Two classes of cysteinyl leukotriene receptor, CysLT$_1$ and CysLT$_2$, have been identified and pharmacologically characterized in human tissues. Although the CysLT$_1$ receptor mediates the proinflammatory effects of leukotrienes in human asthma, the physiological roles of CysLT$_2$ receptor are not defined, and a suitable mouse model would be useful in delineating function. We report here the molecular cloning and characterization of the mouse CysLT$_2$ receptor (mCysLT$_2$R) from heart tissue. mCysLT$_2$R cDNA encodes a protein of 309 amino acids, truncated at both ends compared with the human ortholog (hCysLT$_2$R). The gene resides on the central region of mouse chromosome 14 and is composed of 6 exons with the entire coding region located in the last exon. Two 5'-untranslated region splice variants were identified with the short form lacking exon 3 as the predominant transcript. Although the overall expression of mCysLT$_2$R is very low, the highest expression was detected in spleen, thymus, and adrenal gland by ribonuclease protection assay, and discrete sites of expression in heart were observed by in situ hybridization. Intracellular calcium mobilization in response to cysteinyl leukotriene administration was detected in human embryonic kidney 293T cells transfected with recombinant mCysLT$_2$R with a rank order of potency leukotriene C$_4$ (LTC$_4$) = LTD$_4$ > LTE$_4$. $[^{3}H]$LTD$_4$ binding to membranes expressing mCysLT$_2$R could be effectively competed by LTC$_4$ and LTD$_4$ and only partially inhibited by LTE$_4$ and BAYu9773. The identification of mCysLT$_2$R will be useful for establishing CysLT$_2$R-deficient mice and determining novel leukotriene functions.

Cysteinyl leukotrienes (CysLT)$\cdot$ C$_4$, D$_4$, and E$_4$ have long been known as primary mediators in inflammatory diseases such as asthma and allergic rhinitis (1). These CysLTs are produced predominantly by eosinophils, mast cells, and macrophages in response to a variety of stimuli, including some antigens, which activate the 5-lipoxygenase pathway (2). The major actions of CysLTs in asthma include bronchoconstriction, bronchial hyperresponsiveness, increased pulmonary vascular permeability, parenchyma edema, and impaired mucus clearance (3).

Two types of CysLT receptor, CysLT$_1$ and CysLT$_2$, have been identified to mediate CysLTs effects (4, 5). Both are G protein-coupled receptors. Although pharmacological studies alone have provided valuable information, the recent molecular cloning and functional studies of both human (h) and mouse (m) CysLT$_1$R (6–10) and human CysLT$_2$R (11–13) have provided new avenues for study of leukotriene biological functions. CysLT$_1$R and CysLT$_2$R bind their cognate ligands with high affinity. The human receptor subtypes are only 31% identical at the protein sequence level. They are located on different chromosomes; hCysLT$_1$R is on Xq13-Xq21 and hCysLT$_2$R on 13q14. The rank order affinities of CysLTs for CysLT$_1$R and CysLT$_2$R determined in transfected HEK 293 cells and COS cells are LTD$_4$ > LTC$_4$ > LTE$_4$, and LTD$_4$ = LTC$_4$ > LTE$_4$, respectively, correlating well with what was determined previously pharmacologically (4). Although CysLT$_1$R can be inhibited specifically by the specific antagonists zafirlukast, montelukast, pranlukast, and MK-571, these compounds are unable to compete for binding at CysLT$_2$R, and no specific antagonist for this subtype has been identified to date (14). BAYu9773 is an antagonist at both receptors and also a partial agonist for CysLT$_1$R. The tissue expression patterns of these two receptors are different, indicating distinct roles in mediating leukotriene effects.

In this study we report the molecular cloning of mCysLT$_2$R, alternative transcripts, genomic structure and chromosomal mapping, tissue localization, and CysLT$_2$R functional characterization.
primers (GSP) were 5′-CCGTTCACACGAAGGCTAGGAGGACG (GSP1) and 5′-AGGTGTCGTTCACTAGGACGGCTACACG (GSP2). 5′ and 3′-nested PCRs were performed with nested GSP1, 5′-CTGAAAAAGGACCACTACTCA, and nested GSP2, 5′-ACTGGCGACCTCCACCTTGGCCATAGG. The full-length cDNA sequence was obtained by assembly of the 5′- and 3′-RACE products and the initial PCR-cloned fragment.

