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Research Article

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Leukotriene D_4 Activates a Chloride Conductance in Hepatocytes from Lipopolysaccharide-treated Rats

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Abstract

Endotoxin (LPS) can cause hepatocellular injury under several circumstances, and leukotrienes have been implicated as a contributing factor. Since ion channel activation has been associated with cytotoxicity, the aim of this study was to determine the circumstances under which LPS and/or leukotrienes activate ionic conductances in hepatocytes. LPS treatment of rats increased Cl^- conductance in hepatocytes from 232±42 to 1236±134 pS/pF. Voltage dependence and inhibitor specificity of this conductance were similar to that of a swelling-activated Cl^- conductance, and internal dialysis with nucleoside analogues suggested control by an inhibitory G protein. The lipoxygenase inhibitor nordihydroguaiaretic acid, the specific leukotriene D_4 (LTD_4) receptor antagonist MK-571, and the 5-lipoxygenase activating protein inhibitor MK-886 all significantly inhibited the conductance. Intracellular dialysis with LTD_4 (1.5 μM) elevated intracellular Ca^{2+} from 143±6.5 to 388±114 nM within 6 min and stimulated an outwardly rectifying conductance from 642±159 to 1669±224 pS/pF ($n = 9$, $P < 0.001$). In hepatocytes prepared from untreated rats, this concentration of intracellular LTD_4 neither raised intracellular Ca^{2+} nor activated the conductance. The LTD_4 response could be induced in normal hepatocytes by culture with either conditioned medium from LPS-treated macrophages or purified TNF-α. In conclusion, intracellular LTD_4 activates a chloride conductance in hepatocytes isolated from rats treated with LPS or primed in vitro with TNF-α. Changes in the hepatocellular accumulation of leukotrienes therefore mediate channel activation and may contribute to liver injury during sepsis and other inflammatory conditions. (*J. Clin. Invest.* 1997. 99:2915–2922.) Key words: whole-cell path clamp • inflammation • ion channels • tumor necrosis factor

Introduction

Epithelial chloride channels play a role in both normal cell function and cell injury processes. They are involved in volume regulation, transepithelial fluid transport, and stabilization of membrane potential (1, 2). Activation of chloride channels precedes cell death in several models of hypoxic cell injury (3). In hepatocytes, chloride channels have been identified in canalicular membrane vesicles (4) and isolated hepatocytes (5). The regulation of epithelial chloride channels is complex and examples have been observed for the involvement of intracellular Ca^{2+} elevations, G protein–coupled receptors, and intracellular protein kinase cascades (6). A chloride conductance that is activated by either cell swelling or cAMP has recently been identified in primary isolated hepatocytes (7).

Leukotrienes are metabolites of arachidonic acid produced via the 5-lipoxygense pathway (8). The cysteinyl leukotrienes (LT),[1] LTC_4, LTD_4, and LTE_4, are potent mediators of inflammation but have also been shown to regulate ion channels in a number of systems. These include K^+ currents in cardiac myocytes (9, 10), somatostatin-activated Cl^- currents in hippocampal neurons (11), and Na^+ channel activity in polarized renal epithelial cells (12). Leukotrienes also modulate volume regulation in Ehrlich ascites tumor cells (13–15). Since the liver is the major site of leukotriene clearance from plasma (16), and hepatocytes possess the ability for uptake and secretion of cysteinyl leukotrienes, it is possible that leukotrienes play a role in ion channel regulation in hepatocytes.

Leukotrienes have also been associated with inflammatory or immune liver cell injury (17, 18). In hepatocytes, cysteinyl leukotrienes are taken up into the cells across the sinusoidal membrane and excreted into the bile across the canalicular membrane by the canalicular isoform of the multidrug resistance–associated protein (cMRP) transporter (19–21). During inflammation, this canalicular secretory step is inhibited, but the sinusoidal membrane uptake is unchanged and therefore intracellular concentrations of cysteinyl leukotrienes increase (22). Leukotrienes thus serve as substrates for transport and potentially as modulators of cell function. Leukotriene-induced changes in ion channel activity may contribute to inflammatory cholestasis and cytotoxicity.

In this study, we sought to determine whether ion channels are activated during inflammation and whether leukotrienes modulate this activity. We used whole-cell patch clamp techniques to compare chloride channel activity in hepatocytes derived from control and LPS-treated rats, a model that duplicates some of the systemic and hepatic responses to bacterial infection, and hepatocytes treated in vitro with either cytokine containing conditioned medium or purified TNF-α. Our aims were to identify and characterize LPS-induced ionic conduc-

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1. *Abbreviations used in this paper:* cMRP, canalicular isoform of the multidrug resistance–associated protein; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; I-V, voltage–current relationship; FLAP, 5-lipoxygenase-activating protein; LT, leukotriene; NDGA, nordihydroguaiaretic acid; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid; TMA, tetramethylammonium; UW solution, University of Wisconsin solution.

tances and to determine the role of leukotrienes in channel activation.

Methods

Isolation and primary culture of hepatocytes. Isolated hepatocytes from male Sprague-Dawley rats were prepared by two-step collagenase perfusion of the liver as described previously (23). Trypan blue exclusion was used to assess viability, which ranged from 90 to 95%. Freshly isolated hepatocytes were stored up to 24 h in a modified University of Wisconsin solution at 4°C as described previously (24). Before culturing, stored cells were washed twice with L-15 medium. The hepatocytes were allowed to attach to collagen-coated glass coverslips and were cultured for 2–6 h at 37°C in L-15 medium, containing 10% vol/vol FBS, penicillin (200 U/ml), and streptomycin (0.2 mg/ml).

Conditioned medium was derived from the supernatant of RAW 264.7 cells, a mouse monocyte-macrophage cell line, by the method originally described by Cerami and colleagues (25). They were grown to confluence in RPMI 1640 medium (GIBCO BRL, Gaithersburg, MD) with 10% FBS, and stimulated by replacing the medium with 100 ml of serum-free RPMI medium containing 1 μg/ml LPS and 50 mM Hepes, pH 7.4. After a 22-h incubation the medium was harvested, filtered through a 0.22-μm filter, and stored frozen at −20°C. Conditioned medium was then added to hepatocyte cultures at a dilution of 1:5. Immunoprecipitation of TNF-α was performed by incubating conditioned medium with rabbit anti–mouse TNF-α antiserum (Genzyme, Cambridge, MA) at a dilution of 1:200 for 16 h at 4°C.

Endotoxin treatment of animals. Rats were treated with TCA-precipitated *Escherichia coli* LPS serotype 026:B6 (Sigma Chemical Co., St. Louis, MO), 3 mg/kg in saline, by intraperitoneal administration 18 h before hepatocyte isolation. Animals appeared healthy at this time, and there was no mortality from this treatment.

