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Novel Compounds That Interact with Both Leukotriene B_4 Receptors and Vanilloid TRPV1 Receptors

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ABSTRACT

The aim of this study was to investigate the interaction of a series of novel compounds with leukotriene B_4 receptors (BLT) and vanilloid receptor (TRPV1). First, we characterized leukotriene B_4 (LTB_4) ethanolamide. In guinea pig isolated lung parenchyma, LTB_4 ethanolamide antagonized the contractile action of LTB_4 with an apparent K_B value of 7.28 nM. Using a Boyden chamber assay, we demonstrated that this compound stimulated human neutrophil migration in a similar manner to LTB_4 but with lower efficacy. In rat TRPV1 (rTRPV1)-expressing Chinese hamster ovary (CHO) cells and dorsal root ganglion (DRG) neurons, LTB_4 and LTB_4 ethanolamide acted as low-efficacy agonists, increasing intracellular calcium concentration ($[Ca^{2+}]_i$) in a capsazepine-sensitive manner. These results prompted us to hypothesize that a molecule may possess pharmacophores such that it is capable of dual antagonism of BLT and TRPV1 receptors. Two novel compounds, *N*-{2-fluoro-4-[3-(11 hydroxyheptadec-8-enyl)-thioureio-methyl]-phenyl}-methanesulfonamide (O-3367) and *N*-{4-[3-(11 hydroxyheptadec-8-enyl)-thioureio-methyl]-phenyl}-methanesulfonamide (O-3383), were synthesized. In human neutrophils, both compounds acted as antagonists, significantly attenuating the BLT receptor-mediated ability of LTB_4 to induce migration, with pIC_{50} values of 7.22 ± 0.17 and 5.95 ± 0.16, respectively. In rTRPV1-expressing CHO cells, they caused a significant rightward shift in the log concentration-response curve for the TRPV1 receptor agonist capsaicin (3-methoxy-4-hydroxy)benzyl-8-methyl-6-nonenamide). In DRG neurons O-3367 significantly attenuated the capsaicin-induced increases in $[Ca^{2+}]_i$ with a pIC_{50} value of 5.94 ± 0.004. O-3367 and O-3383 represent novel structural templates for generating compounds possessing dual antagonism at BLT and TRPV1 receptors. In view of the crucial role of both TRPV1 and BLT receptors in the pathophysiology of inflammatory conditions, such compounds may betoken a novel class of highly effective therapeutics.

Leukotriene B_4 (LTB_4) is a potent proinflammatory agent. It has an important role in a number of processes, including neutrophil activation and degranulation, leukocyte migration into the bloodstream, inflammatory pain, and host defense against infection. LTB_4 has been implicated in the pathophysiology of various diseases, including psoriasis, inflammatory bowel disease, arthritis, and asthma (Ford-Hutchison, 1990). In 1997, Yokomizo et al. cloned an LTB_4 G protein coupled receptor (BLT1). This is a high-affinity specific receptor for LTB_4 found predominantly in peripheral leukocytes, with lower expression also found in lung and spleen (Yokomizo et al., 2000a). More recently, a second low-affinity LTB_4 receptor (BLT2) has been cloned (Kamohara et al., 2000; Yokomizo et al., 2000b). The BLT2 receptor seems to be ubiquitously expressed with highest levels in spleen then liver, ovary, and leukocytes (Yokomizo et al., 2000a). The interaction of LTB_4 at these receptors is a contributing factor in the pathogenesis of inflammatory disease (Tager and Luster, 2003). Studies involving the targeted deletion of murine BLT1 and the effect of antagonizing LTB_4

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ABBREVIATIONS: LTB_4, leukotriene B_4, 5(*S*),12(*R*)dihydroxy-6,14-*cis*-8,10 *trans*-eicosatetraenoic acid); BLT, leukotriene B_4 receptor; TRPV1, vanilloid receptor; O-3367, *N*-{2-fluoro-4-[3-(11-hydroxyheptadec-8-enyl)-thioureio-methyl]-phenyl}-methanesulfonamide; O-3383, *N*-{4-[3-(11 hydroxyheptadec-8-enyl)-thioureio-methyl]-phenyl}-methanesulfonamide; CHO, Chinese hamster ovary; LTB_4 ethanolamide, 5(*S*),12(*R*)dihydroxy-6,14-*cis*-8,10 *trans*-eicosatetraenoic ethanolamide; U75302, LTB_4 dimethylamide, 5(*S*),12(*R*)dihydroxy-6,14-*cis*-8,10 *trans*-eicosatetraenoic dimethylamide; DRG, dorsal root ganglion; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; rTRPV1, rat vanilloid receptor; DMSO, dimethyl sulfoxide; ANOVA, analysis of variance; $[Ca^{2+}]_i$, intracellular calcium concentration; FAAH, fatty acid amide hydrolase; LY293111, sodium (2-[2-propyl-3-[2-ethyl-4-(4-fluorophenyl0-5-hydroxyphenoxy]propoxy]phenoxy]benzoic acid sodium salt; U75302, 6-(6-(3-hydroxy-1*E*,5*Z*-undecadienyl)-2-pyridnyl)-1,5-hexane diol.

receptors in animal inflammatory models highlight the therapeutic potential of the BLT receptor with regard to inflammatory disease (Tager and Luster, 2003).

Recently, LTB_4 and other lipoxygenase metabolites of anandamide (arachidonoyl ethanolamide) have been shown to activate the vanilloid TRPV1 receptor (Hwang et al., 2000; Craib et al., 2001; Van Der Stelt and Di Marzo, 2004). The TRPV1 receptor is a ligand-gated, nonselective cation channel, the activation of which leads to excitation of nociceptors and evokes pain-related behavior (Szallasi et al., 1999). TRPV1 expression is largely associated with small diameter primary afferent fibers, which are sensitive to capsaicin (3-methoxy-4-hydroxy)benzyl-8-methyl-6-nonenamide), noxious heat, and low pH (Szallasi et al., 1999). The TRPV1 receptor is necessary for the development of inflammatory hyperalgesia to thermal stimuli (Davis et al., 2000) and important in the pathophysiology of neurogenic inflammation. Furthermore, the development of new TRPV1 receptor antagonists that are effective in the treatment of inflammatory pain is a desirable goal.

The aim of this study was 2-fold: 1) to investigate the pharmacology of the LTB_4 ethanolamide, specifically the interaction with leukotriene (BLT) and vanilloid (TRPV1) receptors; and 2) to investigate the hypothesis that a molecule may possess pharmacophores, such that it is capable of antagonism of both BLT and TRPV1 receptors, with the accompanying potential of potent anti-inflammatory action. To this end, we synthesized two novel compounds, O-3367 and O-3383 (Fig. 1). The models that we have used in this investigation are 1) CHO cells expressing recombinant rat TRPV1 receptors; 2) rat-cultured dorsal root ganglion (DRG) neurons, which express TRPV1 receptors; 3) guinea pig lung parenchyma that express BLT1/2 receptors and TRPV1 receptors; and 4) human neutrophils that express BLT1/2 receptors.

Materials and Methods

Materials

Capsaicin and capsazepine were obtained from Tocris Cookson, Inc. (Bristol, UK). [^{3}H]LTB_4 was obtained from GE Healthcare (Little Chalfont, Buckinghamshire, UK). LTB_4, LTB_4 ethanolamide, and U75302 were obtained from Cayman Chemical (Montingy le Bretoneux, France). Bovine serum albumin, cell culture media, nonenzymatic cell dissociation solution, Fura-2 acetoxymethyl ester, Geneticin (G418), *l*-glutamine, Krebs' salts, penicillin with streptomycin, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), and ionomycin were all obtained from Sigma (Poole, Dorset, UK). Polymorphprep was obtained from Axis-Shield (Oslo, Norway). Diff-Quik was obtained from VWR International (Poole, UK). Boyden chamber and filters were obtained from Neuro Probe (Gaithersburg, MD). Rat TRPV1-transfected CHO cells were a gift from Novartis (London, UK). Polymorphonuclear neutrophils (PMNs) were obtained from healthy volunteers. All studies were carried out in accordance with the Declaration of Helsinki (World Medical Association, 1997).

Cell Culture

TRPV1-Transfected CHO Cells. Rat (r)TRPV1-transfected CHO cells were maintained in minimal essential medium α minus media containing 2 mM L-glutamine supplemented with 10% Hyclone fetal bovine serum (Hyclone Laboratories, Logan, UT), 350 μg ml^{-1} G418, 100 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin. Cells were maintained in 5% CO_2 at 37°C.