**Determination of the mCysLT₂R Gene Structure**—The whole gene structure for mCysLT₂R was determined by PCR. The following primer sets were used to clone each intron starting from the 5′-end of the gene.

1. In set 1 for intron 1, the sense primer was 5′-AGGGCAGCAAGGAGGAGGAC, and the antisense primer was 5′-GGAGGGAGACCACTTTCATAGGC.
2. In set 2 for introns 2 and 3, the sense primer was 5′-GGAAGGAGAAGAGTCCCTATGGAAGGATACTTC, and the antisense primer was 5′-TCTCAAGATCCTTCTTTAGGCAATGATACA.
3. In set 3 for intron 4, the sense primer was 5′-TGTCCTGGGAGGATATTATCAGG, and the antisense primer was 5′-TGTCATATGTCCTTCTTGGAAGGACAACTCTGG.
4. In set 4 for intron 5, the sense primer was 5′-GCCTTAGACTATGGAATTCTTTGGTG.

**Alternative Splicing Pattern in Different Tissues Using RT-PCR**—Total RNAs from various mouse tissues were reverse transcribed using the Superscript First-strand Synthesis System for RT-PCR (Life Technologies, Inc.) with 10% fetal bovine serum, 5′-CTTGGACAACTATTCTTCACTC. Both fragments were verified by SSCP, 0.1% SSPE, 0.01% SDS, 65°C. Fragments of 9.0 and 3.7 kb were detected in BamHI-digested C57BL/6J DNA, and fragments of 8.0 and 3.7 kb were detected in BamHI-digested M. spreus DNA. The presence or absence of the 8.0-kb BamHI M. spreus-specific fragment was followed in backcross mice.

A description of the probes and RFLPs for the loci linked to Cyslt2r including hr and Dct has been reported previously (17). Recombination distances were calculated using Map Manager, version 2.6.5. The gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

**Cell Culture and Transient Expression of the mCysLT₂R Receptor**—HEK 293T cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) with 10% fetal bovine serum, 0.1% penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml fungazole at 37°C in a humidified atmosphere with 5% CO₂. The coding region of mCysLT₂R was subcloned into pcDNA3 vector (Invitrogen). HEK 293 cells were transiently transfected with pcDNA3-mCysLT₂R using Fugene 6 (Roche), and HEK 293T cells were transfected with LipofectAMINE 2000 reagent (Life Technologies, Inc.) following the instructions of the manufacturer.

**Measurement of Agonist-induced Intracellular Calcium Mobilization**—HEK 293T cells were plated onto polystyrene-treated black wall microplates (Biocoat) at 5×10⁵ cells/well 24 h after transfection. 24 h later the cells were loaded with Fluor-4 calcium indicator dye (Molecular Probes, Eugene, Ore.) for 20 min at 37°C in the presence of 20 mM ML-2+ phenol red, and membranes were washed with Hanks’ balanced salt solution containing 20 mM HEPES and 2.5 mM probenecid, the cells were treated with varying concentrations of agonists, LTB₄, LTD₄, LTE₄, and LTB₅, maximum fluorescence indicating the changes in intracellular calcium concentrations following agonist activation was measured in a Molecular Devices Fluorometric Imaging Plate Reader (FLIPR). Data were analyzed by nonlinear regression using PRISM software (GraphPad, San Diego).