Solutions. Most experiments were performed with Cl^- as the only permeant ion. In these circumstances, tetramethylammonium (TMA) was used as an impermeant cation. Cells were bathed in a high Cl^- solution consisting of (in mmol/l) TMA-Cl, 149; $MgSO_4$, 1.0; $CaCl_2$, 1.25; Hepes, 10; NaH_2PO_4, 2.0 adjusted to pH 7.4 with tetramethylammonium hydroxide and having an osmolality of 300 mosmol/kg. Pipet (internal) solution contained TMA-Cl, 140; $MgSO_4$, 3.0; $CaCl_2$, 0.4; EGTA, 2.0; Hepes, 10; and ATP, 3.0 (adjusted to pH 7.2). Osmolality was 270 mosmol/kg. In some experiments, bath solution osmolality was decreased by reduction of TMA-Cl concentration or increased by addition of sucrose. High K^+, low Cl^- bath solution consisted of K-gluconate, 149; NaH_2PO_4, 2.0; $CaCl_2$, 1.25; $MgSO_4$, 1.0; and Hepes, 10 (pH 7.4 adjusted with KOH and having an osmolality of 300 mosmol/kg). High K^+, low Cl^- pipet solution consisted of K-gluconate, 140; $MgSO_4$, 3.0; Hepes, 10; ATP, 3.0; KCl, 1.7; EGTA, 2.0; $CaCl_2$, 0.4 (pH 7.2, 270 mosmol/kg). Osmolality was measured with a vapor-pressure osmometer (model 5500; Wescor, Inc., Logan, UT). The calculated free Ca^{2+} (26) in the standard pipet solution (100 nM) approximated the basal intracellular Ca^{2+} concentration. All solutions were filtered through 0.2-mm diameter membrane filters (Acrodisc, Gelman Sciences, Ann Arbor, MI).

Whole-cell patch clamp. Whole-cell patch clamp of hepatocytes was performed as described previously (7). Borosilicate glass micropipets (catalogue No. 1B150F-6; WP Instruments, Sarasota, FL) were pulled with a multistep pull on a Flaming-Brown micropipet puller (model P-87; Sutter Instrument Co., Novato, CA), and were fire polished to final tip resistance of 2–4 MΩ when filled with filling solution and immersed in bath solution. Coverslips with attached hepatocytes were placed in a polycarbonate chamber on the stage of a Diaphot microscope (Nikon Corporation, Tokyo, Japan) and superfused constantly at 1–2 ml/min by gravity. Solution changes were performed with a six-way valve. Experiments were performed at room temperature. Giga-seals were obtained with gentle suction of the pipet. Seal resistances ranged from 5 to 20 GΩ. After formation of a tight seal between the hepatocyte and the pipet, whole-cell configuration was obtained by applying negative pressure to the pipet.

Whole-cell currents were measured using a patch clamp amplifier (Axopatch-200; Axon Instruments, Foster City, CA) and filtered at 2,000 Hz with the integrated Bessel filter in the amplifier. Cell capacitance and series resistance were measured every few minutes and corrected using the built-in circuits of the Axopatch amplifier. Series resistance never increased by more than 50% during the experiments. Current–voltage (I-V) relationships were determined with a stepwise clamp protocol. From a holding voltage of −20 mV, voltage pulses of 35-ms duration were applied from −60 to +60 mV in 10-mV steps. In some experiments, longer voltage clamp pulses of 750-ms duration were applied over the range −100 to +100 mV. For determination of I-V relationships, current was measured 30 ms after initiation of the voltage step. Outward chord conductance over the range 0 to +60 mV was determined and normalized to total cell capacitance. pCLAMP software (version 5.6; Axon Instruments) was used to generate the command voltages and record the resulting currents. In some experiments, the time course of conductance change was measured by sequentially clamping for 500 ms at −60 and +60 mV at 10-s intervals. Liquid junction potential changes resulting from changes in solution composition were measured with a flowing 3-M KCl junction, and were corrected before plotting I–V curves.

Intracellular Ca^{2+} measurement. Intracellular Ca^{2+} was measured in whole-cell, patch-clamped hepatocytes by monitoring fluorescence of the dye Fluo-3 with a photometer-based system as described previously (24, 27). Hepatocytes were preloaded with the cell-permeant derivative Fluo-3-AM (5 μM) for 20 min at 37°C before patching. In these experiments, the pipet solution contained the cell-impermeant Fluo-3–free acid (10 μM). This kept dye concentration in the cell constant during patch clamp, and it allowed simultaneous current and intracellular Ca^{2+} measurement. Excitation light was provided by a 75-W Xenon arc lamp passed through a 3% transmittance neutral-density filter and a 450–490-nm band-pass filter (Omega Optical, Brattleboro, VT). Emitted light at > 520 nm was measured. The epifluorescence field diaphragm was constricted to illuminate a circular area of 30 μm in diameter, which was slightly larger than one cell. Emitted light was measured from a circle of ∼ 6–7-μm diameter with a photomultiplier tube (model 70706; Oriel, Stratford, CT). The intracellular Ca^{2+} measurement was calibrated with the ionomycin/Mn^{2+} method (28).

Cell size measurements. Cross-sectional areas of single hepatocytes were measured as described previously (7). Bright-field images of hepatocytes in the focal plane of the maximal cross-sectional area were acquired, and the cross-sectional areas were measured with the Image-1 software package (Universal Imaging Corp., West Chester, PA). Previous studies using optical planimetry and three-dimensional reconstruction have shown that although the hepatocytes are not spherical, relative volume changes calculated in this way reflect true changes in cell volume (29).

Materials. Rat tail collagen was obtained from Collaborative Biomedical Products (Bedford, MA); FBS was serum from Biocell Laboratories (Rancho Dominguez, CA); collagenase was from Boehringer Mannheim (Indianapolis, IN); L-15 culture medium was from GIBCO BRL; and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) was from Research Biochemical International (Natick, MA). LTD_4 was from Cayman Chemical Co. (Ann Arbor, MI). TMA-Cl, niflumic acid, nordihydroguaiaretic acid (NDGA), *E. coli* LPS serotype 026:B6, cAMP, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), ATP, EGTA, and Hepes were all obtained from Sigma Chemical Co. HBSS was obtained from Life Technologies, Inc. (Grand Island, NY). MK-571 (L-660,711) and MK-886 (L-663,536) were gifts from Dr. Anthony W. Ford-Hutchinson (Merck Frosst Centre for Therapeutic Research, Quebec, Canada). Fluo-3 (pentapotassium salt) and Fluo-3AM were obtained from Molecular Probes, Inc. (Eugene, OR). Purified mouse TNF-α was a gift from Dr. Allan Green (University of Texas Medical Branch, Galveston, TX).

Statistics. Results are presented as mean±SE unless otherwise stated. Comparisons were made by paired *t* test, unpaired *t* test, or

ANOVA, as indicated using the software package SigmaStat (Jandel Scientific, San Rafael, CA).

Results

Cl^- conductance is activated by LPS treatment. An outwardly rectifying current was present in 19 of 19 hepatocytes that were isolated 18 h after treatment of rats with LPS (day 1 cells). The average conductance of these cells (1,236±134 pS/pF, $n = 19$) was approximately five times greater than that of cells that were similarly isolated from control rats, which had a conductance of 231±41 pS/pF and displayed an outwardly rectifying I-V relationship in 15 of 24 cells. The LPS-activated current slowly inactivated at high positive voltages (Fig. 1). The current magnitude and slow inactivation at +100 mV were similar to those described previously after cell swelling (7).