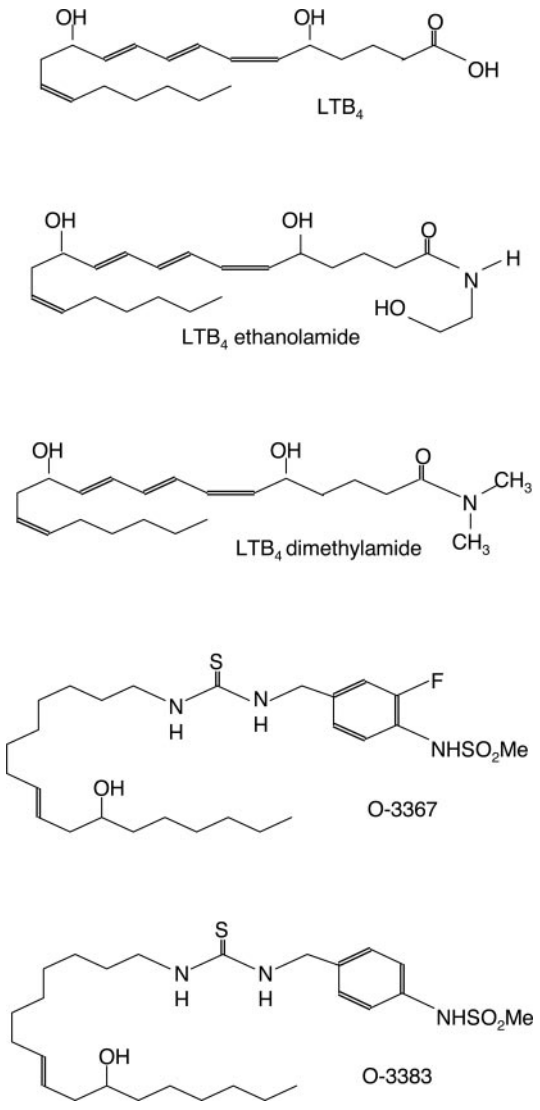

Fig. 1. Structures of LTB_4, LTB_4 ethanolamide, LTB_4 dimethylamide, O-3367, and O-3383.

Primary Cultured Neonatal DRG Neurons. Primary cultures of DRG neurons were prepared following enzymatic (0.125% collagenase, 0.25% trypsin, and 1.6 units ml^{-1} DNAase) and mechanical dissociation of dorsal root ganglia from decapitated 2-day-old Sprague-Dawley rats. The sensory neurons were plated on laminin-polyornithine-coated coverslips and bathed in F-14 culture medium supplemented with 10% fetal bovine serum, penicillin (5000 IU ml^{-1}), streptomycin (5000 mg ml^{-1}), and nerve growth factor (20 ng ml^{-1}). The cultures were maintained for up to 2 weeks at 37°C in humidified air with 5% CO_2 and fed with fresh culture medium every 5 to 7 days.

Isolated Tissue Experiments

Guinea Pig Lung Parenchyma. Lungs were obtained from Dunkin-Hartley guinea pigs weighing 300 to 800 g. Animals were stunned, exsanguinated, and the lungs with attached trachea were quickly removed. The lung parenchyma was cut into strips 1 cm in length. Each strip was mounted in a 4-ml organ bath at an initial tension of 0.5 g and allowed to equilibrate for 1 h. The baths contained Krebs' solution that was kept at 37°C and bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs' solution was 1.29 mM $MgSO_4 \cdot 7H_2O$, 118.2 mM NaCl, 4.75 mM KCl, 1.19 mM KH_2PO_4, 25.0 mM $NaHCO_3$, 11.0 mM glucose, and 2.54 mM $CaCl_2 \cdot 6H_2O$. At the

end of each experiment, the tissue was exposed to 100 μM histamine, and the contractions for each compound were expressed as a percentage of this response. Contractions were monitored by computer (Apple Macintosh LCIII and Performa 475; Apple Computer, Cupertino, CA) using a data recording and analysis system (MacLab; Apple Computer Inc., Burlington, NC) that was linked to either UF1 transducers (Pioden Controls, Canterbury, UK) or model 1030 transducers (UFI, Morro Bay, CA). Values have been expressed as means and variability ± S.E.M. or as 95% confidence limits. The values for pEC_{50} ($-\log EC_{50}$) are defined as the effective concentration producing 50% of the maximum response inducible by that compound. EC_{50} and maximal effects (E_{max}), and the mean ± S.E.M. or 95% confidence limits of these values have been calculated by nonlinear regression analysis using the equation for a sigmoidal concentration-response curve (Prism; GraphPad Software Inc., San Diego, CA). K_B values of LTB_4 ethanolamide or LTB_4 dimethylamide for antagonism of LTB_4 were calculated by substituting a single concentration ratio value into the equation $(x - 1) = B/K_B$, where x (the "concentration ratio") is the concentration of agonist that produced a particular size of effect in the presence of antagonist at a concentration (B) divided by the concentration of agonist that produced an identical effect in the absence of antagonist (Tallarida et al., 1979). Values of the concentration ratio and its 95% confidence limits were determined by symmetrical (2 + 2) dose parallel line assays. This method was also used to establish whether two-point log concentration-response plots deviated significantly from parallelism.

Drug Additions. All agonist additions (10-μl volume) were made noncumulatively with a washout period of 30 min between additions to prevent desensitization. Top stocks of LTB_4 and LTB_4 ethanolamide were dissolved in saline solution and serially diluted in Krebs' solution. Saline top stocks were prepared daily by evaporation of an aliquot of the 100 $\mu g\ ml^{-1}$ top stock of each compound (ethanol) and resuspension in saline. U75302 was diluted in DMSO. In control experiments, the appropriate vehicle was added instead of antagonist. Antagonists/vehicle was added 30 min before the addition of LTB_4.

Calcium Imaging

TRPV1-transfected CHO cells or cultured DRG neurons were incubated for 1 h in NaCl-based extracellular solution (130 mM NaCl, 3.0 mM KCl, 0.6 mM $MgCl_2$, 2.0 mM $CaCl_2$, 1.0 mM $MgHCO_3$, 10.0 mM HEPES, 5.0 mM glucose, pH 7.4, and 310–320 mOsM) containing 10 μM Fura-2 acetoxymethyl ester. The cells were constantly perfused with NaCl-based solution (1–2 ml/min) and viewed under an inverted BX50WI microscope (Olympus, Tokyo, Japan) with a KAI-1001 S/N 5B7890-4201 Olympus camera attached. The fluorescence ratiometric images from data obtained at excitation wavelengths of 340 and 380 nm were viewed and analyzed using UltraView (Orinda, CA) (Merlin morphometry). During acute experiments, the TRPV1-transfected CHO cells were exposed to LTB_4 ethanolamide (1 μM) for 30 s followed by 2.5 min of washout with NaCl solution. This was followed by exposure to ionomycin (4 μM) for 30 s. During chronic experiments the TRPV1-transfected CHO cells were exposed to LTB_4 ethanolamide (1 μM) for 20 min followed by 10 min of washout with NaCl solution. This was followed by exposure to ionomycin (4 μM) for 30 s. The DRG neurons were exposed to LTB_4 ethanolamide (1 μM) for 30 s followed by 2.5 min of washout with NaCl solution. This was followed by exposure to capsaicin (1 μM) for 30 s and washout for 2.5 min and finally exposure to KCl (30 mM) for 30 s. The Ca^{2+} transient (fluorescence ratio after background subtraction) generated by each compound was measured. All data are expressed as means ± S.E.M.

Boyden Chamber Assays

Peripheral PMNs were isolated from normal whole blood by centrifugation over Polymorphprep (Axis-Shield). The isolated cells were resuspended at a concentration of 1×10 cells ml^{-1} in phosphate-buffered saline containing $CaCl_2$ and $MgCl_2$. In vitro cell migration assays were performed using a modified 48-well Boyden chamber. Incubation lasted 30 min in a 5% CO_2 atmosphere at 37°C. After incubation, the migrated adherent cells on the underside of the 3-μm pore filter were stained using a Diff-Quik stain set. Each well was counted in 10 nonoverlapping fields (40× magnification) using a light microscope. Test compounds were placed in the lower wells of the Boyden chamber. fMLP, a well established chemoattractant peptide, was used at a concentration of 1 μM as positive control.

Statistical Analysis of Data

Values have been expressed as means and variability ± S.E.M. or as 95% confidence limits. Mean values have been compared using Student's unpaired t test or analysis of variance (ANOVA) followed by Dunnett's test or the Newman-Keuls test. A P value < 0.05 was considered to be significant.