**Radioligandin Binding Assay**—HEK 293 cell membranes were prepared as described previously (18). Briefly, cells were harvested 48 h after transfection. The cells were dispersed in ice-cold buffer A (10 mM HEPES, 2 mM EDTA, 0.37 mg/ml protease inhibitor mixture (Roche), pH 7.4) by Dounce B homogenization followed by nitrogen cavitation at 1,100 p.s.i. for 15 min. The cell homogenate was centrifuged at 10,000 × g for 10 min, and the supernatant was centrifuged at 100,000 × g for 30 min. The resulting pellet was subjected to a final Dounce A homogenization. For competition studies, membrane preparations (100 µg of protein) were incubated with 0.5 nM [³H]LTDL, together with different concentrations of reagents in buffer containing 10 mM HEPES, 20 mM CaCl₂, pH 7.4, in the presence of 20 mM t-penicillamine at room temperature for 1 h. The reactions were stopped by ice-cold wash buffer (10 mM HEPES, 0.01% bovine serum albumin, pH 8.0), and the bound ligand was captured on Whatman GF/F filters. Radioactivity was quantified by liquid scintillation counting. Specific binding was determined by subtracting nonspecific binding, measured as the radioligand binding in the presence of 1 µM LTD₄, from total binding. Data were analyzed by nonlinear regression using PRISM software.

**RESULTS**

**Identification of Murine CysLT₂R Receptor cDNA, Splice Variants, and Gene**—We sought to identify and characterize the mouse CysLT₂R. Previous data suggested that rat may not possess a functional CysLT₂R because no evidence of signaling could be obtained from the cloned sequence, and its deduced structure was truncated relative to the human receptor (11). We designed a pair of degenerate primers based on the sequence similarity between human and rat CysLT₂R, and PCR was carried out using mouse genomic DNA as template. A single band of 590 bp corresponding to transmembrane domains III–VII was obtained. GSP derived from the fragment’s sequence were used to perform both 5′- and 3′-RACE from mouse heart cDNA. Sequence analysis and assembly of the three products identified a contiguous 1762-bp cDNA. This murine CysLT₂R cDNA has a 5′-untranslated frame of 930 bp, a 620-bp 5′-UTR, and a 212-bp 3′-UTR.

The deduced protein sequence has 309 amino acids with a calculated molecular mass of 35.3 kDa (Fig. 1A). The sequence identities between human and mouse, and rat and mouse are 65 and 84%, respectively. The deduced protein sequence alignment among the three species is shown in Fig. 1B. Interest-
Fig. 1. cDNA and gene structure of mCysLT2R. Panel A, sequence of full-length mCysLT2R cDNA. The deduced amino acid sequence is shown above the open reading frame. The putative polyadenylation signal AATAAA is underlined. The exon-intron junctions are indicated by numbered arrowheads. Exon 3 is shown in italics. * indicates the potential glycosylation site. Panel B, protein sequence alignment among human, rat, and mouse CysLT2Rs. The shaded regions show identity among these orthologs. The consensus sequence is given below the alignment. Putative transmembrane domains (I–VII) are underlined. Panel C, exon-intron organization. Upper section, the boundary sequences of the six exons (in capital letters) and five introns (in lowercase letters). The size of each intron is indicated in parentheses. Lower section, schematic illustration of the mCysLT2R gene. Exons are shown as black boxes and the alternatively spliced exon 3 as a striped box. The open reading frame in exon 6 is depicted by a light gray arrow. Introns are shown as dark gray lines. The gene is drawn to scale, except intron 3 is truncated as indicated by //.

AGAGGGACAG / gaaggttcacctt----(408 bp)----tctctttagctag / ATTCGGTCAT

TGGTTCAGAG / gtaacttggggatt----(258 bp)----tctctttagctag / TCTTTTACAT

GCAAATCAAG / gtaagttcttttt----(13.8 kb)----atgtcttttagctag / TTGCCCTGGA

GAGGATAAAA / gtaagtaacttctc----(1.6 kb)----tctctttagctag / GATGAAGAG

GAGAGATAG / gtaagttctttc----(1.9 kb)----atgtcttttagctag / GCCCATCATG

Exon 1 Intron 1 Exon 2

Exon 2 Intron 2 Exon 3

Exon 3 Intron 3 Exon 4

Exon 4 Intron 4 Exon 5

Exon 5 Intron 5 Exon 6

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ingly, the mouse and rat sequences are both truncated by 16 and 21 residues at the respective N and C termini. Hydrophobicity analysis reveals that each of the proteins has seven transmembrane-spanning domains and six hydrophilic loops, characteristic of G protein-coupled receptors (not shown).

The mCysLT2R gene organization was determined by a series of PCRs using primer sets designed from the cDNA sequence. The gene is composed of six exons and five introns and spans around 20 kb. The coding region is intronless with all introns located in the 5′-UTR and each exon-intron junction following the GT-AG rule (Fig. 1C).

