = 100,000; n_islands = 5; full_crosswt = 0.0; fitness_inc = 0.01; full_mutatewt = 0.0; select_pressure = 1.1; vdw_contact_cutoff = 0.8; popsize = 125; ops_inc = 6,500; allele_mutatewt = 95.0. As summarized in Table 1, 10 alignments were produced with fitness scores ranging from 1,680 to 2,117. The average execution time of the genetic algorithm, was set, at 10 min and 20 s. Among the 10 GASP alignments, alignment 7 was selected for the final alignment of 12-(*S*)-HPETE and capsaicin because of high-fitness score (the second) and low-internal energy (the fourth) of the stable conformation (Table 1). To assure further the applicability of pharmacophore models generated by GASP, the structure of capsaicin in the energy-minimized state was also aligned to a crystal structure of a capsaicin analogue, KR-25003 (21), and the similarity of the two structures was confirmed.

Chemicals. Capsaicin and capsazepine (Research Biomedicals, Natick, MA) were dissolved and stored in 100% ethanol to make 10 mM stock solutions. 12-(*S*)-HPETE and other LO products (Biomol, Plymouth Meeting, PA) were placed in an argon-filled chamber. Immediately before use, solvents of 12-(*S*)-HPETE or other eicosanoids were dried by argon. The lipid metabolites were properly diluted in a solution degassed in advance by argon. The solution was solubilized by a brief sonication.

All values were expressed as means ± SEM. For multiple comparison of means, one-way analysis of variance was used followed by Duncan's test for a post hoc test.

Results

We first tested whether products of LOs could activate capsaicin-activated channels in patch membranes isolated from cultured neurons of dorsal root ganglion. Because capsaicin and its analogues act on channels from the intracellular side of the membrane (8), we applied test chemicals to the cytoplasmic side of the channel. As shown in Fig. 1, application of 1 μM capsaicin to the bath solution of an inside-out patch activated single-channel currents (i_{cap}). In the same patch, 10 μM 12-(*S*)-HPETE also activated similar channels (Fig. 1*A*). The channels activated

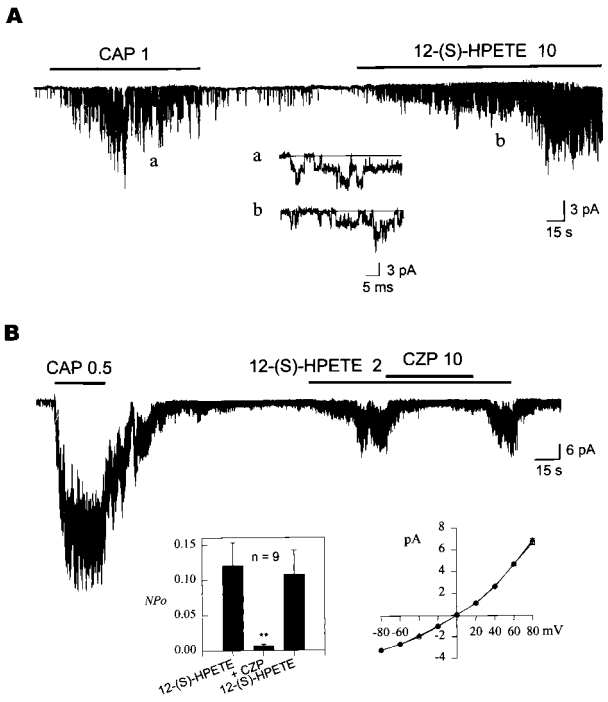

Fig. 1. Direct activation of i_{cap} by 12-(*S*)-HPETE, one of LO products. (*A*) 12-(*S*)-HPETE in 10 μM activates single-channel currents in an inside-out membrane patch of a cultured dorsal root ganglion neuron containing capsaicin (CAP)-activated channels. (*Inset*) Current traces in an expanded time scale. (*B*) Block by 10 μM capsazepine (CZP) of single channel currents activated by 2 μM 12-(S)-HPETE. (*Insets*) A summary of the block by capsazepine (*Left*). Channel current is expressed in channel activity (NP_o). **, $P < 0.01$. (*Right*) Current-voltage relationship of channels activated by capsaicin (open circle, $n = 11$) and 12-(*S*)-HPETE (filled triangle, $n = 5–8$). The two I–V curves are superimposable. Concentrations are expressed in micromolar.

