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The action of the peptidoleukotriene LTD_4 on intracellular calcium in rat mesangial cells

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Abstract. The dose-dependent effect of CGP 45715A on the LTD_4-induced Ca^{2+} response of glomerular mesangial cells has been studied. Our results demonstrate that the LTD_4-dependent increase in the cytosolic Ca^{2+} concentration primarily involves an $InsP_3$-mediated release of Ca^{2+} from intracellular storage sites and to a minor extent an enhanced influx of Ca^{2+} through receptor-operated Ca^{2+} channels located in the plasma membrane. The action of CGP 45715A on the Ca^{2+} response is an inhibitory one and is convincingly explained by a displacement of LTD_4 from its receptor site(s). The contractile effect of LTD_4 on pulmonary smooth muscle is proposed to be mainly caused by a receptor-mediated hydrolysis of phosphatidylinositol-4,5-bisphosphate.
Key words. Asthma; LTD_4; CGP 45715A; LTD_4-receptor antagonist; cytosolic Ca^{2+} transient.

Contraction of airway smooth muscle is largely responsible for the bronchospasm which is one of the characteristic features of the acute phase of an asthmatic attack [1]. It is well accepted today that airways of asthmatic patients are hyper-responsive to a wide variety of provoking stimuli. The underlying pathological process is inflammatory in nature and characterized by bronchial wall oedema, lymphocyte and eosinophil infiltration, and the occurrence of mucus plugs within the airway lumen [2]. International guidelines therefore recommend inhaled corticosteroids or nonsteroidal anti-inflammatory drugs as first-line therapy for the management of asthma [3–5].

However, more specific treatment procedures will soon be available [6]. A substantial body of evidence supports the hypothesis that the peptidoleukotrienes C_4, D_4 and E_4 (LTC_4, LTD_4, LTE_4) play an important role in the pathogenesis of asthma [7, 8]. Generated from membrane-associated arachidonic acid via the 5-lipoxygenase pathway, LTC_4 and LTD_4 are approximately 1000–5000 times more potent than histamine in causing bronchoconstriction in normal and asthmatic subjects [9]. The involvement of the peptidoleukotrienes in the pathophysiology of asthma initiated the search for drugs which control the release or effects of the peptidoleukotrienes and thereby counteract the asthmatic sequel [7]. The major strategies concentrate on inhibitors of phospholipase A_2, of 5-lipoxygenase or of the 5-lipoxygenase-activating protein [7]. Moreover, leukotriene receptor antagonists may prove to be new and potent therapeutic agents [7, 8].

The observation that the binding of LTD_4 to its associated receptor is inhibited by GTP prompted several investigators to postulate an LTD_4-mediated impairment of the activity of adenylate cyclase through an inhibitory guanyl nucleotide binding protein (G_i) [10–13]. Indeed, Andersson [14–16] and Barnes [17] found, independently of each other, that LTD_4 rapidly leads to a reduction in intracellular cAMP concentrations in guinea pig trachea and lung. The decreased generation of cAMP inhibits the activity of cAMP-dependent protein kinase A (PKA), which phosphorylates myosin light chain kinase (MLCK) [18]. Compared to native MLCK, the phosphorylated product exhibits a lower affinity for the calmodulin-Ca_4^{2+} complex, causing a decrease in the phosphorylation of 20-kDa myosin light chains and a reduction in the actin-myosin coupling [19]. A challenge with LTD_4 thus increases the phosphorylation of the myosin light chain and initiates cell contraction. A drop in cAMP also acts to enhance the Ca^{2+} influx through the plasma membrane [5], to delay the sequestration of Ca^{2+} into intracellular storage sites, and to down-regulate the extrusion of Ca^{2+} via the Ca^{2+} ATPase into the extracellular space [20]. In addition, LTD_4 attenuates the inhibitory effect of cAMP on phospholipase C-mediated phosphoinositide hydrolysis [21].

Besides its actions on the cAMP pathway, LTD_4 in guinea-pig lung leads to an allosteric activation of phospholipase C through a specific guanine nucleotide binding protein (G_q) [22, 23]. The subsequent breakdown of phosphoinositides enhances the generation of inositol-1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG) and consequently increases the cytosolic Ca^{2+} concentration. Evidence from the effects of calcium channel blockers [24] and a specific 'intracellular calcium antagonist' [25] suggest that the calcium ions which are responsible for the induction of the LTD_4-induced smooth muscle contraction come from intracellular rather than from extracellular sources [26].