A mouse cDNA sequenced from adult male tongue by the RIKEN Mouse Gene Encyclopedia Project (19; GenBank accession AK008997) is identical to our cDNA sequence except that a 57-bp fragment in the middle of the 5′-UTR is missing. This missing fragment corresponds to the entire exon 3. Hence two potential transcripts were identified: a long form containing all 6 exons and a short form lacking exon 3 (see below).

Expression and Alternative Splicing Pattern of the mCysLT2R Gene in Mouse Tissues—Initial Northern blot analysis of poly(A) RNA from various mouse tissues failed to find a detectable hybridization band indicating low level expression of mCysLT2R. A more sensitive ribonuclease protection assay was tested subsequently. By this technique, the highest expression was found in spleen, thymus, and adrenal gland with weaker expression in kidney, brain, and buffy coat (primarily peripheral blood leukocytes). mCysLT2R expression was not apparent in lung, liver, heart, aorta, skeletal muscle, uterus, and ovary by this technique (Fig. 2A).

A CysLT2R mRNA signal was detected in mouse heart using amplified in situ hybridization. CysLT2R mRNA was detected selectively on larger cells concentrated at the apical portions of the ventricle. This pattern is suggestive of expression on conducting Purkinje cells (Fig. 3C) and is consistent with the CysLT2R expression seen in human heart (11). A CysLT2R signal was also noted on endothelial cells surrounding cardiac vessels (Fig. 3, A and B). No signal was detected using sense control oligonucleotides (Fig. 3D).

RT-PCR was used to delineate the alternative splicing pattern of mCysLT2R. A 270-bp fragment representing the short splice variant was amplified in some tissues with a primer set spanning exon 3 from first strand cDNA. However, an expected 327-bp fragment containing exon 3 representing the long transcript failed to appear by this approach. A primer derived from exon 3 in combination with a downstream primer was used to amplify a fragment containing part of exon 3 which was followed by a second PCR using a nested primer within exon 3 and additional nested downstream primer. Only by two rounds of PCR could a 266-bp band containing part of exon 3 be detected (Fig. 2B). Neither the first primer set nor nested primer set alone was able to amplify a specific PCR product (data not shown). The short splice variant is predominant in every tissue expressing mCysLT2R, whereas the long transcript expression is trivial compared with the short transcript.

Chromosomal Localization of mCysLT2R—The mouse chromosomal location of Cyslt2r was determined by interspecific backcross analysis using progeny derived from matings of ((C57BL/6J × M. spretus) F1 × C57BL/6J) mice. This interspe-
specific backcross mapping panel has been typed for more than 3,200 loci that are well distributed among all of the autosomes as well as the X chromosome (15). C57BL/6J and M. spretus DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using a mouse cDNA Cyslt2r probe. The 8.0-kb BamHI M. spretus RFLP (see “Experimental Procedures”) was used to follow the segregation of the Cyslt2r locus in backcross mice. The mapping results indicated that Cyslt2r is located in the distal region of mouse chromosome 14 linked to hr and Dct. Although 162 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 4), up to 166 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere: hr → 7/166 → Cyslt2r → 37/162 → Dct. The recombination frequencies (expressed as genetic distances in centiMorgans ± the S.E.) are hr → 4.2 ±/− 1.6 → Cyslt2r → 22.8 ± 3.3 → Dct.

The distal region of mouse chromosome 14 shares regions of homology with human chromosomes 8p and 13q (summarized in Fig. 4), consistent with the assignment of CysLT2R to 13q14 in humans.