by 12-(*S*)-HPETE and capsaicin showed an identical I–V relationship (Fig. 1*B*) (4, 8). The channels activated by capsaicin and 12-(*S*)-HPETE were equally permeable to Na^+ and K^+ because the reversal potential under 140 mM Na^+/K^+ bi-ionic solution condition was near zero (-1.71 ± 0.49 mV, $n = 6$). We also used capsazepine, a relatively nonselective vanilloid receptor antagonist (16, 22, 23), to test whether capsaicin-activated channels are involved. Activation of the channels by 12-(*S*)-HPETE (2 μM) was blocked by addition of 10 μM capsazepine, suggesting that both 12-(*S*)-HPETE and capsaicin act on the same receptor. In isolated membrane patches, application of 10 μM 12-(*S*)-HPETE activated i_{cap} in the majority of patches tested (32 of 37 patches having i_{cap}) with variable current responses. In 63 patches that did not exhibit i_{cap}, application of 10 μM 12-(*S*)-HPETE did not activate any channels. In sympathetic ganglion neurons that are capsaicin insensitive, neither 0.5 μM capsaicin nor 10 μM 12-(*S*)-HPETE activated any channels ($n = 22$). 12-(*S*)-HPETE also activated outwardly rectifying i_{cap} in HEK 293 cells transfected with VR1 ($n = 15$) in a capsazepine-sensitive manner (Fig. 2). In patches from control ($n = 37$) or mock-transfected (transfected with vectors only) HEK 293 cells ($n = 38$), both 0.5 μM capsaicin and 10 μM 12-(*S*)-HPETE failed to activate i_{cap}. Because 12-(*S*)-HPETE contains a peroxide moiety, it can act as a donor of oxygen-free radicals that could directly activate i_{cap}. To test whether oxygen-free radicals can activate i_{cap}, *t*-butylperoxide was applied intracellularly to inside-out patches containing capsaicin channels. *t*-Butylperoxide failed to activate i_{cap} ($n = 12$) or inhibit it when i_{cap} was first activated with capsaicin ($n = 4$). Taken together, these results

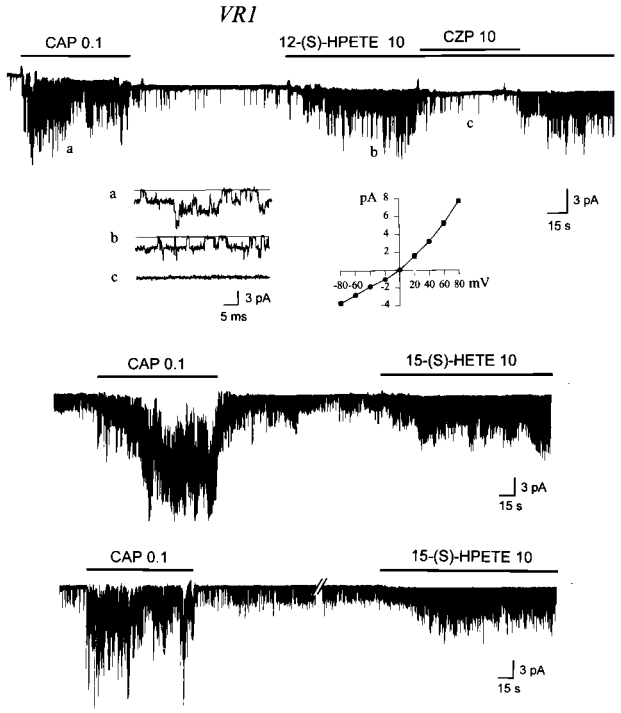

Fig. 2. Activation of VR1 by products of LOs in an inside-out membrane patch of a VR1-transfected HEK 293 cell. (*Inset*) Current traces in an expanded time scale (*Left*) and current-voltage relationship of channels (*Right*) activated by 12-(*S*)-HPETE in HEK 293 cells transfected with VR1. 15-(*S*)-HETE and 15-(*S*)-HPETE are also capable of activating VR1 expressed in HEK 293 cells (lower traces).

indicate that 12-(*S*)-HPETE and capsaicin activate the same nonselective cation channel.