We further assessed the ionic selectivity of the LPS-activated conductance by determining if it was present in high K^+ solutions. When K^+ was the only permeant ion, with K-gluconate instead of TMA-Cl in both pipet and bath, there was no difference in conductance between LPS-treated and control cells (357±122 pS/pF, $n = 9$ vs. 340±171 pS/pF, $n = 5$). This result demonstrates that the conductance in LPS cells is anion selective.

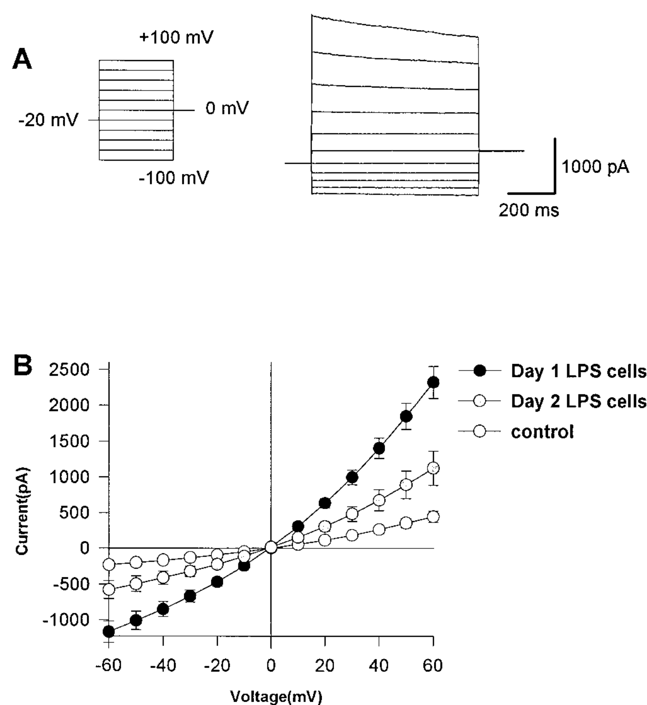

Figure 1. Whole-cell conductance in hepatocytes from LPS-treated rats. Whole-cell patch clamp was performed in hepatocytes isolated from control and LPS-treated rats. (*A*) Voltage step protocol for the ±100 mV range is displayed on the left. Example of currents obtained 3 min after formation of the whole-cell configuration in LPS day 1 cell is displayed on the right, demonstrating slow inactivation at positive voltage. (*B*) I-V relationships determined 3 min after formation of the whole-cell configuration in day 1 LPS cells (freshly isolated from LPS-treated rats, *filled circles*, $n = 19$), day 2 LPS cells (after 24-h storage at 4°C in UW solution, *shaded circles*, $n = 16$), and control cells (*open circles*, $n = 26$). Currents were measured 30 ms after voltage steps performed over the ±60 mV range as described in Methods. Points represent mean±SE.

To evaluate whether cell swelling could account for the conductance activation in these cells, we measured the cross-sectional area in populations of isolated control and LPS hepatocytes. Cell cross-sectional area increased from 468±8 ($n = 76$) to 521±12 μm² ($n = 71$, $P < 0.001$) after LPS treatment, suggesting that cell swelling could contribute to conductance activation. LPS-treated cells were then exposed to bath osmolality changes to determine if swelling and LPS-induced conductance changes were independent. Fig. 2 demonstrates that unlike the situation in control cells (7), hyposmotic bath solution (250 mosmol/kg) failed to significantly increase conductance in LPS-treated cells. Cell shrinkage with a hyperosmotic bath (350 msomol/kg), however, reduced conductance from 1,610±248 to 807±342 pS/pF ($n = 4$, Fig. 2). These results suggest that cell swelling contributes to the conductance activation present in LPS-treated cells, since cell swelling and LPS treatment are not additive, and cell shrinkage partially reverses the LPS-induced conductance.

Inactivation of conductance after cell storage. When hepatocytes derived from LPS-treated rats were preserved for 24 h at 4°C in UW solution (day 2 cells), the conductance was reduced. In approximately half of the cells, it returned to the control value (114±32 pS/pF, $n = 7$), and in the remainder, it was reduced to 919±181 pS/pF ($n = 9$). The effect varied between rats, but was relatively constant within a hepatocyte preparation from a single rat. The average conductance of these LPS day 2 cells was 566±144 pS/pF ($n = 16$), significantly lower than that of day 1 cells (Fig. 1). The results indicate that channel activation induced by LPS was gradually diminished with in vitro cell storage.

Role of intracellular Ca^{2+} in conductance activation in LPS cells. The Ca^{2+} dependence of the LPS-activated conductance was examined by simultaneous Ca^{2+} removal from the bath

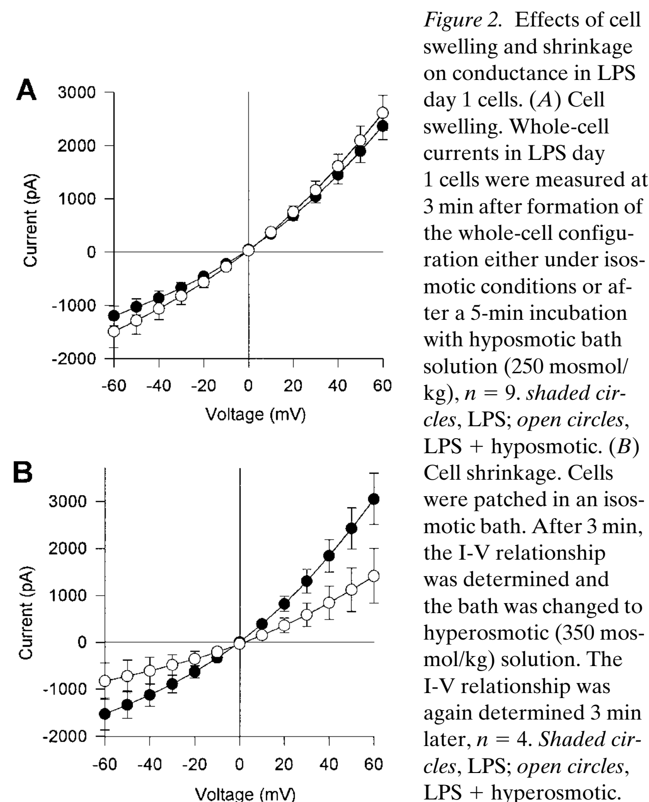

Figure 2. Effects of cell swelling and shrinkage on conductance in LPS day 1 cells. (*A*) Cell swelling. Whole-cell currents in LPS day 1 cells were measured at 3 min after formation of the whole-cell configuration either under isosmotic conditions or after a 5-min incubation with hyposmotic bath solution (250 mosmol/kg), $n = 9$. *shaded circles*, LPS; *open circles*, LPS + hyposmotic. (*B*) Cell shrinkage. Cells were patched in an isosmotic bath. After 3 min, the I-V relationship was determined and the bath was changed to hyperosmotic (350 mosmol/kg) solution. The I-V relationship was again determined 3 min later, $n = 4$. *Shaded circles*, LPS; *open circles*, LPS + hyperosmotic.