Results

LTB_4 Ethanolamide Results

Isolated Guinea Pig Lung Parenchyma. Initially, we performed isolated tissue experiments with strips of guinea pig lung parenchyma to determine the pharmacological effect of replacing the carboxylic acid group of LTB_4 with an ethanolamide group. LTB_4 produced concentration-dependent contraction of isolated lung parenchymal strips with pEC_{50} and E_{max} values of 8.02 ± 0.13 and 77.0 ± 5.34% ($n = 7$) (Fig. 2A). In contrast, LTB_4 ethanolamide produced only very modest contraction of the tissue, the maximum being 13.23 ± 2.66% at 300 nM ($n = 7$) (Fig. 2A). In view of the high degree of structural similarity displayed by LTB_4 and LTB_4 ethanolamide (Fig. 1), we theorized that this compound may behave as an antagonist in this tissue. In line with this hypothesis, we found that preincubation with LTB_4 ethanolamide produced a concentration-related rightward shift in the log concentration-response curve of LTB_4 (Fig. 2A), such that, in the presence of 30, 100, and 300 nM LTB_4 ethanolamide, the pEC_{50} values for LTB_4 were 7.25 ± 0.18, 6.90 ± 0.12, and 6.86 ± 0.14, respectively ($n = 7$). These values are significantly lower than the pEC_{50} for LTB_4 in the absence of antagonist ($P < 0.05$; one-way ANOVA). The E_{max} values for LTB_4 in the presence of LTB_4 ethanolamide at 30 and 100 nM were 69.7 ± 6.50 and 66.7 ± 6.12%, respectively; these values were not significantly different from that of LTB_4 in the absence of antagonist ($P > 0.05$; one-way ANOVA). However, in the presence of 300 nM LTB_4 ethanolamide, the E_{max} value for LTB_4 of 44.0 ± 5.30% was significantly lower than of that of LTB_4 alone ($P < 0.01$; one-way ANOVA). In this series of experiments, LTB_4 ethanolamide alone elicited contractions of 2.8 ± 2.3 and 9.2 ± 6.2% ($n = 7$) at 100 and 300 nM, respectively. To determine whether LTB_4 ethanolamide was acting nonspecifically to inhibit parenchymal contraction elicited by LTB_4, we investigated whether it could attenuate the effects of histamine. We found that LTB_4 ethanolamide did not affect the contraction of the tissue in response to histamine (Fig. 2B); the pEC_{50} values for histamine in the absence and presence of 300 nM LTB_4 ethanolamide were 5.89 ± 0.14 and 6.02 ± 0.11 ($n = 5$). Next, for the sake of comparison, we investigated the effects of U75302. At 1 μM, U75302 caused a rightward shift in the log concentration-response curve of LTB_4 (Fig. 2C); the pEC_{50} values of 8.05 ± 0.15, for LTB_4 in the presence of DMSO, and 7.01 ± 0.27, in the presence of U75302, were significantly different ($P <$

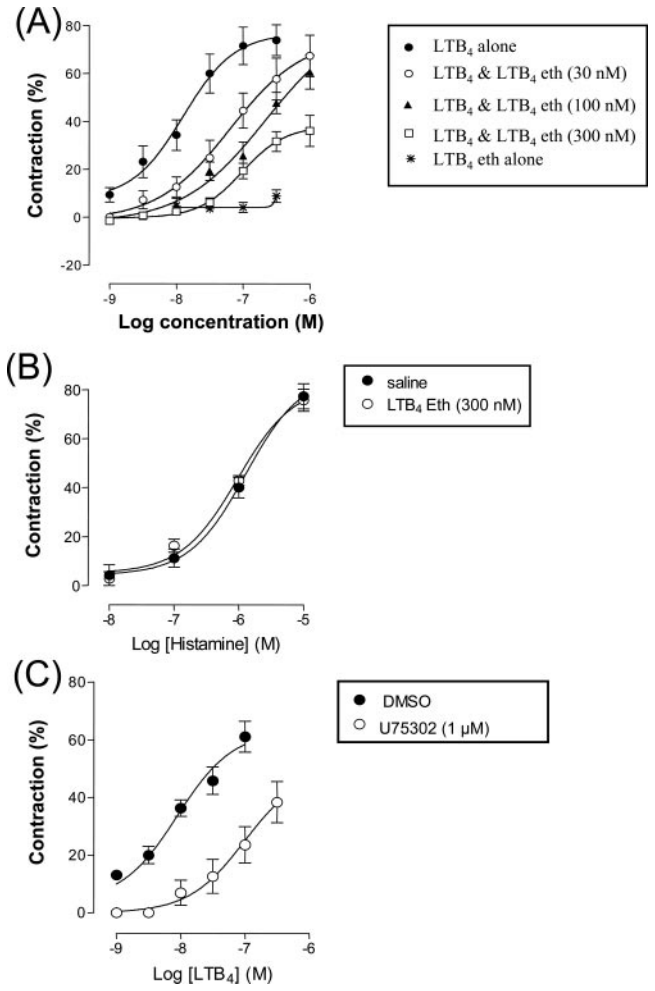

Fig. 2. Log concentration-response curves for contraction of the guinea pig-isolated lung parenchymal strip by LTB_4, LTB_4 ethanolamide, and LTB_4 in the presence of LTB_4 ethanolamide (100 and 300 nM) (A); by histamine in the absence and presence of LTB_4 ethanolamide (300 nM) (B); and by LTB_4 in the presence of DMSO or U75302 (1 μM) (C). Log concentration-response curves were noncumulative to prevent desensitization. Data are from the tissues of five to seven animals. Each symbol represents the contraction calculated as a percentage of the maximum contraction induced by 100 μM histamine ± S.E.M.

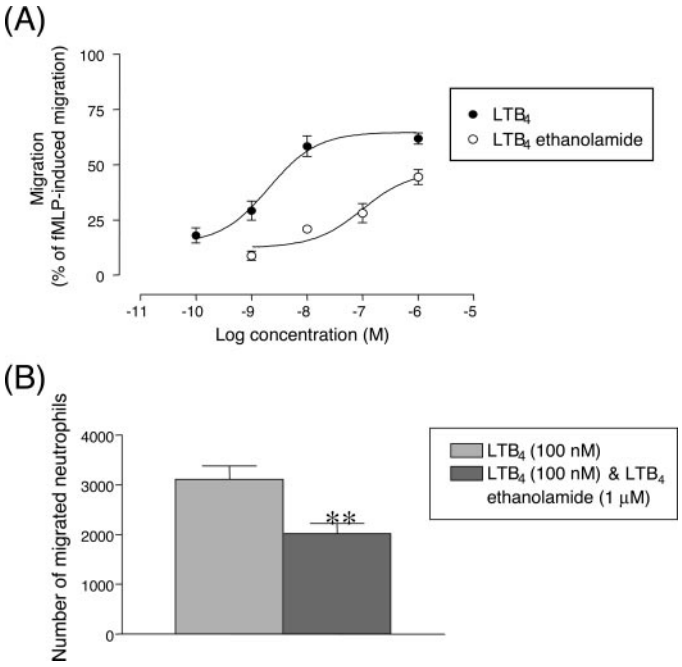

Fig. 3. Log concentration-response curve (A) shows the migration of neutrophils induced by LTB_4 and LTB_4 ethanolamide. The data represent the mean number of PMNs migrated ± S.E.M. (n = 3–6) as a percentage of the migration induced by 1 μM fMLP. The histogram (B) shows the number of neutrophils induced to migrate by LTB_4 (100 nM) in the presence and absence of LTB_4 ethanolamide (1 μM). The data represent the mean number of PMNs migrated ± S.E.M. (n = 12). The number of neutrophils that migrated in response to LTB_4 (100 nM) in the presence of LTB_4 ethanolamide (1 μM) was significantly lower ($P < 0.001$; Student's unpaired t test) than for LTB_4 (100 nM) alone.

0.01; Student's unpaired t test). In this series of experiments, U75302 alone elicited small contractions of 4.3 ± 2.8%.

Using the 2 + 2 assay (see *Materials and Methods*), the apparent K_B values (with 95% confidence limits) for LTB_4 ethanolamide (100 nM) and U75302 (1 μM) were calculated to be 7.28 nM (1.23–23.1) and 53.6 nM (20.2–445.91), respectively. In both cases, the rightward shift in the log concentration-response curve of LTB_4 did not differ significantly from parallelism.