Products of LOs other than 12-(*S*)-HPETE were also capable of activating the channel. Among eicosanoids, 12-(*S*)-HPETE, 15-(*S*)-HPETE, 5-(*S*)-hydroxyeicosatetraenoic acid (5-(*S*)-HETE), leukotriene B_4 (LTB_4), and 15-(*S*)-HETE were most effective in activating i_{cap} (Fig. 3). Other LO products such as hepoxilin B_3, 5-(*S*)-HPETE, 8-(*R*)-15-(*S*)-dihydroxyeicosatetraenoic acid, 12-(*S*)-HETE, hepoxilin A_3, and LTC_4 were nearly without effect. The current responses produced by eicosanoids (10 μM) were generally smaller than that produced by 0.5 μM capsaicin. For example, the averaged channel activity (NP_o) after 0.5 μM capsaicin application was 1.38 $\pm$ 0.26 (n = 14). In the same patches, 10 μM 12-(*S*)-HPETE activated i_{cap} with NP_o of 0.62 $\pm$ 0.24. Anandamide, an endogenous agonist of cannabinoid receptor, is now known to activate VR1 (24). Therefore, we tested whether anandamide could also activate native i_{cap}. As shown in Fig. 3*B*, 10 μM anandamide was also able to activate i_{cap}. The efficacy of anadamide was, however, much lower than that of 12-(*S*)-HPETE (Fig. 4*B*). In addition, anandamide exhibited an apparent desensitization during the application period in 10 of 13 patches (Fig. 3*B*).

Because 12-(*S*)-HPETE and other LO products have chiral centers having stereoisomers, we tested the effects of *R* and *S* isomers of 5-HETE and 12-HETE to determine the stereospecificity of LO products. As shown in Fig. 3*C*, 5-(*R*)-HETE showed a much lower effect ($P < 0.05$) in activating i_{cap} than the *S* form of 5-HETE. Both *R* and *S* isomers of 12-HETE showed weak activation compared with that by 5-HETE isomers. Concentration–response relationships for 12-(*S*)-HPETE, 15-(*S*)-HPETE, 5-(*S*)-HETE, LTB_4, and anandamide were obtained by applying increasing concentrations of each lipid to the bath solution (Fig.