In the course of the continuing search for novel and effective antagonists of the peptidoleukotrienes, a compound, CGP 45715A, has been synthesized at Ciba-Geigy which potently inhibited LTD_4-induced broncho-

constrictions in guinea-pig lung in vitro and in vivo [27, 28]. Recently, it has also been shown that in healthy volunteers the drug efficiently antagonized bronchospasms triggered by a challenge with LTD_4 [29].

In the present paper the dose-dependent effect of CGP 45715A on the LTD_4-induced Ca^{2+} response of glomerular mesangial cells, a specialized type of vascular smooth muscle cell [30], has been studied to understand the mechanisms leading to an increase in the cytosolic Ca^{2+} concentration. Mesangial cells are known to show a good response to a number of smooth muscle agonists and were therefore used as models of human pulmonary smooth muscle cells [30].

Materials and methods

CGP 45715A [(1*R*,2*S*)-(3*E*,5*Z*)-7-[1-(3-trifluoromethylphenyl)-1-hydroxy-10-(4-acetyl-3-hydroxy-2-propylphenoxy)-3,5-decadien-2-yl-thio]-4-oxo-4*H*-1-benzopyran-2-carboxylic acid sodium salt] and LTD_4 were synthesized at Ciba-Geigy Ltd. (Basle, Switzerland). Fluo-3:AM was obtained from Molecular Probes (Eugene, OR, USA), nifedipine and bovine insulin from Sigma (St. Louis, MO, USA), RPMI 1640 medium, penicillin and streptomycin sulphate from Boehringer Mannheim (Germany), and pertussis toxin from List Biological Laboratories (Campbell, CA, USA); all other chemicals were from Fluka (Buchs, Switzerland).

Cell culture. Primary cultures of rat mesangial cells were kindly provided by Professor J. Pfeilschifter (Biocentre, University of Basle, Switzerland). Grown in an RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin sulphate (100 µg/ml) and bovine insulin (0.66 U/ml), mesangial cells were cultivated as monolayers on glass plates (5×10^4 cells/cm^2) at 37 °C in air/CO_2 (19:1) essentially as described previously [31, 32]. The cells were subcultured every 5–6 days and the culture media renewed every 2–3 days. Since mesangial cells lose their ability to contract isotonically after multiple passages in culture [33], only early cell passages (No. 6–9) were used throughout the study. Finally, each cell line was extensively characterized using the following criteria: morphological analysis by phase-contrast light microscopy; positive staining for the intermediate filaments desmin and vimentin which is considered to be specific for myogenic cells [34]; negative staining for factor VIII-related antigen and cytokeratin, excluding endothelial and epithelial contaminations [34], respectively; and contraction in response to angiotensin II (20 nM).

Cell labeling with fluo-3. All our fluorescence assays were based on the spectroscopic determination of the free and Ca^{2+}-bound fluo-3 concentration [35]. Upon complexation of Ca^{2+}, the fluorescence intensity of fluo-3 increases by a factor of ca. 80 without major spectral shifts [36]. The introduction of a membrane permeant, esterasehydrolyzable, penta-acetoxymethylester derivative, fluo-3:AM, has provided a sound basis for extensive measurements of the cytosolic Ca^{2+} concentration, $[Ca^{2+}]_i$ [35, 36].

The cell plates were incubated with fluo-3:AM (10 µM, 37 °C, 30 min) in the presence of 1.3 mM Ca^{2+}. After the incubation period, the cells were washed twice and stored in a saline buffer solution which contained the following ingredients in physiological concentrations: 1.3 mM $CaCl_2$, 135 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 1 mM $MgCl_2$ and 5 mM D-glucose, buffered to a pH of 7.4 with 10 mM HEPES/HCl [37, 38]. In order to analyze the effects of CGP 45715A on the LTD_4-induced Ca^{2+} transient, fractions of the mesangial cells were pretreated with different concentrations of CGP 45715A (37 °C, 30 min) prior to agonist challenge.

Prior to starting the Ca^{2+} measurements, the viability of the mesangial cells was assessed by the trypan blue exclusion method, which indicated a survival rate of $95 \pm 1\%$. Moreover, an aliquot of the LTD_4 solution was analyzed on a standard UV/VIS-absorption spectrophotometer (Perkin Elmer, Lambda 19) to verify that the oxygen-sensitive LTD_4 molecules were not yet degraded [39].