Neutrophil Migration Experiments. Because LTB_4 ethanolamide antagonized the ability of LTB_4 to elicit contraction of guinea pig lung parenchyma, we performed Boyden chamber assays to determine whether the novel analog antagonized the ability of LTB_4 to induce migration of human neutrophils. Both LTB_4 and LTB_4 ethanolamide induced PMN migration with pEC_{50} values of 8.69 ± 0.06 (n = 3–6) and 7.00 ± 0.18 (n = 3), respectively (Fig. 3A); the latter being significantly lower ($P < 0.0001$; Student's unpaired t test). The E_{max} value for LTB_4 ethanolamide was significantly lower ($P > 0.05$; Student's unpaired t test) than that of LTB_4, the values being 47.10 ± 3.23% (n = 3) and 64.74 ± 1.08% (n = 3–6) of that induced by fMLP, respectively. To further test the hypothesis that LTB_4 ethanolamide has lower efficacy than LTB_4, we investigated the effect of this compound on LTB_4-induced migration. In the presence of LTB_4 ethanolamide (1 μM), the mean number of neutrophils induced to migrate by LTB_4 was 2020 ± 201 (n = 12), which was significantly lower ($P < 0.01$; Student's unpaired t test) than 3110 ± 271 (n = 12) for LTB_4 alone (Fig. 3B). These results indicate that LTB_4 ethanolamide behaves as a partial agonist at BLT receptors in PMNs.

Calcium Imaging. *rTRPV1-transfected CHO cells.* Previous studies suggest that TRPV1 receptors can be activated by LTB_4 (Hwang et al., 2000). We therefore performed calcium imaging experiments to characterize LTB_4 and LTB_4 ethanolamide at this receptor. In cultured rTRPV1-transfected CHO cells, 20-min exposure to LTB_4 and LTB_4 ethanolamide produced a concentration-dependent increase in $[Ca^{2+}]_i$ (Fig. 4A). When normalized against the change in fluorescence produced by ionomycin (4 μM), the pEC_{50} value for LTB_4 was 7.87 ± 0.01 (n = 14–21), whereas that for LTB_4 ethanolamide was significantly lower ($P < 0.0001$; Student's unpaired t test) at 7.28 ± 0.03 (n = 5–30). The E_{max} values were 52.3 ± 0.10% (n = 14–21) and 55.1 ± 0.55% (n = 5–30) for LTB_4 and LTB_4 ethanolamide, respectively, the latter being significantly higher ($P < 0.0001$; Student's unpaired t test). The E_{max} values for these compounds were significantly lower than those of capsaicin ($P < 0.0001$; Student's unpaired t test), which had an E_{max} of 88 ± 1.2%. We investigated the time course of the calcium response to peak and found that

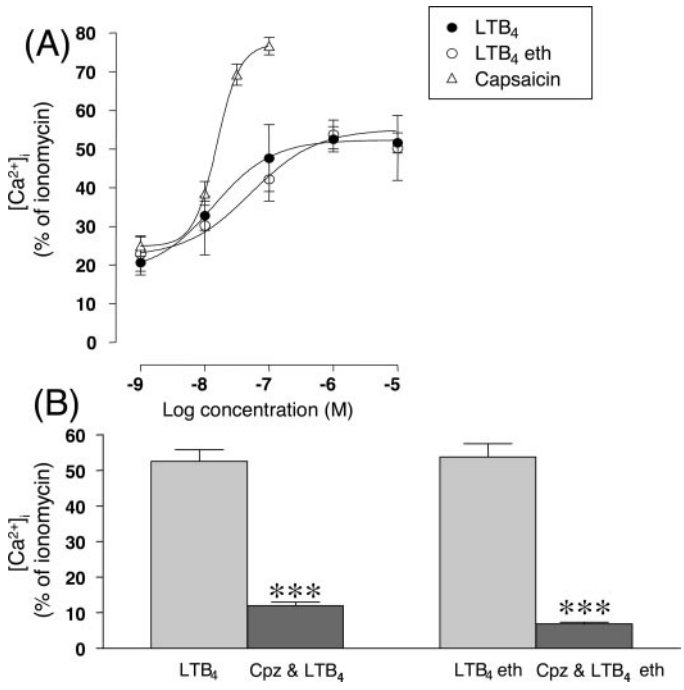

Fig. 4. Log concentration-response curve (A) showing $[Ca^{2+}]_i$ evoked in rTRPV1-transfected CHO cells by LTB_4 (n = 14–21), LTB_4 ethanolamide (n = 5–30), and capsaicin (n = 53–81). Each symbol represents the mean as a percentage of $[Ca^{2+}]_i$ evoked by 4 μM ionomycin ± S.E.M. The histogram (B) shows $[Ca^{2+}]_i$ evoked in rTRPV1-transfected CHO cells by LTB_4 (1 μM) in the presence and absence of capsazepine (1 μM) and by LTB_4 ethanolamide (1 μM) in the presence and absence of capsazepine (1 μM). The data represent the mean as a percentage of $[Ca^{2+}]_i$ evoked by 4 μM ionomycin ± S.E.M. (n = 19–30). The $[Ca^{2+}]_i$ evoked by LTB_4 (1 μM) in the presence of capsazepine (1 μM) was significantly lower ($P < 0.0001$; Student's unpaired t test) than for LTB_4 (1 μM) alone. The $[Ca^{2+}]_i$ evoked by LTB_4 ethanolamide (1 μM) in the presence of capsazepine (1 μM) was significantly lower ($P < 0.0001$; Student's unpaired t test) than for LTB_4 ethanolamide (1 μM) alone.

the time required to achieve maximum $[Ca^{2+}]_i$ response was 3.79 ± 0.67 (n = 21) and 2.13 ± 0.31 (n = 30) min for LTB_4 (1 μM) and LTB_4 ethanolamide (1 μM), respectively, compared with that of 0.76 ± 0.03 (n = 30) for capsaicin. The exposure time required to achieve maximum $[Ca^{2+}]_i$ response was significantly longer for LTB_4 and LTB_4 ethanolamide than for capsaicin ($P < 0.0001$; Student's unpaired t test). The responses produced by 1 μM LTB_4 and LTB_4 ethanolamide were both significantly reduced ($P < 0.0001$; Student's unpaired t test) in the presence of capsazepine (1 μM), an established antagonist of the TRPV1 receptor (Fig. 4B), indicating that both compounds are acting via TRPV1. The increase in $[Ca^{2+}]_i$ for LTB_4 (1 μM) and LTB_4 ethanolamide (1 μM) in the presence of capsazepine (1 μM) was 11.99 ± 0.95% (n = 30) and 6.87 ± 0.48% (n = 19), respectively.

DRG Neurons in Culture. *LTB_4 experiments.* LTB_4 ethanolamide displayed TRPV1 agonism similar to LTB_4, but both compounds were less potent than capsaicin in CHO cells that had been transfected to highly express TRPV1 receptors. To determine whether this behavior was consistent in tissue that natively expresses TRPV1 receptors we performed calcium imaging experiments with neonatal rat DRG neurons. DRG neurons consist of subpopulations of cells, and the size of the cell soma gives an indication of the fibers arising from these cells. The populations are Aα/β cells that are large/medium sized with myelinated axons; Aδ cells that are medium sized with finely myelinated axons, and C cells that are small and have unmyelinated axons.

Of a total of 161 DRG neurons from six separate cultures, there were three groups of responses observed (Fig. 5A). In all the cells analyzed, high K^+ (30 mM) evoked an increase in $[Ca^{2+}]_i$, thus confirming the neuronal phenotype. One population (group 1) of neurons (64/161) responded only to KCl (30 mM); this group constituted 40% of the total population. A second population (group 2) (84/161) responded to capsaicin (1 μM) and KCl, and this group constituted 52% of the total population. A third subpopulation (group 3) of capsaicin-sensitive cells also responded to LTB_4, and this group constituted 13% of the total capsaicin-sensitive population (13/97). LTB_4 did not elicit a Ca^{2+} transient in any capsaicin-insensitive cells. The Ca^{2+} transient evoked LTB_4 (1 μM) was not significantly different ($P > 0.05$; one-way ANOVA) from that of evoked by capsaicin (1 μM) in the same group of cells, the change in fluorescence being 0.469 ± 0.146 for LTB_4 and 1.067 ± 0.244 for capsaicin (Fig. 5A). In cells that did not respond to LTB_4, the response to capsaicin (0.972 ± 0.090) was not significantly different ($P > 0.05$; one-way ANOVA) from that obtained in those that responded to LTB_4 (Fig. 5A). The area (square micrometers) of the DRG neurons that responded to KCl only (group 1) (488.95 ± 31.62 μm^2) was significantly greater than the area of those that responded to capsaicin and KCl (group 2) (261.22 ± 12.69 μm^2) ($P < 0.001$; one way ANOVA) as well as the area of those that responded to LTB_4, capsaicin, and KCl (group 3) (227.61 ± 18.6 μm^2) ($P < 0.001$; one-way ANOVA). The areas of the latter two groups were not significantly different.