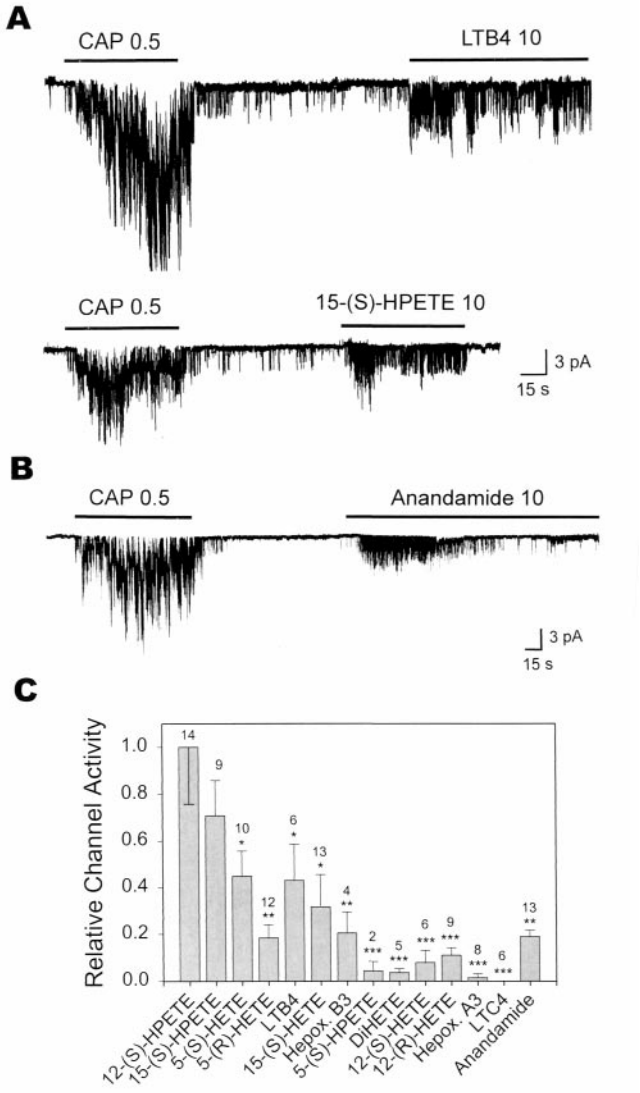

Fig. 3. Various LO products (*A*) and anandamide (*B*) in activating i_{cap} in inside-out patches of cultured dorsal root ganglion neurons. (*C*) A summary of effects of various LO products on activating i_{cap}. Relative channel activities of each 10 μM LO product are normalized to the channel activity (NP_o) obtained with 10 μM 12-(*S*)-HPETE. DiHETE, 8-(*R*)-15-(*S*)-dihydroxyeicosatetraenoic acid; Hepox. A3 or B3, hepoxilin A_3 or B_3. Numbers above the bars represent the number of experiments. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ compared with NP_o of 12-(*S*)-HPETE.

4). Half-maximal concentrations of 12-(*S*)-HPETE, 15-(*S*)-HPETE, 5-(*S*)-HETE, LTB_4, and anandamide for activating the channel were 8.0 $\pm$ 0.6, 8.7 $\pm$ 0.7, 9.2 $\pm$ 0.1, 11.7 $\pm$ 1.2, and 11.7 $\pm$ 0.3, respectively, as compared with 1.1 μM for capsaicin (4). Hill coefficients for 12-(*S*)-HPETE, 15-(*S*)-HPETE, 5-(*S*)-HETE, LTB_4, and anandamide were 2.1, 2.2, 1.9, 1.6, and 2.0, respectively, suggesting the presence of two binding sites for the lipids. Hill coefficient of 1.8 was previously obtained with capsaicin (4, 25).

Prostaglandins (PGs) are other major metabolic products of arachidonic acid (AA) and targeted for the development of non-steroidal anti-inflammatory analgesics. Therefore, we tested whether PGs could also activate i_{cap}. As shown in Fig. 5*A*, bath application of PGE_2 in inside-out patches failed to activate i_{cap}, whereas 12-(*S*)-HPETE activated i_{cap} in the same patch. Similar negative results were obtained when 10 μM PGD_2, PGI_2,