Intracellular Calcium Mobilization in Response to CysLTs—To detect the function of mCysLT2R, intracellular calcium mobilization was measured by FLIPR assay upon agonist activation in HEK 293T cells transiently transfected with pcDNA3-mCysLT2R vector. The cells responded to LTC4, LTD4, and LTE4 with marked, dose-dependent elevations of intracellular Ca2+ (Fig. 5). LTC4 and LTD4 were equipotent agonists for this receptor with EC50 values of 13.4 and 14.4 nM, respectively. LTE4 behaved as a partial agonist. LTE4 failed to elicit a significant Ca2+ response at concentrations up to 3 μM. A Ca2+ mobilization response to CysLTs using hCysLT2R in parallel gave comparable elevations. However, CysLTs seemed to have higher efficacy at the mCysLT2R, but the difference was not significant (p > 0.1). The EC50 values of these reagents for both mouse and human CysLT2R are shown in Table I. pcDNA3 vector-alone transfected cells did not respond to leukotrienes. BAYu9773 could elicit a partial Ca2+ mobilization response by itself. Pretreatment with BAYu9773 decreased the response to LTC4 and LTD4 (data not shown).

Radioligand Binding Characterization—Cell membranes from HEK 293 cells transiently transfected with pcDNA3-mCysLT2R were used for radioligand binding assays. [3H]LTD4 bound specifically to these membranes. Specific binding represented ~60% of the total binding, whereas membranes from vector-alone transfected cells showed no specific binding (data not shown). Competition assays with CysLTs, LTB4, CysLT2R-specific antagonist MK-571, and partial agonist/antagonist BAYu9773 were performed in three separate experiments with three different membrane preparations (Fig. 6). The rank order potency of leukotriene agonists to compete with [3H]LTD4 (0.5 nM) binding was LTC4 = LTD4 > BAYu9773 > LTE4 with IC50 values of 8.5, 17.6, 325, and 1,985 nM, respectively. LTB4 and MK-571 did not compete with [3H]LTD4 even at 10 μM.

**DISCUSSION**

In this report we have described the cloning and functional characterization of the mouse CysLT2 receptor. Contrary to previous data that implicate a nonfunctional rat CysLT2R (11), our functional experiments with mCysLT2R in parallel with the human and rat homologs revealed that all three CysLT2Rs had similar responses to CysLTs in raising intracellular calcium levels (Fig. 5 and data not shown).

Both mouse and rat CysLT2R are truncated by 16 and 21 residues at the respective N and C termini relative to the human sequence. However, this truncation is not significant in terms of ligand binding and intracellular signaling because the binding affinity of CysLTs to mCysLT2R is similar to hCysLT2R, and the ligand-induced intracellular Ca2+ mobilization is similar in both intensity and EC50 values in each of the human, rat, and mouse CysLT2R-transfected cell preparations. The significance of the extra sequences in hCysLT2R merits further investigation.

We report the first determination of a CysLT2R gene structure. It is composed of 6 exons and 5 introns with all introns located in the 5′-UTR and the coding region intronless like many other G protein-coupled receptor genes. We identified two splice variants for mCysLT2R mRNA. The 57-bp exon 3 is spliced out from the short transcript. Even though the long transcript was isolated during the initial cloning step from
Murine CysLT$_2$ Receptor

FIG. 4. Cyslt2r maps in the distal region of mouse chromosome 14. Cyslt2r was placed on mouse chromosome 14 by interspecific backcross analysis. The segregation patterns of Cyslt2r and flanking genes in 162 backcross animals that were typed for all loci are shown at the top of the figure. For individual pairs of loci, more than 162 animals were typed (see “Experimental Procedures”). Each column represents the chromosome identified in the backcross progeny which was inherited from the (C57BL/6J × M. spretus) F$_1$ parent. The shaded boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of a M. spretus allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 14 linkage map showing the location of Cyslt2r in relation to linked genes is shown at the bottom of the figure. Recombination distances between loci in centimorgans are shown on the left of the chromosome, and the positions of loci in human chromosomes, where known, are shown on the right. References for the human map positions of loci cited in this study can be obtained from GDB (Genome Data Base), a computerized data base of human linkage information maintained by the William H. Welch Medical Library of The Johns Hopkins University (Baltimore).

Heart cDNA, later RT-PCR results revealed that the short transcript was the predominant form in every tissue where the mCysLT$_2$R gene was expressed. Thus, PCR using a primer set spanning exon 3 could only amplify the short transcript because of the relative rareness of the long transcript. The mCysLT$_2$R gene also has long and short splice variants (8, 9) resulting in two mCysLT$_2$R receptors that differ at the N terminus. However, because the alternative splicing of mCysLT$_2$R occurs in the 5'-UTR region there should be no difference in protein structure. The significance of the alternative splicing remains to be clarified. The long transcript expression pattern is different from the short form, indicating that exon 3 might play some role in transcriptional or translational regulation.