LTB_4 ethanolamide experiments. As for the LTB_4 DRG experiments mentioned above, we were able to categorize 120 DRG neurons from six separate cultures into three groups based on the responses observed (Fig. 5B). High K^+ (30 mM) evoked, in all the cells analyzed, an increase in $[Ca^{2+}]_i$, indicating the neuronal phenotype. Forty-four percent of the total population (53/120) reacted only to KCl (30 mM) (group 1). The population (group 2) that reacted only to capsaicin (1 μM) constituted 28% (34/120) of the total. Last, capsaicin-sensitive cells that also responded to LTB_4 ethanolamide (group 3) formed 49% of the capsaicin-sensitive population (33/67). This is notably higher than the small number (13%) of capsaicin-sensitive cells that responded to LTB_4. LTB_4 ethanolamide did not elicit a Ca^{2+} transient in any capsaicin-insensitive cells. LTB_4 ethanolamide (1 μM) evoked a significantly lower ($P < 0.05$; one-way ANOVA) Ca^{2+} transient than that of capsaicin (1 μM) in the same group of cells, the change in fluorescence being 0.352 ± 0.052 for LTB_4 ethanolamide and 0.777 ± 0.101 for capsaicin (Fig. 5B). The response to capsaicin (0.826 ± 0.126) was not significantly different ($P > 0.05$; one-way ANOVA) in cells that did not respond to LTB_4 ethanolamide from those that did (Fig. 5B). Again, the area (square micrometers) of the DRG neurons that responded to KCl (group 1) (433.82 ± 29.8 μm^2) was significantly greater than those that responded to capsaicin only (group 2) (272.20 ± 18.68 μm^2) ($P < 0.001$; one way ANOVA) as well as those that responded to both LTB_4 and capsaicin (group 3) (234.79 ± 18.16 μm^2) ($P < 0.001$; one-way ANOVA). The latter two groups were not significantly different in terms of their areas.

Of 71 DRG neurons that responded to KCl, four cells (6%) responded to capsaicin in the presence of the TRPV1 receptor

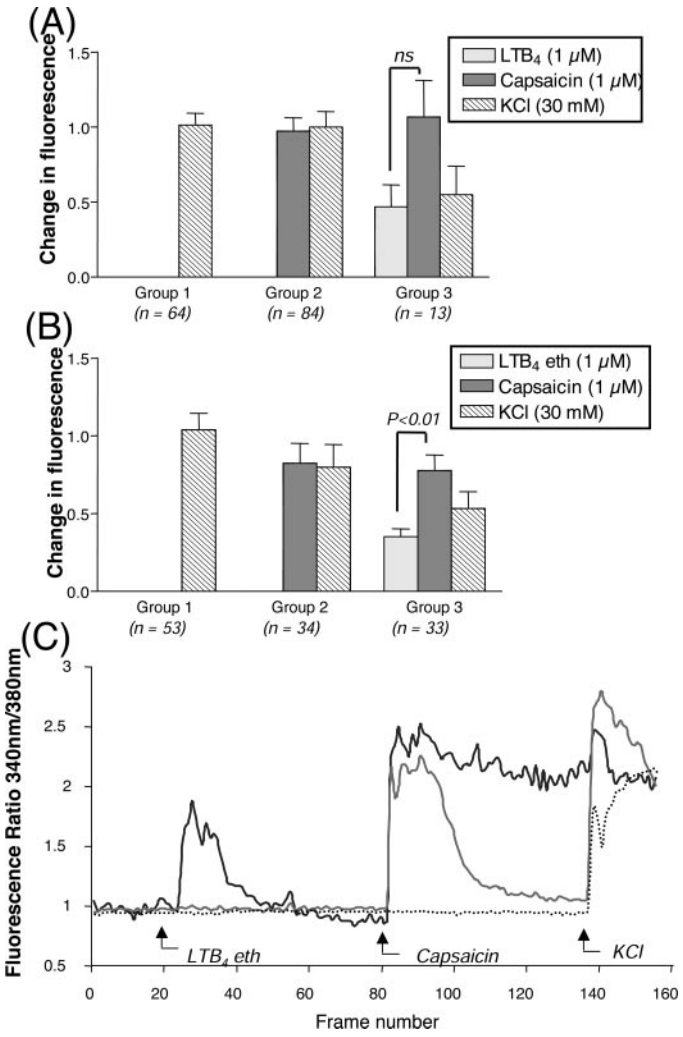

Fig. 5. The histogram (A) shows $[Ca^{2+}]_i$ evoked in 161 DRG neurons obtained from six separate cultures by capsaicin (1 μM), LTB_4 (1 μM), and KCl (30 mM). The data represent the mean change in fluorescence ratio ± S.E.M. All cells analyzed responded to KCl, confirming the neuronal phenotype. A population of DRG neurons responded to KCl only (group 1), another responded to capsaicin and KCl (group 2), and a third responded to LTB_4 and capsaicin (group 3). In group 3, the change in fluorescence induced by LTB_4 was not significantly different from that elicited by capsaicin. The number of cells in each group is shown in italics. The histogram (B) shows $[Ca^{2+}]_i$ evoked in 120 DRG neurons obtained from six separate cultures by capsaicin (1 μM), LTB_4 ethanolamide (1 μM), and KCl (30 mM). The data represent the mean change in fluorescence ratio ± S.E.M. All cells analyzed responded to KCl, confirming the neuronal phenotype. A population of DRG neurons responded to KCl only (group 1), another responded to capsaicin only (group 2), and a third responded to LTB_4 ethanolamide and capsaicin (group 3). In group 3, the change in fluorescence induced by LTB_4 ethanolamide was significantly lower ($P < 0.01$; one-way ANOVA) than that elicited by capsaicin. The number of cells in each group is shown in italics. A trace (C) shows examples of $[Ca^{2+}]_i$ responses (F_{340}/F_{380} ratio) evoked by LTB_4 ethanolamide (1 μM), capsaicin (1 μM), and KCl (30 mM) in single neonatal DRG neurons. The compounds were applied at the points indicated by arrows. Data were captured at a rate of one frame every 3 s. The dotted line shows a DRG neuron that only responds to KCl (group 1), the gray line shows a DRG neuron that responds to both capsaicin and KCl (group 2), and the black line shows a DRG neuron that responds to LTB_4 ethanolamide, capsaicin, and KCl (group 3).

antagonist capsazepine (10 μM) (change in fluorescence, 1.36 ± 0.08), but no cells responded to LTB_4 (1 μM). Likewise, from a total of 70 cells that responded to KCl, four cells (6%) responded to capsaicin in the presence of capsazepine (10 μM) (change in fluorescence, 1.21 ± 0.42), and of these, three cells also responded to LTB_4 ethanolamide (change in fluorescence, 0.38 ± 0.27).

O-Compound Results

We extended the study to investigate the hypothesis that a molecule may possess pharmacophores such that it is capable of antagonism of both BLT and TRPV1 receptors, with the accompanying potential of potent anti-inflammatory action. To this end, we synthesized two novel compounds, O-3367 and O-3383 (Fig. 1).

rTRPV1-Transfected CHO Cells. We performed calcium imaging experiments with rTRPV1-transfected CHO cells to determine whether the two novel compounds, O-3367 and O-3383, were capable of antagonizing TRPV1 receptors. O-3367 alone produced no change in basal $[Ca^{2+}]_i$ (data not shown). Perfusion with capsaicin in the presence of O-3367 (10 μM) produced a rightward shift in the log concentration-response curve of capsaicin (Fig. 6A), which did not differ significantly from parallelism. The control capsaicin pEC_{50} value was 7.52 ± 0.15 (n = 53–81), whereas in the presence of O-3367 (10 μM) the capsaicin pEC_{50} was 6.81 ± 0.17 (n = 28–74). This value is significantly lower than the pEC_{50} for capsaicin in the absence of antagonist ($P < 0.05$; Student's unpaired t test). A significant reduction ($P < 0.0001$; Student's unpaired t test) in $[Ca^{2+}]_i$ response was observed at 1, 10, 30, and 100 nM capsaicin in the presence of 10 μM O-3367 (Fig. 6A). The estimated dissociation constant, K_e (with 95% confidence limits), for O-3367 (10 μM) was calculated to be 2.43 μM (1.87–3.19).

Likewise, in rTRPV1 CHO cells, O-3383 alone produced no change in basal $[Ca^{2+}]_i$. Perfusion with O-3383 (10 μM) produced a rightward shift in the log concentration-response curve of capsaicin (Fig. 6B), which did not differ significantly from parallelism. The control capsaicin pEC_{50} value was 7.86 ± 0.22 (n = 53–81), whereas in the presence of O-3383 (10 μM) the capsaicin pEC_{50} was 7.43 ± 0.20 (n = 21–57). These values were not significantly different ($P > 0.05$; Student's unpaired t test). However, a significant reduction in $[Ca^{2+}]_i$ response was observed at 10, 30 ($P < 0.0001$; Student's unpaired t test), and 100 nM ($P < 0.05$; Student's unpaired t test) concentrations of capsaicin in the presence of O-3383 (Fig. 6B). The K_e for O-3383 (10 μM) was calculated to be 5.92 μM.