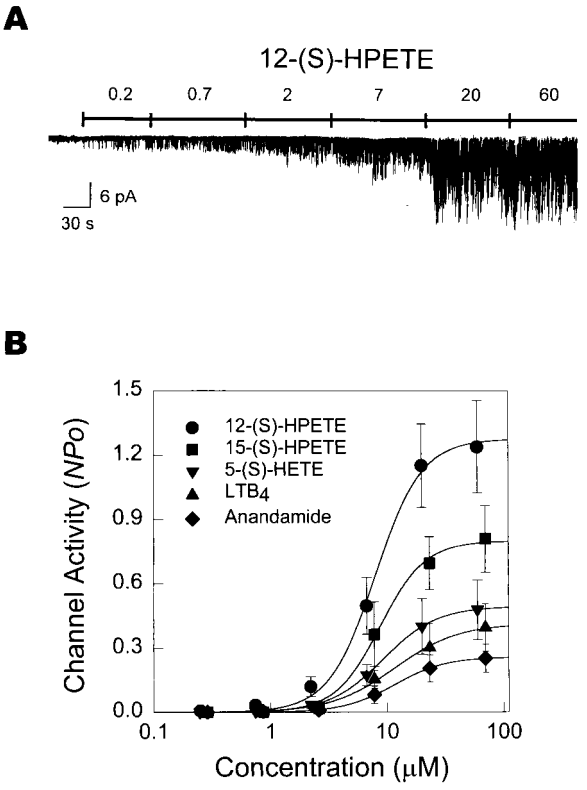

Fig. 4. (*A*) An example trace showing a dose-response relationship of 12-(*S*)-HPETE in activating i_{cap}. (*B*) Channel activity (NP_o) obtained after application of 12-(*S*)-HPETE ($n = 13$), 15-(*S*)-HPETE ($n = 9$), 5-(*S*)-HETE ($n = 11$), LTB_4 ($n = 9$), and anandamide ($n = 8$) in different concentrations is plotted against concentrations of the lipids. Each data point is fitted to the Hill equation: NP_o = maximal $NP_o \times [1/\{1 + (K_d/[\text{lipid}])^n\}]$.

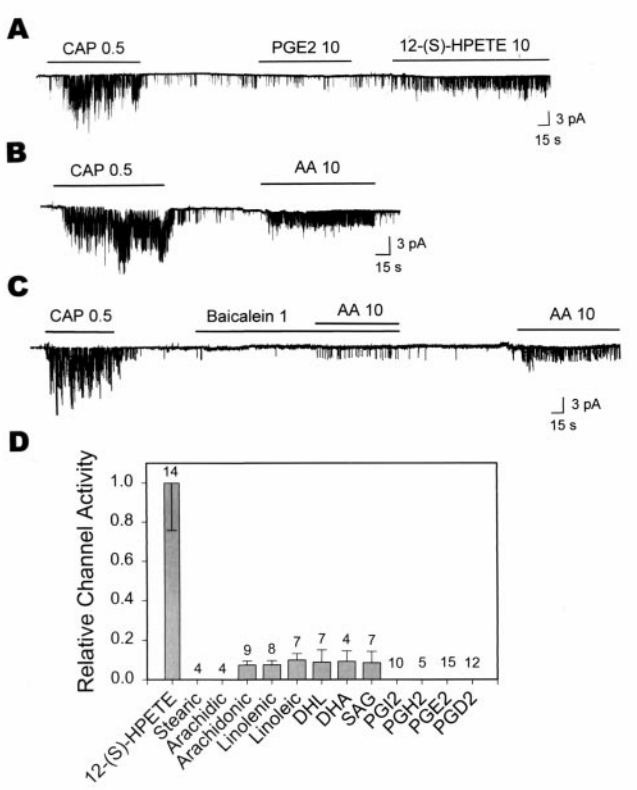

Fig. 5. Effects of various lipids other than products of LOs on i_{cap} in cultured dorsal root ganglion neurons. (*A*) PGE_2 fails to activate i_{cap} in inside-out membrane patch. (*B*) Current response of i_{cap} to AA. (*C*) Block by baicalein, a 12-LO inhibitor, of the i_{cap} activated by AA. (*D*) Relative channel activities of the various lipids expressed as normalized to the channel activity (NP_o) obtained with 10 μM 12-(*S*)-HPETE. DHA, docosahexaenoic acid; DHL, dihomo-γ-linolenic acid; SAG, 1-stearyl-2-arachidonyl-*sn*-glycerol. Numbers above the bars represent the number of experiments. Relative channel activities of the lipids are significantly ($P < 0.001$) different from the channel activity (NP_o) of 10 μM 12-(*S*)-HPETE.

or PGH_2 was used (Fig. 5*D*). These results indicate that AA metabolites activate i_{cap} via the lipoxygenase, but not the cyclooxygenase, pathway.