Previously, CysLT$_2$R was described pharmacologically in guinea pig trachea and ileum, ferret trachea and spleen, sheep bronchus, and pulmonary arteries and veins and vasculature of various species (4, 20–23). hCysLT$_2$R mRNA was identified mainly in heart, spleen, adrenal gland, and various regions of the brain. Despite the identification of CysLT$_2$R expression sites, its function is not well defined. We cloned the mCysLT$_2$R cDNA from mouse heart, but this tissue exhibits very low overall expression as detected by Northern blotting, ribonuclease protection assay, and RT-PCR. These results are consistent with mCysLT$_2$R expression limited to Purkinje and endothelial cells by tyramide-enhanced in situ hybridization. CysLT$_2$s were shown to reduce coronary blood flow and myocardial contractility in guinea pig and rat (24). Human CysLT$_2$R was cloned from vascular endothelial cells, and LTD$_4$-induced vascular leakage was shown in mice (10). The identification of CysLT receptors in both human and mouse Purkinje and endothelial cells indicates multifunctional roles for these receptors in modulating cardiovascular function.

Consistent with hCysLT$_2$R distribution, we found the relative highest mCysLT$_2$R expression in spleen and adrenal gland. Thymus also has high expression, and kidney, brain, and buffy coat leukocytes show weaker evidence of expression. CysLT$_2$R may also mediate pulmonary vessel relaxation possibly through nitric oxide release (22). CysLT$_2$R might also take part in the neuroendocrine regulation of hormones (25) and identification of its expression in many brain regions and adrenal gland (Fig. 2; 11) might broaden our current perspective.

FIG. 5. Agonist-induced intracellular Ca$^{2+}$ mobilization measured by FLIPR assay. HEK 293T cells were transiently transfected with either human or mouse CysLT$_2$ receptor cDNAs and 48 h later were challenged with LTD$_4$ (●), LTE$_4$ (▲), LTE$_4$ (▼), and LTB$_4$ (●). Results are expressed as fluorescence counts and are the mean ± S.E. of duplicate determinations from three separate experiments. pcDNA3 vector-alone transfected cells were used as negative control and were challenged with LTD$_4$ ( ○) and LTE$_4$ (○). The results are the mean of duplicate determinations from one representative experiment (n = 3).

| Agonist | EC$_{50}$ value (nM) for CysLT$_2$R | EC$_{50}$ value (nM) for mCysLT$_2$R |
|---------|----------------------------------|----------------------------------|
| LTD$_4$ | 21.2                             | 13.4                             |
| LTE$_4$ | 26.6                             | 14.4                             |
| LTB$_4$ | >1,000                           | 728.3                            |
| LTE$_4$, PCDNA3 | >1,000 | 1,000                            |

TABLE I

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of CysLTs as inflammatory mediators toward other important physiological roles. CysLTs were able to elicit intracellular Ca²⁺ mobilization in CysLT₂R-transfected HEK 293T cells in a dose-dependent manner, indicating that CysLT₂R couples to the G₉/₁₁ family as does CysLT₁R. Coupling of CysLT₂R to other members in this family was also tested, but only G₉ signaled effectively (data not shown). Pertussis toxin did not affect the CysLT-induced Ca²⁺ response in CysLT₂R-transfected cells (7). However, it could partially block the response in U937 cells (26), which might only express CysLT₁R because the binding of CysLTs could be totally inhibited by CysLT₁R-specific antagonists (18), indicating the potential for differential CysLT₁R-mediated G protein coupling. Whether CysLT₂R might also couple to the G₉ class in certain tissues in unknown.

mCysLT₂R binds CysLTs in a rank order of affinity LT₄ = LTD₄ ≫ LTE₄, as does hCysLT₂R. BAYu9773 works at this receptor as both antagonist and partial agonist. Pretreatment with BAYu9773 partially abolished cellular reaction to LT₄ and LTD₄, indicating desensitization of the receptor. With cloned CysLT₂ receptors available, it should be feasible to develop new pharmaceutical agents specific to CysLT₂R.