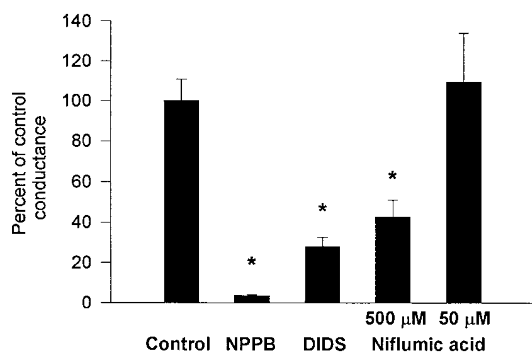

Figure 3. Effect of Cl^- channel blockers on conductance in LPS day 1 cells. Conductance was measured before (control) and after exposure to NPPB (200 μM), DIDS (150 μM), or niflumic acid (50 or 500 μM). Bars represent the percent of control conductance. $n = 4$–19. *Significantly different than control by one-way ANOVA.

and intracellular dialysis with 10 mM EGTA without added Ca^{2+}. Although this method reduces intracellular Ca^{2+} to less than 10 nM (unpublished observations), it had no effect on the conductance in LPS day 1 cells. Conductance was 1,228±144 pS/pF ($n = 5$) for control vs. 1,279±431 pS/pF ($n = 6$) for Ca^{2+} chelation.

Effects of chloride channel blockers and guanine nucleotides on the LPS-activated conductance. We further characterized the conductance activated in LPS-treated cells by examining its inhibition by Cl^- channel blockers and involvement of G proteins in conductance activation. The conductance of LPS day 1 cells was completely and reversibly inhibited by the anion channel blocker NPPB (200 μM). The stilbene derivative Cl^- channel inhibitor DIDS (150 μM) partially inhibited the conductance. Niflumic acid, an inhibitor of Ca^{2+}-dependent and other chloride channels, inhibited the conductance at a concentration of 500 μM but not at 50 μM (Fig. 3).

The effects of intracellular dialysis with GTP and the slowly metabolizable GDP analogue GDP-β-S were measured to determine if G proteins are involved in conductance activation (Fig. 4). Intracellular GTP (100 μM) reduced conductance from 1,144±214 to 509±245 pS/pF ($n = 4$). GDP-β-S (200 μM) in the pipet solution increased the conductance from 729±97 pS/pF to 1826±371 pS/pF ($n = 6$). These results are consistent with the involvement of an inhibitory G protein in the conductance activation pathway.

Involvement of leukotrienes in LPS-activated currents. We used leukotriene inhibitors and LTD_4 to determine if leukotrienes are involved in the LPS-activated current. Freshly isolated hepatocytes from LPS-treated animals were treated with three different inhibitors: NDGA, a direct lipoxygenase inhibitor (30); MK-571, an LTD_4 receptor antagonist (31); and MK-886, a 5-lipoxygenase activating protein (FLAP) inhibitor that prevents leukotriene biosynthesis by preventing the interaction of 5-lipoxygenase with FLAP (32). The results, presented in Fig. 5, demonstrate that treatment of LPS day 1 cells for 10 min with each of these inhibitors reduced membrane conductance.

Effects of LTD_4 on membrane conductance. The ability of exogenous leukotrienes to directly activate the conductance was tested in both control hepatocytes and hepatocytes derived from LPS-treated rats. Freshly isolated LPS day 1 cells had maximal current activation, and neither cell swelling nor exogenous LTD_4 further increased the conductance. After 24 h of storage (LPS day 2 cells), however, cell conductance was lower (see above). We tested whether LTD_4 altered membrane conductance in this condition.

Intracellular dialysis with LTD_4 (0.5–3.0 μM) produced significant current activation in LPS cells (Fig. 6). Current activation was saturable with a half-maximal effect at 370 nM. In control cells, intracellular LTD_4 produced a significant conductance activation only at 3 μM. LTD_4 addition to the bath had no effect on the conductance of control cells. It increased conductance of LPS cells slightly, but this effect did not reach statistical significance.

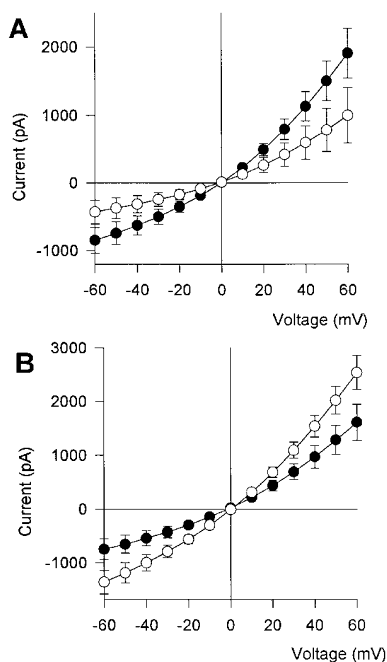

Figure 4. Effects of guanine nucleotides on conductance in LPS day 1 cells. (*A*) GTP. Whole-cell currents in LPS day 1 cells were measured at 3 min after formation of the whole-cell configuration either under control conditions or with inclusion of GTP (100 μM) in the pipet. $n = 4$. *shaded circles*, control; *open circles*, GTP. (*B*) GDP-β-S. Conditions identical to above, except that pipet solution in experimental condition contained GDP-β-S (200 μM). $n = 6$–7. *closed circles*, control; *shaded circles*, GDP-β-S.

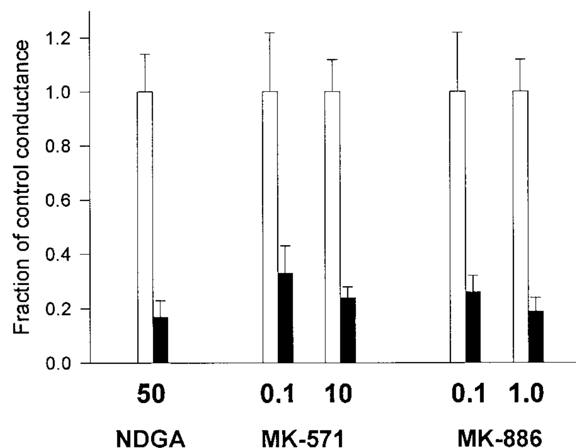

Figure 5. Effect of leukotriene inhibitors on conductance in LPS day 1 cells. Data were obtained 3 min after formation of the whole-cell configuration either in the absence of inhibitor (control) or after 10 min bath exposure to NDGA (50 μM), MK-571 (0.1 or 10 μM), or MK-886 (0.1 or 1 μM). $n = 5$–18, all inhibitor effects $P < 0.05$. *open bars*, control; *shaded bars*, inhibitor.