DRG Neurons in Culture. To determine whether the antagonism of TRPV1 receptors observed with O-3367 was consistent in tissue that natively expresses TRPV1 receptors, we performed calcium imaging experiments with neonatal rat DRG neurons. Exposure of DRGs to capsaicin (100 nM) and increasing concentrations of O-3367 concomitantly resulted in the reduction of capsaicin-induced intracellular calcium increase with a pIC_{50} of 5.94 ± 0.004 (n = 10–37) (Fig. 7A). The percentage of DRGs responding to capsaicin (100 nM) was reduced by the presence of O-3367 (Fig. 7B). O-3367 produced no change in basal $[Ca^{2+}]_i$ when exposed to DRG neurons alone (data not shown).

Neutrophil Migration Experiments. Having observed that O-3367 and O-3383 behaved as TRPV1 receptor antagonists, we performed Boyden chamber assays to investigate whether these compounds could also antagonize LTB_4-induced migration of human neutrophils, which is BLT receptor-mediated.

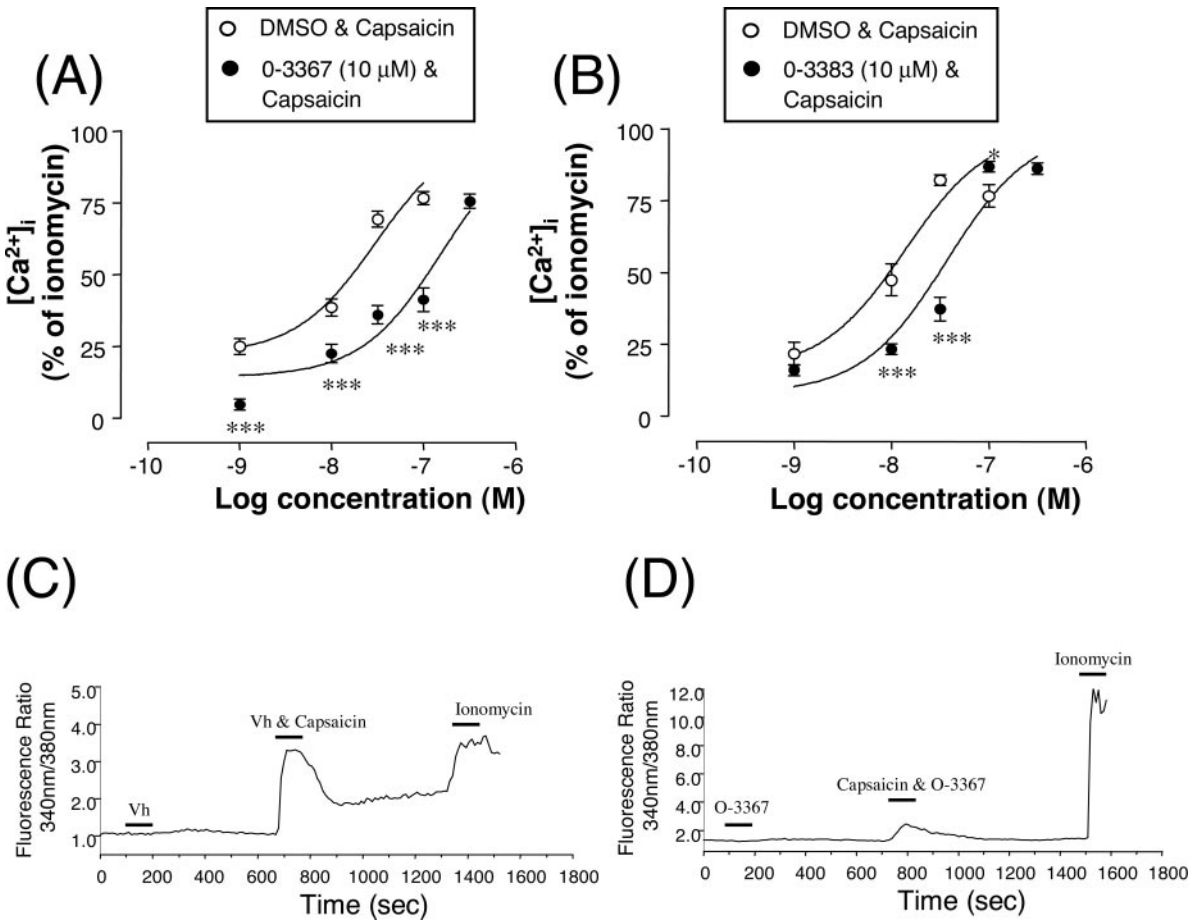

Fig. 6. Log concentration-response curves for $[Ca^{2+}]_i$ evoked in rTRPV1-transfected CHO cells by DMSO (0.1%) and capsaicin (n = 53–81); O-3367 (10 μM) and capsaicin (n = 28–74) (A) and DMSO (0.1%) and capsaicin (n = 21–30); and O-3383 (10 μM) and capsaicin (n = 21–57) (B). Each symbol represents the mean as a percentage of $[Ca^{2+}]_i$ evoked by 4 μM ionomycin ± S.E.M. Traces showing examples of $[Ca^{2+}]_i$ responses (F_{340}/F_{380} ratio) evoked by vehicle and capsaicin (30 nM) (C) and capsaicin and O-3367 (30 nM) (D) in rTRPV1-transfected CHO cells. The compounds were applied at the points indicated by bars. Data were captured at a rate of one frame every 3 s.

Both compounds significantly antagonized chemotaxis induced by LTB_4 (Fig. 8, A and B). Exposure to LTB_4 (100 nM) and increasing concentrations of O-3367, or of O-3383, resulted in the reduction of LTB_4-induced neutrophil migration with a pIC_{50} value of 7.22 ± 0.17 (n = 3) for O-3367 (Fig. 8A) and of 5.95 ± 0.16 (n = 3) for O-3383 (Fig. 8B). Neither O-3367 nor O-3383 acted as chemoattractants in human neutrophils (Fig. 8C). Thus, when placed in the lower wells of the Boyden chamber at a concentration of 1 μM, the migrated cells were 852 ± 21.6 (n = 3) for O-3367 and 566 ± 7.95 (n = 3) for O-3383 compared with the vehicle alone of 539 ± 5.67 and fMLP (1 μM) of 3284 ± 19.1. The compounds did not inhibit the migration induced by fMLP (1 μM): fMLP in the presence of O-3367 (1 μM) stimulated 4704 ± 105.3 (n = 3) neutrophils to migrate, fMLP in the presence of O-3383 (1 μM) stimulated 4644 ± 42.0 (n = 3) neutrophils to migrate, whereas fMLP in the presence of vehicle (0.01% DMSO) stimulated 4671 ± 42.1 (n = 3) neutrophils to migrate (Fig. 8D). In the presence of O-3367 (1 μM) (Fig. 8E), the pEC_{50} of LTB_4 was significantly lower ($P < 0.001$; Student's unpaired t test), being 6.61 ± 0.04 compared with 8.88 ± 0.14 in the presence of vehicle (n = 6). In the presence of O-3383 (1 μM) (Fig. 8F), the pEC_{50} of LTB_4 was significantly lower ($P < 0.001$; Student's unpaired t test), being 7.03 ± 0.08 compared with 9.44 ± 0.15 in the presence of vehicle (n = 6).

Discussion

The first aim of this study was to characterize the interaction of a novel eicosanoid, LTB_4 ethanolamide, with BLT and TRPV1 receptors, comparing its effects with those of LTB_4. In the guinea pig lung parenchyma, LTB_4 produced concentration-related contraction with an EC_{50} of 10 nM, which is consistent with the values obtained by Jackson et al. (1999) and Lawson et al. (1989). This tissue is thought to express both BLT1 and BLT2 receptors; however, the ability of U75302, which has low affinity for the BLT2 receptor (Yokomizo et al., 2000b, 2001), to antagonize the action of LTB_4 in the lung suggests that the contractile response is BLT1 receptor-mediated. In the lung parenchyma, LTB_4 ethanolamide produced negligible contraction, but rather behaved as a BLT receptor antagonist in this tissue, with a similar K_B value to that of LY293111 (~4 nM) (Jackson et al., 1999). In human peripheral PMNs, we found that the EC_{50} of LTB_4 of ~1 nM was significantly lower than that of LTB_4 ethanolamide of ~100 nM. LTB_4 ethanolamide behaved as a partial agonist and significantly antagonized the ability of LTB_4 to induce migration (Fig. 3, A and B). Quantitative real-time polymerase chain reaction techniques and Northern blotting assays suggest that the expression of BLT1 is significantly higher in human peripheral blood leukocytes