It has been reported recently that polyunsaturated fatty acids such as arachidonic, linoleic, or linolenic acid directly activate *Drosophila* retinal TRP channels that share sequence homology with VR1 (26). In addition, some mammalian homologues of TRP (TRPC3 and TRPC6) are activated by diacylglycerol (27). We therefore tested the effects of the lipids on capsaicin receptors. Both saturated (stearic and arachidic) and unsaturated fatty acids with different carbon length or number of double bond such as linoleic (18:2), linolenic (18:3), dihomo-γ-linolenic (20:3), or docosahexaenoic acid (22:6) were ineffective in activating i_{cap}. 1-Stearyl-2-arachinonyl-*sn*-glycerol, an analogue of diacylglycerol, did not activate i_{cap} when applied to patches containing capsaicin receptors (Fig. 5*D*). However, AA (20:4) was found to activate i_{cap} in ≈50% of patches tested (37 of 69 patches containing capsaicin-activated channels) (Fig. 5*B*). We reasoned that the frequent activation of the channel by AA resulted from the metabolic conversion of AA to LO products by LOs that were possibly present in the patch membrane (14, 28, 29). To determine whether activation by AA was due to the action of LOs, LO inhibitors such as nordihydroguaiaretic acid or eicosatetraynoic acid were applied to the bath along with AA. Pretreatment with 10 μM nordihydroguaiaretic acid or 5 μM eicosatetraynoic acid caused 88 ± 4% ($n = 4$) or 75 ± 18% ($n = 5$) inhibition, respectively, of i_{cap} activated by AA. Because these inhibitors are generally nonspecific, more specific inhibitors for 5-LO (REV5901) and 12-LO (baicalein) were applied along with AA. In this experiment, 1 μM baicalein (Fig. 5*C*), 10 μM REV5901, and a mixture of the two inhibitors reduced the AA-induced currents by 67 ± 22% ($n = 5$), 42 ± 12% ($n = 5$), and 73 ± 6%, ($n = 9$), respectively. These results suggest that capsaicin receptors are activated preferentially by LO products among lipid messengers.

Results of the present study indicate that capsaicin and various eicosanoids act on the capsaicin receptor, suggesting a structural similarity between capsaicin and eicosanoids. Thus, structures of eicosanoids and capsaicin in the energy-minimized state in the gas phase were superimposed to compare three-dimensional structures. Three-dimensional structures of 12-(*S*)-HPETE, 15-(*S*)-HPETE, 5-(*S*)-HETE, and LTB_4 were compared with that of capsaicin. To do this, we extracted structures in the energy-minimized state in the gas phase first using molecular mechanics (SYBYL molecular modeling program; Tripos Associates) (17) and then aligned the structures using a GASP (Tripos Associates) (18). As shown in Fig. 6*B*, capsaicin in the energy-minimized state fits well to the S-shaped 12-(*S*)-HPETE. In particular, the phenolic hydroxide and amide moieties in capsaicin overlap precisely with the carboxylic acid and hydroperoxide moieties in 12-(*S*)-HPETE, respectively. The two key regions in capsaicin or 12-(*S*)-HPETE are known to have dipolar property that allows hydrogen bond interactions with the capsaicin receptor (30). In addition, the aliphatic chain region of the 12-(*S*)-HPETE fits well with the alkyl chain of capsaicin (C region of capsaicin, Fig. 6*A*).