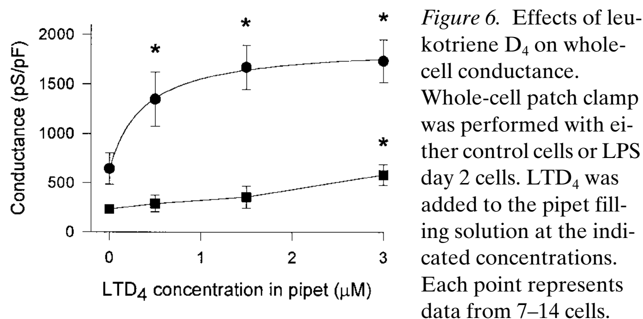

Figure 6. Effects of leukotriene D_4 on whole-cell conductance. Whole-cell patch clamp was performed with either control cells or LPS day 2 cells. LTD_4 was added to the pipet filling solution at the indicated concentrations. Each point represents data from 7–14 cells. $^*P < 0.05$. For LPS cells, the curve represents the best fit to the Michaelis-Menten equation ($K_m = 371$ nM, $V_{max} = 1{,}887$ pS/pF). *shaded circles*, LPS; *shaded squares*, control.

Ca^{2+} dependence of LTD_4-activated conductance. When internal Ca^{2+} activity was reduced by intracellular introduction of 10 mM EGTA without added Ca^{2+} in pipet or bath, the LTD_4-activated conductance was decreased from 1,889±221 pS/pF ($n = 4$) to 654±129 pS/pF ($n = 5$, $P < 0.001$). This result suggests that direct LTD_4-mediated conductance activation is partially Ca^{2+} dependent, unlike the conductance in day 1 LPS cells.

The possibility that LTD_4 raises intracellular Ca^{2+} and subsequently activates a Ca^{2+}-dependent Cl^- conductance was investigated by measuring intracellular Ca^{2+} in patch-clamped hepatocytes while LTD_4 was introduced through the pipet. Fig. 7 shows that when control hepatocytes were patched without LTD_4 in the pipet, a constant intracellular Ca^{2+} was maintained (138±17 nM at 3 min). When LTD_4 was added to the pipet, there was no change in intracellular Ca^{2+} in control hepatocytes.

Intracellular Ca^{2+} was then measured in LPS day 2 cells under identical conditions. When LTD_4 was omitted from the pipet, intracellular Ca^{2+} was 127±27 nM at 3 min, and there was no change over 6 min. When LTD_4 was added to the pipet, intracellular Ca^{2+} was initially the same as in control cells, but it increased as LTD_4 diffused from the pipet into the cell. Intracellular Ca^{2+} was 223±25 nM at 3 min, and further increased during the 6 min of observation (Fig. 7). Although relatively slow to develop, these increases are within the range of intracellular Ca^{2+} changes shown to regulate ion channels (33).

Cytokine and conditioned medium effects on conductance. We tested if the priming effect of LPS, i.e., induction of LTD_4 responsiveness of conductance, could be reproduced in vitro with soluble mediators. Hepatocytes were isolated from untreated rats and cultured for 4 h either with no addition, with LPS (10 μg/ml) added directly to the culture medium, with conditioned medium prepared from an LPS-simulated monocyte-macrophage cell line, with conditioned medium that had first been neutralized with anti–TNF-α antiserum, or with purified TNF-α. Fig. 8 demonstrates that although direct LPS treatment had no effect, conditioned medium–primed hepatocytes demonstrated a LTD_4 responsive conductance. This in-

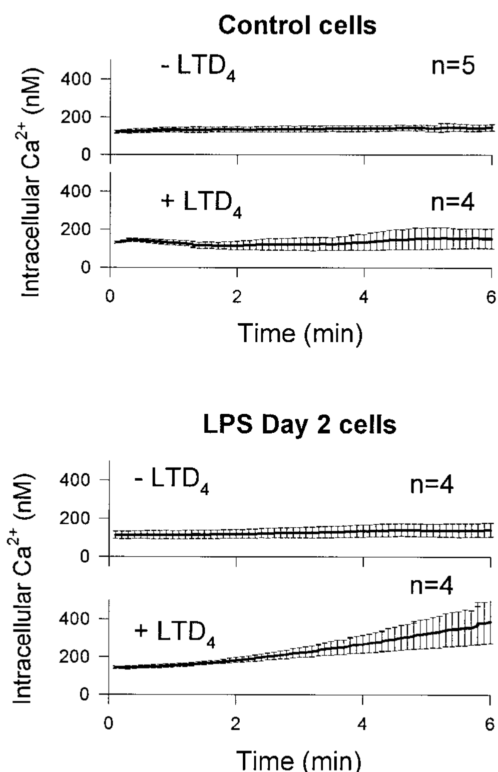

Figure 7. Effects of intracellular LTD_4 on intracellular Ca^{2+}. Intracellular Ca^{2+} was measured in whole-cell patch clamped hepatocytes by Fluo-3 fluorescence after preloading the cells with the permeant ester and including the impermeant form of the dye in the pipet. In each case, the whole-cell configuration was established at $t = 0$.

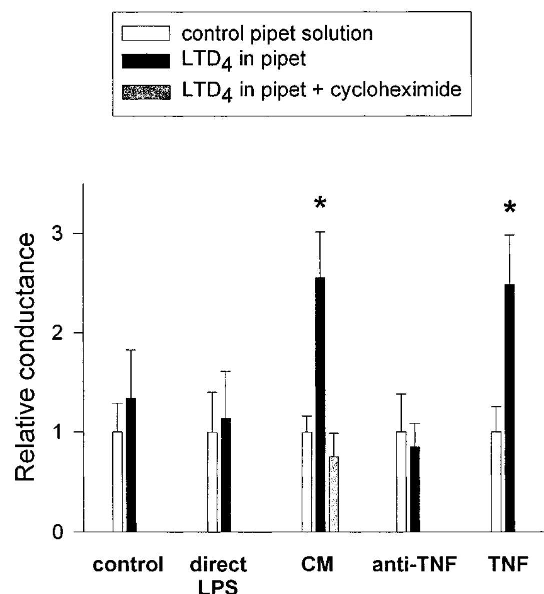

Figure 8. Effect of conditioned medium and TNF-α on LTD_4 responsiveness of conductance. Isolated hepatocytes were cultured for 4 h either in L-15 medium alone (*control*), L-15 medium containing 10 μg/ml LPS (*direct LPS*), L-15 containing a 1:5 dilution of supernatant from an LPS-stimulated monocyte-macrophage cell line (conditioned medium, *CM*), conditioned medium that had been incubated for 16 h with anti-TNF antiserum (*anti-TNF*), or with 100 ng/ml purified TNF-α (*TNF*). Hepatocytes were patched either with or without 1.5 μM LTD_4 in the pipet. Where indicated for conditioned medium cells, cycloheximide (50 μM) was added at the same time as the conditioned medium. $^*P \leq 0.01$ for the comparison of conductance with and without internal LTD_4.

duction of LTD_4-responsiveness was abolished by inclusion of cycloheximide (50 μM) with the conditioned medium during the 4-h incubation. Furthermore, the priming effect of conditioned medium was abolished by anti–TNF-α antiserum, and could be duplicated by purified TNF-α.