In summary, we identified mCysLT₂R as a functional CysLT receptor that is derived from 5'-UTR alternative transcripts, and which functions similar to hCysLT₁R. The establishment of a CysLT₂R-deficient mouse model would potentially yield new insights into biological and pathophysiological roles of this receptor.

Acknowledgments—We thank Anna Myklebust for excellent technical assistance and Drs. Mark Abramovitz, Jilly Evans, and Xin-Sheng Chen for helpful discussions on CysLT receptors.

REFERENCES

1. Busse, W. F., and Gaddis, J. N. (1991) Am. Rev. Respir. Dis. 143, S103–S107
2. Dahlen, S. E., Hansson, G., Hedqvist, P., Bjorck, T., Granstrom, E., and Dahlen, B. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1712–1716
3. Bisgaard, H. (2001) Pediatrics 107, 381–390
4. Coleman, R. A., Eglein, R. M., Jones, R. L., Narumiya, S., Shimizu, T., Smith, W. L., Dahlen, S. E., Dzave, J. M., Gardiner, P. J., Jackson, W. T., Jones, T. R., Krell, R. D., and Nicosia, S. (1995) Adv. Prostaglandin Thromboxane Leukotriene Res. 23, 283–285
5. Metters, K. M. (1995) J. Lipid Mediat. Cell Signal. 12, 413–427
6. Lynch, K. R., O’Neill, G. P., Liu, Q., Im, D. S., Sawyer, N., Metters, K. M., Coulombe, N., Abramovitz, M., Figueroa, D. J., Zeng, Z., Connolly, B. M., Bai, C., Austin, C. P., Chateauneau, F., Stocco, R., Greig, G. M., Karmgar, S., Hooke, S. B., Hosfield, E., Williams, D. L., Jr., Ford-Hutchinson, A. W., Caskey, C. T., and Evans, D. J. (1999) Nature 399, 789–793
7. Sarau, H. M., Ames, R. S., Chambers, J., Ellis, C., Elshourbagy, N., Foley, J. L., Schmidt, D. B., Musciatti, R. M., Jenkins, O., Murdock, P. R., Herrity, N. C., Halsey, W., Sathe, G., Murer, A. I., Nuthalapati, P., Dyrek, G. M., Buckley, P. T., Wilson, S., Bergsma, D. J., and Hay, D. W. (1999) Mol. Pharmacol. 56, 657–663
8. Maekawa, A., Kanoaka, Y., Lam, B. K., and Austen, K. F. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 2556–2561
9. Martin, V., Sawyer, N., Stocco, R., Unett, D., Lerner, M. R., Abramovitz, M., and Funk, C. (2001) Biochem. Pharmacol. 62, 1193–1200
10. Gronert, K., Martinsson-Niskanen, T., Rivas, S., Chiang, N., and Serhan, C. N. (2001) Am. J. Pathol. 158, 3–9
11. Heise, C. E., O'Dowd, B. F., Figueroa, D. J., Sawyer, N., Nguyen, T., Im, D. S., Stocco, R., Bellefeuille, J. N., Abramovitz, M., Cheng, R., Williams, D. L., Jr., Zeng, Z., Liu, Q., Ma, L., Clements, M. K., Coulombe, N., Liu, Y., Austin, C. P., George, S. R., O’Neill, G. P., Metters, K. M., Lynch, K. R., and Evans, J. F. (2000) J. Biol. Chem. 275, 30531–30536
12. Takasaki, J., Kaminohara, M., Matsumoto, M., Saito, T., Sugimoto, T., Okishi, T., Ishii, H., Ota, T., Nishikawa, T., Kawai, Y., Mano, Y., Iosiga, T., Suzuki, Y., Sugano, S., and Furuichi, K. (2000) Biochem. Biophys. Res. Commun. 274, 316–322
13. Nohacke, H. P., Wang, Z., Zhu, Y., Reinscheid, R. K., Lin, S. H., and Civelli, O. (2000) Mol. Pharmacol. 58, 1601–1608
14. Dahlen, S. E. (1999) Clin. Rev. Allergy Immunol. 17, 179–191