Measurement of LTD_4-induced Ca^{2+} transients. To avoid any exposure of the cells to the protein-degrading enzyme trypsin, which may lead to a complete or partial hydrolysis of membrane-associated leukotriene receptors, an experimental set-up has been devised to measure LTD_4-induced Ca^{2+} transients directly on cell plates without bringing the cells into suspension by any harvesting method. As shown in figure 1, the cell plates were mounted in a fixed position in a thermostated (37 °C) and magnetically stirred 2-cm-wide sample cuvette, which was filled with the saline buffer solution.

Since the signal-to-noise ratio increases with the square root of the number of cells under observation, a widely expanded argon-ion laser (Spectra Physics, SP 2045-15S, $\lambda = 488$ nm, 0.5 W, irradiated area ≈ 3 cm^2) was used to excite fluo-3 and to record LTD_4-induced changes in $[Ca^{2+}]_i$. The light photons emitted from the Ca^{2+} indicator fluo-3 (Corion, IF-filter, $\lambda = 535$ nm, FWHM = 10 nm) were collected at an angle of 90°, detected by two photomultiplier tubes operating in the single photon-counting mode (Hamamatsu, R 928) and monitored by a two channel single photon-counting unit (Stanford Research System, SR 400). A disadvantage of the photon-counting method is the limited range of intensity over which count rates are linear. To retain the advantages of the single photon-counting versus analog detection mode without reducing the emission intensity (using neutral density filters), the fluorescence emitted from free and Ca^{2+}-complexed fluo-3 was divided by a beam splitter cube (Laser components, PCBD10) and registered by two separate photomultipli-

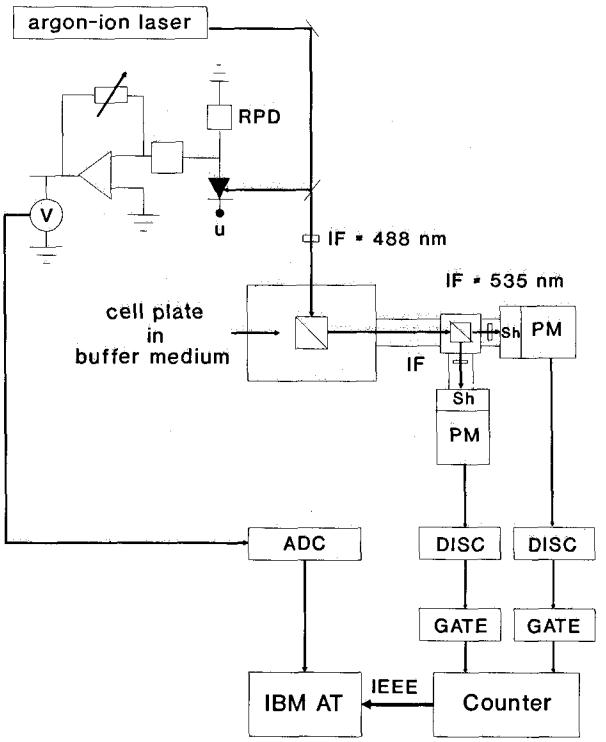

Figure 1. Experimental set-up used to measure LTD_4-induced Ca^{2+} transients of mesangial cells cultivated on glass plates and loaded with fluo-3:AM. The abbreviations have the following meaning: IF = interference filter; RPD = reference photodiode; Sh = shutter; PM = photomultiplier; DISC = discriminator; ADC = analog-to-digital converter.

ers. To improve the signal-to-noise ratio further, the photon counter was equipped with adjustable discriminators which allowed us to differentiate between real pulses and background noise. Typically, the incoming pulses were accumulated within a time interval (=gate) of 0.5 s. At the end of each measurement cycle, the count rates (photons/s) were transferred via an IEEE interface to an IBM-AT computer and averaged over the two detection channels used.

Fluctuations in the power of the argon-ion laser were continuously monitored by a dedicated photodiode (EG&G, UV-250-BQ) for subsequently normalizing the fluorescence signals obtained (see fig. 1). The photodiode signal was processed by a low-noise current-to-voltage converter (UDC, 101C), digitized by a variable gain analog-to-digital I/O interface (Metrabyte, DAS-8 PGA) and stored on the IBM-AT computer, which also controlled the photon counter.

To obtain reliable Ca^{2+} measurements, antifluorescein-IgG antibodies were used to quench extracellular fluorescence, since a leakage of fluo-3 through the cell membrane and/or disintegration of cells increases fluorescence intensity and falsifies the results. The fluorescence signal was calibrated at the end of each individual scan essentially as described elsewhere [40, 41]. However, digitonin (50 μM) was added to equilibrate extra- and intracellular Ca^{2+} concentrations. Agents such as ionomycin or triton X-100 led to at least a partial detachment of the cells from the glass surface and falsified the results.