Discussion

This study has demonstrated that exposure to LPS in vivo activates an outwardly rectifying chloride conductance in hepatocytes. There appear to be two mechanisms of conductance activation in these cells. The first depends on cell swelling, and the second involves a leukotriene-mediated conductance activation that requires cytokine priming of hepatocytes.

Several observations support these conclusions. First, the large outwardly rectifying conductance present in LPS day 1 hepatocytes has identical rectification, voltage-dependent inhibition kinetics, Cl^- channel blocker sensitivity, and Ca^{2+} independence as the swelling activated conductance from control cells (7). LPS day 1 cells are larger than control cells, hyposmotic bath no longer activates additional conductance, and cell shrinkage with hyperosmotic bath solution partially reduces the conductance (Fig. 2). These observations support the conclusion that most of the conductance activation in LPS day 1 cells is related to cell swelling. However, the conductance in LPS day 1 cells is inhibited by two different leukotriene synthesis inhibitors, as well as by a leukotriene receptor antagonist. Leukotrienes may therefore modulate this conductance.

Role of leukotrienes in conductance activation. Leukotriene effects on chloride conductance were studied in several different hepatocyte preparations. Freshly isolated LPS day 1 cells proved unsuitable for examination of conductance activation since conductance was already maximal and could not be further increased by hyposmotic solutions or leukotrienes. For this reason, we examined cells derived from LPS-treated rats but stored at 4° for 1 d (day 2 cells). Chloride conductance in LPS day 2 cells was half of that in day 1 cells, possibly because of changes in cell volume, intracellular leukotriene concentrations, or other intracellular components that occurred during the cold storage period. Day 2 cells responded to intracellularly introduced LTD_4 with a twofold increase in chloride conductance and a slow increase in intracellular Ca^{2+} reaching approximately twofold over control (Fig. 7). The magnitude of this increase in intracellular Ca^{2+} is similar to that associated with channel activation in smooth muscle (33) and induced by LTD_4 in human monocytic leukemia cells (34). The partial Ca^{2+} dependence of the LTD_4-induced conductance suggests that the Ca^{2+} rise may be involved in channel activation.

Unlike the day 2 LPS hepatocytes, control hepatocytes did not respond to intracellular LTD_4. However, direct culture of the cells in vitro with either conditioned medium from LPS-stimulated RAW 264.7 cells (25) or purified TNF-α produced an identical LTD_4 responsiveness (Fig. 8). This priming effect was inhibited by cycloheximide, and anti–mouse TNF-α antibodies abolished the effect in conditioned medium. It thus appears that priming is a direct result of TNF-α–induced events in hepatocytes. Chloride channel activation in hepatocytes after LPS treatment of the animal therefore results from both cell swelling and a TNF-α–mediated priming event that confers a leukotriene dependence of chloride conductance.

Possible explanations for the LTD_4 effect. There are two general explanations that could explain LTD_4 stimulation of chloride conductance, either a direct intracellular signaling cascade or an indirect effect in which leukotrienes alter the function of a channel regulator.

LTD_4-mediated signal transduction in multiple target cells involves a G protein–coupled receptor and a rise in intracellular Ca^{2+} (34–36). In hepatocytes, the mechanism of LTD_4 action is uncertain. A similar mechanism is supported by the observations that channel activation is blocked by an LTD_4 receptor antagonist (MK-571), there is an associated rise in intracellular Ca^{2+}, and the stimulatory effects of GDP-β-S and inhibitory effects of GTP suggest inhibitory G protein involvement, as has been shown for outwardly-rectifying chloride channels in airway epithelial cells (37, 38).

However, several characteristics of the LTD_4-induced channel activation do not suggest a classical receptor-mediated signaling pathway. These include the intracellular site of LTD_4 action, the lack of any previous demonstration of high affinity leukotriene receptors in hepatocytes, the relatively high concentrations required for channel activation ($K_{1/2}$ = 370 nM), and inhibition by several leukotriene synthesis inhibitors—not just leukotriene receptor antagonists.

Although inhibition of channel activation by leukotriene synthesis inhibitors suggests that hepatocytes might be able to synthesize leukotrienes from arachidonic acid, this may not be the case. Hepatocytes have been shown to have LTC_4 synthase activity, the ability to produce LTC_4 from LTA_4 (39, 40), and have been shown to produce cysteinyl leukotrienes from 5-hydroperoxyeicosatetraenoic acid (41, 42), but they have not been shown to have 5-lipoxygenase activity or to produce leukotrienes directly from arachidonic acid.

An alternative explanation that could account for LTD_4-induced channel regulation and modification of this effect by both leukotriene receptor antagonists and synthesis inhibitors is the possibility that each of these molecules could be interacting directly with a channel regulatory protein. This hypothesis is suggested by the observation that cMRP, the canalicular membrane multispecific organic ion transport protein (20, 21, 43), is a high affinity cysteinyl leukotriene transporter (19) and binds leukotriene analogues including MK-571 (44). Outwardly rectifying chloride conductances appear to be regulated by several related proteins, notably the multidrug resistance P-glycoprotein (45, 46) and the cystic fibrosis transmembrane conductance regulator (47). MRP, the closely related multidrug resistance–associated protein observed in resistant small cell lung cancer cells (48), has itself been shown to alter ionic conductances (49). In addition, Van Kuijck et al. have recently cloned an endogenous epithelial channel regulatory protein with a close structural homology to MRP (50). Taken together, these observations suggest that interaction with a channel regulatory protein such as cMRP is a reasonable explanation for the LTD_4 effect.

Several aspects of cMRP-regulated ion channels in hepatocytes would be novel. Ion channel regulation by related ABC transport proteins has previously been demonstrated only in cells that express these proteins inappropriately, such as multidrug-resistant cancer cells or transfected cells. It has not been shown to be a physiological phenomenon in normal cells. Second, substrate-induced modulation of the effect has not been observed for P-glycoprotein. We observed stimulation with internally added LTD_4 and inhibition with external MK-571 or MK-886. There would have to be either a sidedness to the effect or an agonist/antagonist character to ligand interactions. TNF-α may serve to prime cells for this effect by altering the

expression levels, membrane localization, or channel coupling of the cMRP protein. These questions are currently under investigation.

Leukotrienes and liver injury. It has previously been shown that leukotriene production is necessary for liver injury in several models of LPS-induced hepatotoxicity (18, 51). Chloride channels have been shown to be involved in cell injury from hypoxia, and chloride channel blockers have been shown to prevent lactate dehydrogenase release from renal proximal tubules under anaerobic conditions (3). In hepatocytes, LPS exposure inhibits leukotriene export while cellular uptake is preserved and intracellular leukotriene concentrations increase (22). Chloride channel activation under this circumstance could contribute to cytotoxicity.