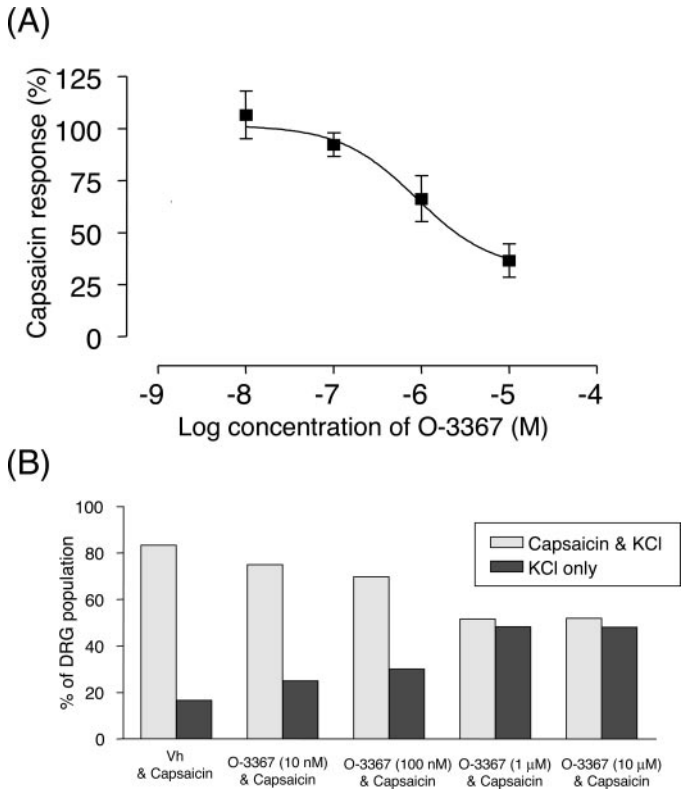

Fig. 7. Log concentration-response curve (A) for $[Ca^{2+}]_i$ evoked in DRG neurons by capsaicin (100 nM) in the presence of increasing concentrations of O-3367. Each symbol represents the mean as a percentage of $[Ca^{2+}]_i$ evoked by 1 M KCl ± S.E.M. (n = 10–37). The histogram (B) shows the percentage of the DRG population that is sensitive to either capsaicin and KCl or to KCl alone in the presence of capsaicin (100 nM) and increasing concentrations of O-3367 (n = 10–37).

than other human tissues (Owman et al., 1996; Yokomizo et al., 1997). The higher expression level of BLT1 receptors in leukocytes, compared with the lung, provides an explanation for LTB_4 ethanolamide behaving as a partial agonist in neutrophils and as an antagonist in the lung parenchyma. The receptor reserve in a given tissue or cell line will influence the efficacy of a low intrinsic efficacy agonist whereby such a compound may behave as a full agonist or a partial agonist/antagonist in situations of high or low receptor reserve, respectively.

The hypothesis that LTB_4 ethanolamide, as an antagonist of BLT receptors, may represent a putative endogenous "anti-inflammatory" metabolite of anandamide is a significant discussion point. However, conflicting observations surround this possibility. Maccarrone et al. (2000) have observed that specific inhibitors of 5-lipoxygenase markedly enhance the level of hydrolysis of anandamide by FAAH in human mast cells, implicating a physiological role for the 5-lipoxygenase metabolites of anandamide. Indeed, this group suggests that 5-lipoxygenase metabolites of anandamide may be acting as endogenous inhibitors of FAAH (Maccarrone et al., 1998, van der Stelt et al., 2002). However, the prospect of the production of LTB_4 ethanolamide in vivo has to be entertained with caution: Ueda et al. (1995) have shown that porcine leukocyte 5-lipoxygenase does not metabolize anandamide. Indeed, the weight of evidence suggests that anandamide is a poor substrate for 5-lipoxygenase (De Petrocellis et al., 2004).

TRPV1 receptors play a role in inflammation, and they can be activated/sensitized by various immune response products (Di Marzo et al., 2001; Piomelli, 2001). In inside-out patch-clamp experiment products of lipoxygenase metabolism activate vanilloid receptors in TRPV1-transfected human embryonic kidney 293 cells and DRG neurons (Hwang and Oh, 2002), the most potent metabolite being 12(S)-hydroperoxy-eicosatetraenoic acid followed by 15(S)-hydroperoxy-eicosatetraenoic acid and LTB_4. However, at a concentration of 10 μM, the response elicited with lipoxygenase products was significantly less than that obtained with 500 nM capsaicin (Hwang et al., 2000). In line with these findings, in this investigation, LTB_4 and LTB_4 ethanolamide produced an increase in $[Ca^{2+}]_i$ in rTRPV1-transfected CHO cells when exposed to the compounds for 20 min. Our studies suggest that these compounds have low efficacy at the TRPV1, the E_{max} values being significantly lower than that of capsaicin. The actions of both LTB_4 and LTB_4 ethanolamide are significantly antagonized by the TRPV1 antagonist capsazepine, suggesting that the $[Ca^{2+}]_i$ response is indeed TRPV1 receptor-mediated.

Jung et al. (1999), De Petrocellis et al. (2001), and Jordt and Julius (2002) have published studies indicating that the agonist-binding site of TRPV1 may be intracellular, and anandamide has been reported to be more potent at the TRPV1 receptor when applied intracellularly (Evans et al., 2004), presumably a consequence of the putative intracellular binding location. Conversely, Vyklicky et al. (2003) reported that TRPV1 is not activated by vanilloids applied intracellularly, and a study by Chou et al. (2004) suggests that the ultrapotent TRPV1 agonist resinaferatoxin interacts with extracellular residues. The complexity of TRPV1 ligand binding is reflected in a study by Gavva et al. (2004) who identify key residues in the transmembrane regions of rat and human TRPV1 that confer vanilloid sensitivity. They demonstrate that these residues differentially affect ligand recognition as well as the functional response and propose that the vanilloid-binding pocket can be fluid, moving across the membrane, easily accessible by both intra- and extracellular sides. In this study, we found that the exposure time required to achieve maximum $[Ca^{2+}]_i$ response was significantly longer for LTB_4 and LTB_4 ethanolamide than for capsaicin. This slow time course may be indicative of slow diffusion across the cell membrane to reach an intracellular/transmembrane binding site. An alternative explanation is a slow rate of activation of TRPV1 by these ligands, as has been demonstrated previously for other TRPV1 agonists: using Fura-2 calcium imaging in CHO cells expressing rTRPV1, Toth et al. (2004) demonstrated that different agonists show distinct patterns of modulation of the $[Ca^{2+}]_i$ and that there is significant difference in the latencies of response between agonists.

In line with the finding that these compounds are TRPV1 agonists in rTRPV1-expressing cells, we demonstrate that LTB_4 and LTB_4 ethanolamide increase $[Ca^{2+}]_i$ in a subpopulation of small-diameter capsaicin-sensitive DRG neurons. LTB_4 ethanolamide induces $[Ca^{2+}]_i$ increase in a significantly larger population of neurons than LTB_4. A similar structure-activity relationship is observed with anandamide, which is a more potent TRPV1 agonist than arachidonic acid, which lacks the ethanolamide group (Hwang et al., 2000). The data presented here concur with the hypothesis that lipoxygenase metabolites of anandamide are TRPV1 ago-