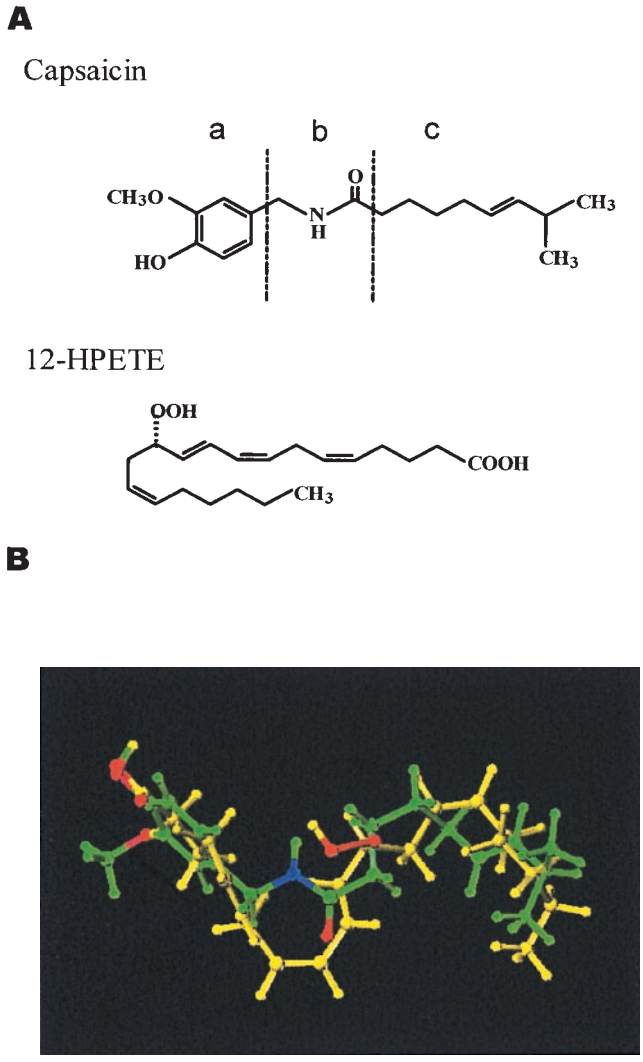

Fig. 6. (*A*) Chemical structures of capsaicin and 12-(*S*)-HPETE. Capsaicin is divided into three functional regions as described by others (30). (*B*) Molecular modeling overlay of minimum-energy conformations of capsaicin (green) and 12-(*S*)-HPETE (yellow). Oxygen and nitrogen atoms are colored in red and blue, respectively.

In contrast, 15-(*S*)-HPETE, 5-(*S*)-HETE, and LTB_4, shared less structural similarity with capsaicin (data not shown).

Discussion

Capsaicin receptors are ligand-gated, nonselective cation channels activated by capsaicin and other vanilloids (2, 4, 5). The capsaicin receptors are present in small sensory neurons and implicated in a primary role in nociception because activation of these receptors by capsaicin causes severe pain or pain-related reflexes (31, 32). In analogy with morphine receptors, the presence of capsaicin receptors in sensory neurons strongly suggests the existence of an endogenous activator. Although the capsaicin receptor and its homologues have been cloned and the molecular property of the channel has been characterized (5, 33), an endogenous activator has not been identified. In the present study, we provide several lines of evidence that products of LOs are candidates for the endogenous activator of the capsaicin receptor. (*i*) Products of LOs evoked single-channel currents with the conductance, ion selectivity, and I–V relationship nearly identical to those evoked by capsaicin. (*ii*) Activation of the channels by products of LOs was blocked by capsazepine, an antagonist of the capsaicin receptor. (*iii*) The three-dimensional structure of 12-(*S*)-HPETE, one of the LO products, exactly superimposed that of capsaicin.

PG has long been a prime target for the development of nonsteroidal anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs such as aspirin or indomethacin inhibit the production of PGs. The analgesic action of nonsteroidal anti-inflammatory drugs leads to an assumption that PGs play a role in mediating inflammatory pain. However, mechanisms underlying the pain causing actions of PGs are not clearly understood (34, 35). PGs are metabolites of AA and known to augment the current response of sensory neurons to capsaicin (36). These results along with the implication of PGs in nociception suggest a possible link between PGs and a capsaicin receptor. In the present study, however, PGs failed to activate the capsaicin receptors. Thus, the hyperalgesic action of PGs results from mechanisms other than a direct activation of the capsaicin receptor, possibly via interplay with other inflammatory mediators.