In a number of examples, including glioma cells (52) and skate hepatocytes (53), a swelling-activated chloride conductance has been identified with similar kinetics and blocker sensitivity to that observed in this study. Although the molecular identity of these channels is still uncertain, they appear to serve as a leak pathway for taurine and other organic osmolytes, and have been shown to be permeable to lactate, glucose, amino acids, and a number of other metabolites and metabolic intermediates (52). If this is also true for the LTD_4-activated ion channels in hepatocytes, they could create an organic substrate leak, alter intracellular energy metabolism and redox potential, and thus affect cell viability. This possibility could explain why cysteinyl leukotriene toxicity to hepatocytes is particularly prevalent during hypoxia (54). This hypothesis requires further study.

In conclusion, our studies have demonstrated chloride channel activation by LPS and have shown that it consists of a swelling-mediated process and an intracellular leukotriene-stimulated process. This later requires hepatocyte priming with TNF-α. Channel activation may be mediated by leukotriene binding to its canalicular membrane transport protein but the precise mechanism by which leukotrienes activate the conductance is unknown. Since leukotrienes serve both as substrates for transport and channel regulators, intracellular accumulation of leukotrienes may alter hepatocyte function. Further studies will be necessary to precisely define the details of the signal transduction, as well as the role of the channel activation in hepatotoxicity.

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References

1. Dubinsky, W.P. 1989. The physiology of epithelial chloride channels. *Hospital Prac.* 24:69–82.

2. Paulmichl, M., M. Gschwentner, E. Wöll, A. Schmarda, M. Ritter, G. Kanin, H. Ellemunter, W. Waitz, and P. Deetjen. 1993. Insight into the structure-function relation of chloride channels. *Cell Physiol. Biochem.* 3:374–387.

3. Waters, S.L., and R.G. Schnellmann. 1996. Extracellular acidosis and chloride channel inhibitors act in the late phase of cellular injury to prevent death. *J. Pharmacol. Exp. Ther.* 278:1012–1017.

4. Sellinger, M., S.A. Weinman, R.M. Henderson, A. Zweifach, J.L. Boyer, and J. Graf. 1992. Anion channels in rat liver canalicular plasma membranes reconstituted into planar lipid bilayers. *Am. J. Physiol.* 262:G1027–G1032.

5. Koumi, S., R. Sato, and T. Aramaki. 1994. Characterization of the calcium-activated chloride channel in isolated guinea-pig hepatocytes. *J. Gen. Physiol.* 104:357–373.

6. Jentsch, T.J. 1994. Molecular physiology of anion channels. *Curr. Opin. Cell Biol.* 6:600–606.

7. Meng, X.-J., and S.A. Weinman. 1996. Cyclic AMP and swelling-activated chloride conductance in rat hepatocytes. *Am. J. Physiol.* 271:C112–C120.

8. Samuelsson, B., S. Dahlén, J. Lindgren, C. Rouzer, and C. Serhan. 1987. Leukotrienes and lipoxins: structures, biosynthesis and biological effects. *Science (Wash. DC).* 237:1171–1176.

9. Kim, D., D.L. Lewis, L. Graziadei, E.J. Neer, D. Bar-Sagi, and D.E. Clapham. 1989. G-protein β-subunits activate the cardiac muscarinic K^+-channel via phospholipase A_2. *Nature (Lond.).* 337:557–560.

10. Kurachi, Y., H. Ito, T. Sugimoto, T. Shimizu, I. Miki, and M. Ui. 1989. Arachidonic acid metabolites as intracellular modulators of the G protein–gated cardiac K^+ channel. *Nature (Lond.).* 337:555–557.

11. Schweitzer, P., S. Madamba, and G.R. Siggins. 1990. Arachidonic acid metabolites as mediators of somatostatin-induced increase of neuronal M-current. *Nature (Lond.).* 346:464–467.

12. Cantiello, H.F., C.R. Patenaude, J. Codina, L. Birnbaumer, and D.A. Ausiello. 1990. $G_{\alpha i\text{-}3}$ regulates epithelial Na^+ channels by activation of phospholipase A_2 and lipoxygenase pathways. *J. Biol. Chem.* 265:21624–21628.

13. Lambert, I.H., E.K. Hoffmann, and P. Christensen. 1987. Role of prostaglandins and leukotrienes in volume regulation by Ehrlich ascites tumor cells. *J. Membr. Biol.* 98:247–256.

14. Lambert, I.H. 1989. Leukotriene-D_4 induced cell shrinkage in Ehrlich ascites tumor cells. *J. Membr. Biol.* 108:165–176.

15. Lambert, I.H., and E.K. Hoffmann. 1993. Regulation of taurine transport in Ehrlich ascites tumor cells. *J. Membr. Biol.* 131:67–79.

16. Keppler, D. 1992. Leukotrienes: biosynthesis, transport, inactivation and analysis. *Rev. Physiol. Biochem. Pharmacol.* 121:1–30.

17. Keppler, D., M. Huber, and T. Baumer. 1988. Leukotrienes as mediators in diseases of the liver. *Sem. Liver Dis.* 8:357–366.

18. Keppler, D., W. Hagmann, S. Rapp, C. Denzlinger, and H. Koch. 1985. The relation of leukotrienes to liver injury. *Hepatology.* 5:883–891.

19. Leier, I., G. Jedlitschky, U. Buchholz, S.P.C. Cole, R.G. Deeley, and D. Keppler. 1994. The MRP gene encodes an ATP-dependent export pump for leukotriene C_4 and structurally related conjugates. *J. Biol. Chem.* 269:27807–27810.

20. Büchler, M., J. König, M. Brom, J. Kartenbeck, H. Spring, T. Horie, and D. Keppler. 1996. cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. *J. Biol. Chem.* 271:15091–15098.

21. Paulusma, C., P. Bosma, G. Zaman, C. Bakker, M. Otter, G. Scheffer, R. Scheper, P. Borst, and R. Elferink. 1996. Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science (Wash. DC).* 271:1126–1128.

22. Wettstein, M., B. Noe, and D. Haussinger. 1995. Metabolism of cysteinyl leukotrienes in the perfused rat liver: the influence of endotoxin pretreatment and the cellular hydration state. *Hepatology.* 22:235–240.

23. Weinman, S.A., and R.P. Weeks. 1993. Electrogenicity of Na-coupled bile salt transport in isolated rat hepatocytes. *Am. J. Physiol.* 265:G73–G80.

24. Maglova, L.M., A.M. Jackson, X.-J. Meng, M.W. Carruth, C.D. Schteingart, H.-T. Ton-Nu, A.F. Hofmann, and S.A. Weinman. 1995. Transport characteristics of three fluorescent conjugated bile acid analogs in isolated rat hepatocytes and couplets. *Hepatology.* 22:637–647.

25. Beutler, B., J. Mahoney, N. Le Trang, P. Pekala, and A. Cerami. 1985. Purification of cachectin, a lipoprotein lipase–suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *J. Exp. Med.* 161:984–995.

26. Fabiato, A. 1988. Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods Enzymol.* 157:378–414.

27. Grüne, S., and S.A. Weinman. 1995. Cyclic AMP stimulation of bile acid uptake results from membrane hyperpolarization. *Gastroenterology.* 108:A1076 (Abstr.).