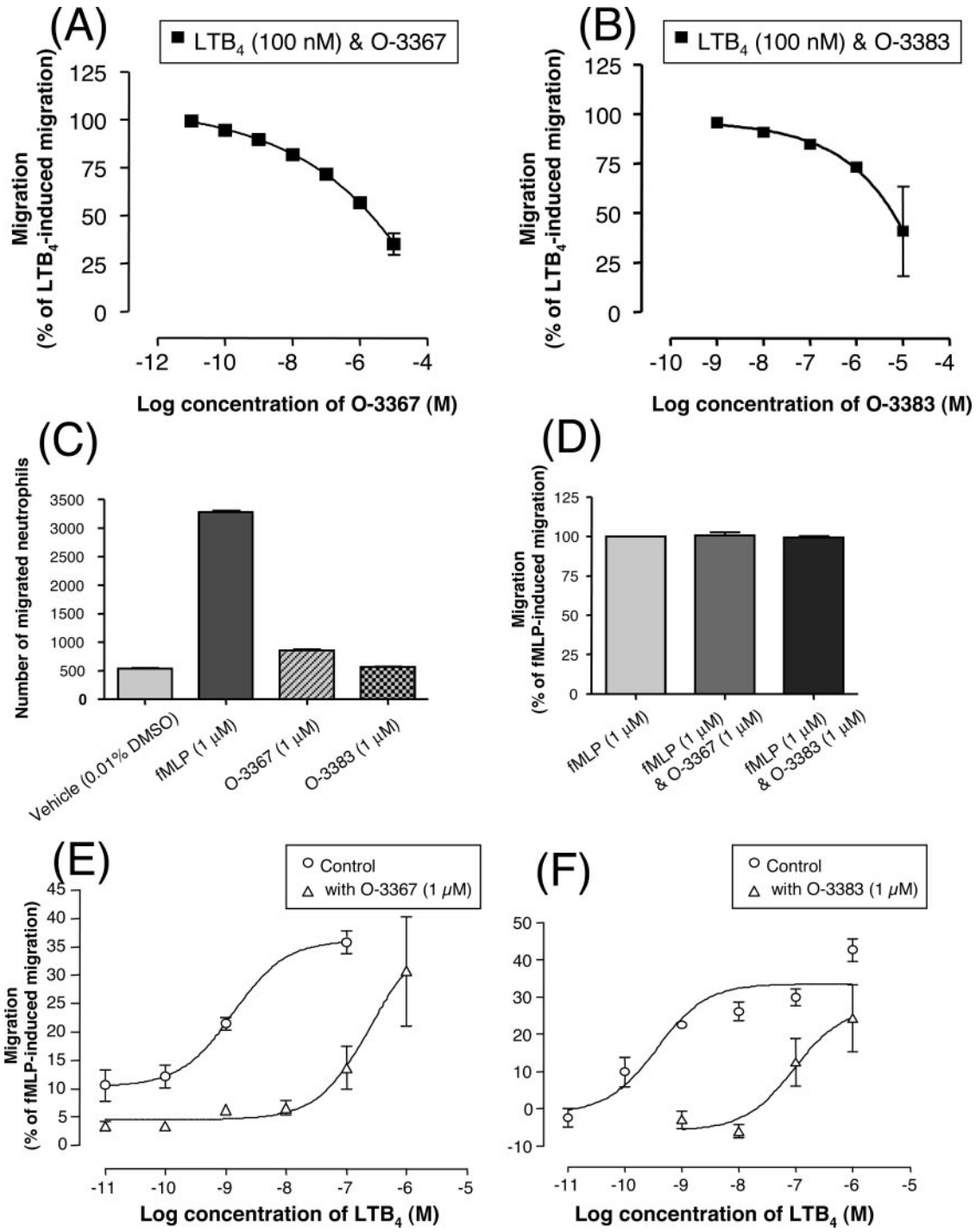

Fig. 8. Log concentration-response curves showing the migration of human neutrophils in response to LTB_4 in the presence of vehicle (control, 0.01% DMSO) and either O-3367 (1 μM) (A) or O-3383 (1 μM) (B). Migration is represented as a percentage of that induced by fMLP (1 μM). Each symbol represents the mean ± S.E.M. ($n = 6$). Histogram (C) shows the number of neutrophils induced to migrate by vehicle (0.01% DMSO), fMLP (1 μM), O-3367 (1 μM), and O-3383 (1 μM). The data represent the mean number of PMNs migrated ± S.E.M. ($n = 3$). The number of neutrophils that migrated in response to fMLP (1 μM) was significantly higher ($P < 0.0001$, Student's unpaired t test) than for vehicle (0.01% DMSO), O-3367 (1 μM), and O-3383 (1 μM). Histogram (D) shows the number of neutrophils induced to migrate by fMLP alone (1 μM), fMLP (1 μM) and O-3367 (1 μM), and fMLP (1 μM) and O-3383 (1 μM). The data represent the mean number of PMNs migrated ± S.E.M. ($n = 3$) as a percentage of the migration induced by 1 μM fMLP. There was no significant difference ($P > 0.05$; Student's unpaired t test) in the number of neutrophils that migrated in response to fMLP alone (1 μM) compared with fMLP (1 μM) in the presence of O-3367 (1 μM) or O-3383 (1 μM). Log concentration-response curves showing the migration of neutrophils induced by LTB_4 in the presence and absence of O-3367 (1 μM) (E) or by LTB_4 in the presence and absence of O-3383 (1 μM) (F). The data represent the mean number of PMNs migrated ± S.E.M. ($n = 6$) as a percentage of the migration induced by 1 μM fMLP.

nists, as evidenced by the observation that (in the presence of FAAH inhibitors) lipoxygenase enzyme inhibitors prevent the TRPV1 receptor-mediated contraction of the guinea pig isolated bronchus by anandamide (Craib et al., 2001). The metabolite(s) involved has yet to be isolated. In view of the evidence suggesting that anandamide is a poor substrate for

5-lipoxygenase, it is tempting to speculate that anandamide metabolism by 12- or 15-lipoxygenase may generate endogenous TRPV1 agonists. It is notable that capsaicin is known to contract the guinea pig lung parenchyma (Holzer, 1988). In this study, however, concentrations of LTB_4 ethanolamide that produced TRPV1 receptor-mediated effects in DRG, produced negligible contraction of guinea pig lung parenchymal strips. Thus, the low TRPV1 receptor efficacy of LTB_4 ethanolamide would explain the lack of a TRPV1-mediated contraction in this tissue compared with the high expressing recombinant CHO cell line and DRG neurons. It is known that anandamide acts as a full TRPV1 receptor agonist in isolated blood vessels, but it acts as a low-efficacy agonist in guinea pig bronchus and trigeminal neurons where it antagonizes the action of capsaicin (Andersson et al., 2002; Roberts et al., 2002; Ross, 2003).

On the basis of the preceding studies demonstrating that LTB_4 ethanolamide is a low-efficacy TRPV1 receptor agonist and a BLT receptor antagonist/partial agonist, we formed the hypothesis that it should be possible to synthesize a compound possessing pharmacophores such that it is capable of both BLT and TRPV1 receptor antagonism. Structure-activity studies on capsaicin analogs have shown that the 4-phenolic hydroxyl group is an essential pharmacophore (Wrigglesworth et al., 1996). Lee et al. (2003) theorized that isosteric replacement of this group in TRPV1 agonist compounds might modify the biological characteristic of such ligands and found that the isosteric replacement resulted in compounds that were effective TRPV1 receptor antagonists. Yagaloff et al. (1995) conducted a series of experiments to test the hypothesis that certain unsaturated fatty acids were capable of inhibiting LTB_4 receptor binding and LTB_4-induced leukocyte activation. The most potent fatty acid LTB_4 receptor antagonists were those with chain lengths of 16–22 and either *cis*- or *trans*-saturation at the *n*-9 or *n*-6 positions, e.g., ricinelaidic acid, eicosadienoic acid, and homo-γ-linolenic acid. The findings of these studies led us to design ligands that theoretically possess pharmacophores that confer antagonism of both TRPV1 and BLT receptors. This combination may be particularly effective in conditions such as inflammatory hyperalgesia (Rice et al., 2002) and airway hyperactivity (Hwang and Oh, 2002) where both receptors are influential proinflammatory target sites. We synthesized two novel compounds, O-3367 and O-3383.

Our data demonstrate that these compounds are antagonists of the TRPV1 receptor: the compounds significantly attenuated the effect of the TRPV1 receptor agonist capsaicin. O-3367 and O-3383 produced no change in basal $[Ca^{2+}]_i$ in rTRPV1-transfected CHO cells, but 10 μM produced a significant rightward shift in the log concentration-response curve for capsaicin. In DRG neurons that express native TRPV1 receptors, the IC_{50} for inhibition of capsaicin-induced increases in $[Ca^{2+}]_i$ by O-3367 was ~1 μM. The compounds also behaved as BLT receptor antagonists: in human neutrophils, which express both BLT1 and BLT2 receptors but not TRPV1 (Heiner et al., 2003), O-3367 and O-3387 significantly attenuated LTB_4-induced neutrophil migration with IC_{50} values of 60 nM and 1 μM, respectively (Fig. 8). Thus, O-3367 seems to be approximately 20-fold more potent as an antagonist of the BLT receptor-mediated effects in neutrophils compared with antagonism of TRPV1 receptor in DRG neurons. The relative potency of these compounds at BLT1 versus BLT2 remains to be established.

In summary, in this investigation we have demonstrated that LTB_4 ethanolamide acts as a BLT receptor antagonist in the lung and a BLT receptor partial agonist in human neutrophils and that it is a low-efficacy TRPV1 receptor agonist. Our studies with LTB_4 ethanolamide lead us to hypothesize that the overlap in structure-activity relationship between BLT and TRPV1 receptors may lend itself to the synthesis of compounds that antagonize both receptor types. Thus, in the second part of this study we demonstrate that O-3367 and O-3383 represent novel structural templates for generating compounds that possess dual antagonism of BLT and TRPV1 receptors. In view of the crucial role of both TRPV1 and BLT receptors in the pathophysiology of inflammatory conditions, such compounds may represent a novel class of highly effective therapeutics.

Acknowledgments

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