Structural Comparison. In the present study, we show that the three-dimensional structures of capsaicin and 12-(*S*)-HPETE can be superimposed in the energy-minimized state. The structural similarity between capsaicin and 12-(*S*)-HPETE may explain why capsaicin mimics 12-(*S*)-HPETE in activating the channel or vice versa. Furthermore, the structural analysis also explains why various capsaicin analogues possess agonistic activity (30). In particular, phenolic hydroxide and amide moieties appear to be critical for inducing capsaicin responses. Removal of the phenolic hydroxide or amide bond in capsaicin analogues leads to reduction of potency. As structural comparison shows (Fig. 6), the phenolic hydroxide and amide moieties in capsaicin, that share potential multiple hydrogen bond interactions with the capsaicin receptor, clearly overlap with the carboxylic acid and hydroperoxide moieties in 12-(*S*)-HPETE, respectively. In addition, the optimal length of alkyl chain in capsaicin congeners for agonistic activity is about 8–10 carbon atoms (30), which fits well with the length of the aliphatic chain of 12-(*S*)-HPETE. Therefore, the structural similarity further suggests that 12-(*S*)-HPETE is a likely candidate for an endogenous capsaicin-like substance. Other LO products, such as 15-(*S*)-HPETE, 5-(*S*)-HETE, or LTB_4, that have current responses similar to that produced by 12-(*S*)-HPETE, share less structural similarity with capsaicin than 12-(*S*)-HPETE, although they all have a carboxyl group and an aliphatic chain that grossly correspond to phenolic hydroxide and the aliphatic tail of capsaicin, respectively. Our results show that although 12-(*S*)-HPETE, 15-(*S*)-HPETE, 5-(*S*)-HETE, and LTB_4 have slightly different structures, they activate i_{cap} with similar potency. This contrasts with the strict structural requirement among capsaicin and its analogues (30). One explanation might be that the eicosanoids are structurally more flexible whereas capsaicinoids are relatively rigid. Therefore, different eicosanoids may be able to activate the capsaicin channel.

Activation by LO Products. LO products were less efficacious in activating i_{cap} than capsaicin. Thus, the maximal channel current activated by LO products was lower than that evoked by capsaicin. It is difficult to determine the actual concentration of LO products generated in sensory neurons. Nevertheless, the concentration of 12-(*S*)-HPETE required to activate i_{cap} was similar to those found to activate other channels (12, 14). Although the activation of i_{cap} by 12-(*S*)-HPETE in isolated membrane patches was weak, it is possible that different subtypes of capsaicin receptors could have different sensitivities to capsaicin and 12-(*S*)-HPETE, as suggested by others (37). The slow activation of the channel by 12-(*S*)-HPETE also contrasts with the rapid activation by capsaicin. At present, it is difficult to know the

reasons for the apparent kinetic difference between the effects of capsaicin and 12-(*S*)-HPETE, but it may result from differences in the physicochemical property of the ligands such as solubility in solution. For example, resiniferatoxin, a potent analogue of capsaicin, also activates the channel at a much slower rate (≈5 min for full activation; data not shown).

A Possible Signaling Pathway. Bradykinin generates diacylglycerol and inositol 1,4,5-tris-phosphate by activating phospholipase C (38, 39). Bradykinin also activates phospholipase A_2 and generates AA (40, 41). There are several lines of evidence indicating that LO products are involved in the bradykinin-induced biological effects. For example, responses of sensory neurons to bradykinin and capsaicin are highly correlated (42) and bradykinin-induced hyperalgesia is blocked by mepacrine, an inhibitor of phospholipase A_2 (43). The excitation of sensory neurons by bradykinin is also reduced by the LO inhibitor (44). We also observed that the excitation of sensory nerve fibers of the adult rat skin by bradykinin was greatly inhibited by mepacrine, nordihydroguaiaretic acid, and capsazepine, the inhibitors of phospholipase A_2, LOs, and capsaicin receptors, respectively (our unpublished data). In a recent study, inflammatory mediators containing bradykinin at low pH are found to cause capsazepine-sensitive excitation in cultured dorsal root ganglion neurons (45). These results suggest that bradykinin may produce hyperalgesia via the phospholipase A_2/LO pathway and activation of capsaicin channels by LO products.

In summary, we show that 12-(*S*)-HPETE and other LO products are capable of activating the capsaicin receptor. 12-(*S*)-HPETE is structurally similar to capsaicin, possibly endowing it the structural properties to bind the capsaicin receptor and activate i_{cap}. Since the role of capsaicin receptor in mediating nociception is now well known, results of the present study should greatly aid in designing and developing novel nonsteroidal analgesics.

We thank Su-Yeon Kim at T & J Tech Inc. for her technical advice for SYBYL and GASP computer analysis. This work was supported by National Creative Research Initiatives of the Korean Ministry of Science and Technology.

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