28. Kao, J.P.Y. 1994. Practical aspects of measuring $[Ca^{2+}]$ with fluorescent indicators. *Methods Cell Biol.* 40:155–181.

29. Haddad, P., J.S. Beck, J.L. Boyer, and J. Graf. 1991. Role of chloride ions in liver cell volume regulation. *Am. J. Physiol.* 261:G340–G348.

30. Egan, R.W., and P.H. Gale. 1985. Comparative biochemistry of lipoxygenase inhibitors. *In* Prostaglandins, Leukotrienes, and Lipoxins. Biochemistry, Mechanism of Action, and Clinical Applications. J.M. Bailey, editor. Plenum Press, New York. 593–607.

31. Jones, T., R. Zamboni, M. Belley, E. Champion, L. Charette, A. Ford-Hutchinson, R. Frenette, J. Gauthier, S. Leger, P. Masson, et al. 1988. Pharmacology of L-660,711 (MK-571): a novel potent and selective leukotriene D_4 receptor antagonist. *Can. J. Physiol. Pharmacol.* 67:17–28.

32. Gillard, J., A. Ford-Hutchinson, C. Chan, S. Charleson, D. Denis, A. Foster, R. Fortin, S. Leger, C. McFarlane, H. Morton, H. Piechuta, et al. 1989. L–663,536 (MK-886) (3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2 -dimethylpropanioc acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.* 67:456–464.

33. Carl, A., K. Lee, and K.M. Sanders. 1996. Regulation of ion channels in smooth muscles by calcium. *Am. J. Physiol.* 271:C9–C34.

34. Chan, C.-C., P. Ecclestone, D.W. Nicholson, K.M. Metters, D.J. Pon, and I.W. Rodger. 1994. Leukotriene D_4-induced increases in cytosolic calcium in THP-1 cells: dependence on extracellular calcium and inhibition with selective leukotriene D_4 receptor antagonists. *J. Pharmacol. Exp. Ther.* 269:891–896.

35. Crooke, S.T., H. Sarau, D. Saussy, J. Winkler, and J. Foley. 1990. Signal transduction processes for the LTD_4 receptor. *Adv. Prost. Thomb. Leuko. Res.* 20:127–137.

36. Prié, S., G. Guillemette, G. Boulay, P. Borgeat, and P. Sirois. 1995. Leukotriene C_4 receptors on guinea pig tracheocytes. *J. Pharmacol. Exp. Ther.* 275: 312–318.

37. Schwiebert, E.M., D.C. Gruenert, W.B. Guggino, and B.A. Stanton. 1995. G protein Ga_{i-2} inhibits outwardly rectifying chloride channels in human airway epithelial cells. *Am. J. Physiol.* 269:C451–C456.

38. Ismailov, I.I., B. Jovov, C.M. Fuller, B.K. Berdiev, D.A. Keeton, and D.J. Benos. 1996. G-protein regulation of outwardly rectified epithelial chloride channels incorporated into planar bilayer membranes. *J. Biol. Chem.* 271:4776–4780.

39. Fukai, F., Y. Suzuki, H. Ohtaki, and T. Katayama. 1993. Rat hepatocytes generate peptide leukotrienes from leukotriene A_4. *Arch. Biochem. Biophys.* 305:378–384.

40. Fukai, F., Y. Suzuki, Y. Nishizawa, and T. Katayama. 1996. Transcellular biosynthesis of cysteinyl leukotrienes by Kupffer cell-hepatocyte cooperation in rat liver. *Cell Biol. Int.* 20:423–428.

41. Huwyler, J., M. Bürgin, T. Zeugin, and J. Gut. 1990. Transformation of 5-hydroperoxyeicosatetraenoic acid into dihydroxy- and cysteinyl-leukotrienes by rat hepatocytes: effects of glutathione. *Mol. Pharmacol.* 39:314–323.

42. Gut, J. 1994. Homeostasis of eicosanoids in the liver critically depends on partial O_2 concentrations. *J. Hepatol.* 20:563–566.

43. Mayer, R., J. Kartenbeck, M. Büchler, G. Jedlitschky, I. Leier, and D. Keppler. 1995. Expression of the MRP gene–encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. *J. Cell Biol.* 131:137–150.

44. Gekeler, V., W. Ise, K. Sanders, W. Ulrich, and J. Beck. 1995. Leukotriene LTD_4 receptor antagonist MK571 specifically modulates MRP associated multidrug resistance. *Biochem. Biophys. Res. Commun.* 208:345–352.

45. Hardy, S.P., H.R. Goodfellow, M.A. Valverde, D.R. Gill, F.V. Sepulveda, and C.F. Higgins. 1995. Protein kinase C–mediated phosphorylation of the human multidrug resistance P-glycoprotein regulated cell volume-activated chloride channels. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:68–75.

46. Han, E.S., C.G. Vanoye, G.A. Altenberg, and L. Reuss. 1996. P-glycoprotein-associated chloride currents revealed by specific block by an anti-P-glycoprotein antibody. *Am. J. Physiol.* 270:C1370–C1378.

47. Jovov, B., I.I. Ismailov, and D.J. Benos. 1995. Cystic fibrosis transmembrane conductance regulator is required for protein kinase A activation of an outwardly rectified anion channel purified from bovine tracheal epithelia. *J. Biol. Chem.* 270:1521–1528.

48. Cole, S., G. Bhardwaj, J. Gerlach, J. Mackie, C. Grant, K. Almquist, A. Stewart, E. Kurz, A. Duncan, and R. Deeley. 1992. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science (Wash. DC).* 258:1650–1654.

49. Gollapudi, S., T. McDonald, P. Gardner, N. Kang, and S. Gupta. 1992. Abnormal chloride conductance in multidrug resistant HL60/AR cells. *Cancer Lett.* 66:83–89.

50. Van Kuijck, M.A., R.A.M.H. Van Aubel, A.E. Busch, F. Lang, F.G.M. Russel, R.J.M. Bindels, C.H. Van Os, and P.M.T. Deen. 1996. Molecular cloning and expression of a cyclic AMP-activated chloride conductance regulator: a novel ATP-binding cassette transporter. *Proc. Natl. Acad. Sci. USA.* 93:5401–5406.

51. Nagai, H., T. Shimazawa, I. Yakuo, M. Aoki, A. Koda, and M. Kasahara. 1989. Role of peptide-leukotrienes in liver injury in mice. *Inflammation.* 13:673–680.

52. Strange, K., and P. S. Jackson. 1995. Swelling-activated organic osmolyte efflux: a new role for anion channels. *Kidney Int.* 48:994–1003.

53. Ballatori, N., T.W. Simmons, and J.L. Boyer. 1994. A volume-activated taurine channel in skate hepatocytes: membrane polarity and role of intracellular ATP. *Am. J. Physiol. Gastrointest. Liver Physiol.* 30:G285–G291.

54. Trudell, J.R., M. Bendix, and B. Bosterling. 1984. Hypoxia potentiates killing of hepatocyte monolayers by leukotrienes, hydroperoxyeicosatetraenoic acids, or calcium ionophore A23187. *Biochim. Biophys. Acta.* 803:338–341.