15. Copeland, N. G., and Jenkins, N. A. (1991) Trends Genet. 7, 113–118
16. Jenkins, N. A., Copeland, N. G., Taylor, B. A., and Lee, B. K. (1992) J. Virol. 66, 43–56
17. Jackson, I. J., Chambers, D. M., Tsukamoto, K., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., and Hearing, V. (1992) EMBO J. 11, 527–535
18. Frey, E. A., Nicholson, D. W., and Metters, K. M. (1993) Eur. J. Pharmacol. 244, 239–250
19. Kawai, J., Shinagawa, A., Shibata, K., Yoshino, M., Ishi, H., Otsuji, Y., Turat, H., Fukuda, S., Aizawa, K., Iwazawa, M., Kishi, K., Ikikawa, H., Kondo, S., Yamanaka, I., Saito, T., Okazaki, Y., Gejocki, T., Bono, H., Kasukawa, T., Saito, R., Kodota, K., Matsuda, H. A., Ashburner, M., Bataev, S., Cassavant, T. F., Fleischmann, W., Gaasterland, T., Glisi, C., King, B., Koichi, H., Koebi, P., Lewis, S., Matsu, N., Naido, I., Pesele, G., Quackenbush, J., Schreiber, L. M., Staubli, F., Suzuki, R., Tomita, M., Wagner, L., Washio, T., Sakai, K., Okido, T., Furuno, M., Ano, H., Baldarelli, R., Grish, B., Blake, J., Boffelli, D., Bojunga, N., Carninci, P., de Bonalde, M. F., Brownstein, M. J., Bult, C., Fletcher, C., Fujita, M., Gaboriobi, M., Grottisch, S., Hill, D., Hofmann, M., Hume, D. A., Kamiji, M., Lee, N. H., Lyons, P., Marchionni, L., Mabusha, J. M., MacRae, D., Menken, P., Nordone, P., Ring, B., Ringwald, M. Rodriguez, I., Sakamoto, N., Sasaki, H., Saito, K., Schonbach, C., Seya, T., Shibata, Y., Storch, K. F., Suzuki, H., Togo-oka, K., Wang, K. H., Weitz, C., Wiberg, C., Wilming, L., Woyhrauch-Boris, A., Yoshida, K., Hasegawa, Y., Kawai, H., Kohsuzuki, S., and Hayashizaki, Y. (2001) Nature 409, 685–690
20. Labat, C., Ortiz, J. L., Norel, X., Gereigne, I., Verley, J., Abrams, T. S., Cuthbert, N. J., Ludzhe, S. R., Norman, P., Gardner, P., and Brink, C. (1992) J. Pharmacol. Exp. Ther. 266, 803–805
21. Ortiz, J. L., Gereigne, I., Cortijo, J., Seller, A., Labat, C., Sarria, B., Abrams, T. S., Gardner, P. J., Morcillo, E., and Brink, C. (1995) Br. J. Pharmacol. 115, 1582–1586
22. Walch, L., Norel, X., Gascard, J. P., and Brink, C. (2000) Am. J. Respir. Crit. Care. Med. 161, S107–S111
23. Back, M., Norel, X., Walch, L., Gascard, J., Mazmanian, G., Dahlen, S., and Brink, C. (2000) Eur. J. Pharmacol. 401, 381–388
24. Letts, G. L., and Piper, J. P. (1983) Adv. Prostaglandin Thromboxane Leukotriene Res. 11, 391–395
25. Saadil, M., Gerzinovitz, K., Rouget, C., Minard, P., and Dray, F. (1990) Life Sci. 46, 1857–1865
26. Saussy, D. L., Jr., Sarau, H. M., Foley, J. L., Mong, S., and Crouse, S. T. (1989) J. Biol. Chem. 264, 19845–19855

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The Murine Cysteinyl Leukotriene 2 (CysLT2) Receptor: cDNA AND GENOMIC CLONING, ALTERNATIVE SPLICING, AND IN VITRO CHARACTERIZATION

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J. Biol. Chem. 2001, 276:47489-47495. doi: 10.1074/jbc.M107556200 originally published online October 8, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107556200

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