Principle of analysis. A modified Bateman function has been used to fit the experimental data and to obtain physiologically meaningful constants which characterize the time dependency of the Ca^{2+} signal after agonist challenge with LTD_4. The model takes an exponential behavior into account as follows; equation (1):

$$[Ca^{2+}]_i(nM) = C_1[2^{-t/\tau_{el}} - 2^{-t/\tau_{in1}}] + C_2[1 - 2^{-t/\tau_{in2}}] + C_3 \quad (1)$$

The parameters C_1 and C_2 (nM) correspond to the maximal increase in $[Ca^{2+}]_i$ caused by an intracellular release and an influx of Ca^{2+}, respectively. τ_{el} (s) characterizes the Ca^{2+} half-life elimination constant and τ_{in1}, τ_{in2} (s) the half-life rise times of the individual Ca^{2+} channel increases. Finally, C_3 (nM) gives the basal Ca^{2+} concentration.

The fitting function is based on the idea that two different processes are dominating the release and reuptake of Ca^{2+}. The first term in equation (1) describes the $InsP_3$-mediated intracellular release and sequestration of Ca^{2+}. The second term corresponds to a rise in $[Ca^{2+}]_i$ caused by a stimulus-induced increase in the permeability of Ca^{2+} channels located in the plasma membrane. The subdivision of the Ca^{2+} transient into two distinct mathematical functions does not mean that the mechanisms act independent of each other. On the contrary, the covariance matrix implied strong correlations between the various fitting constants and especially between the two Ca^{2+} channels involved.

Results

Measurements in the presence of 1.3 mM $CaCl_2$ or 1 mM EGTA (see figs 2a and 2b; tables 1a and 1b), respectively, demonstrated that the transient phase of the LTD_4-induced Ca^{2+} signal (first term in equation 1) was not attenuated by pretreatment of the cells with EGTA, suggesting a mobilization of Ca^{2+} from intracellular storage sites. In contrast, the sustained phase of the cytosolic Ca^{2+} signal (second term in equation 1) was completely abolished in Ca^{2+}-free media, indicating the requirement of extracellular Ca^{2+}, i.e. reflecting an enhanced influx of Ca^{2+} through the plasma membrane. Pretreatment of the cells with nifedipine (1 μM, 37 °C, 5 min), a substance known to block specifically voltage-operated Ca^{2+} channels of the L-type [42], showed no effect on the LTD_4-induced Ca^{2+} timecourses in Ca^{2+}-containing buffer solutions (data not shown) in either control cells or in cells pretreated with 50 nM CGP 45715A.

As displayed in figures 2a and 4 and verified by the fitting constants given in tables 1a and 1d, the LTD_4-induced Ca^{2+} response was much higher in control cells

Table 1. Parameters characterizing the Ca^{2+} timecourses in figures 2, 3 and 4; [the function used to fit the Ca^{2+} transients is given in the text, equation (1)].

	a	b	c	d
Constant	Mean ± (σ)	Mean ± (σ)	Mean ± (σ)	Mean ± (σ)
C_3	158 (2) nM	157 (2) nM	157 (2) nM	144 (3) nM
Peak response[1]	91 (2) nM	78 (3) nM	51 (2) nM	49 (2) nM
C_2	21 (2) nM	1 (1) nM	12 (1) nM	4 (1) nM
τ_{el}	70 (2) s	71 (2) s	46 (2) s	45 (2) s
τ_{in1}	7 (1) s	7 (1) s	7 (1) s	8 (2) s
τ_{in2}	15 (2) s	17 (3) s	22 (2) s	24 (3) s

a Stimulation of control cells with 40 nM LTD_4 in the presence of 1.3 mM $CaCl_2$
b Stimulation of control cells with 40 nM LTD_4 in the absence of Ca^{2+}, in a buffer medium containing 1 mM EGTA
c Stimulation of control cells with 25 nM LTD_4 in the presence of 1.3 mM $CaCl_2$
d Stimulation of the cells with 40 nM LTD_4 in the presence of 1.3 mM $CaCl_2$ after pretreatment with CGP 45715A (50 nM, 37 °C, 30 min).
[1] C_1 corresponds to the maximal increase in $[Ca^{2+}]_i$ triggered by an intracellular release of $InsP_3$ in the absence of any elimination processes. If $\tau_{in1} \ll \tau_{el}$ and $\tau_{in1} \ll \tau_{in2}$, C_1 is equal to the peak response.

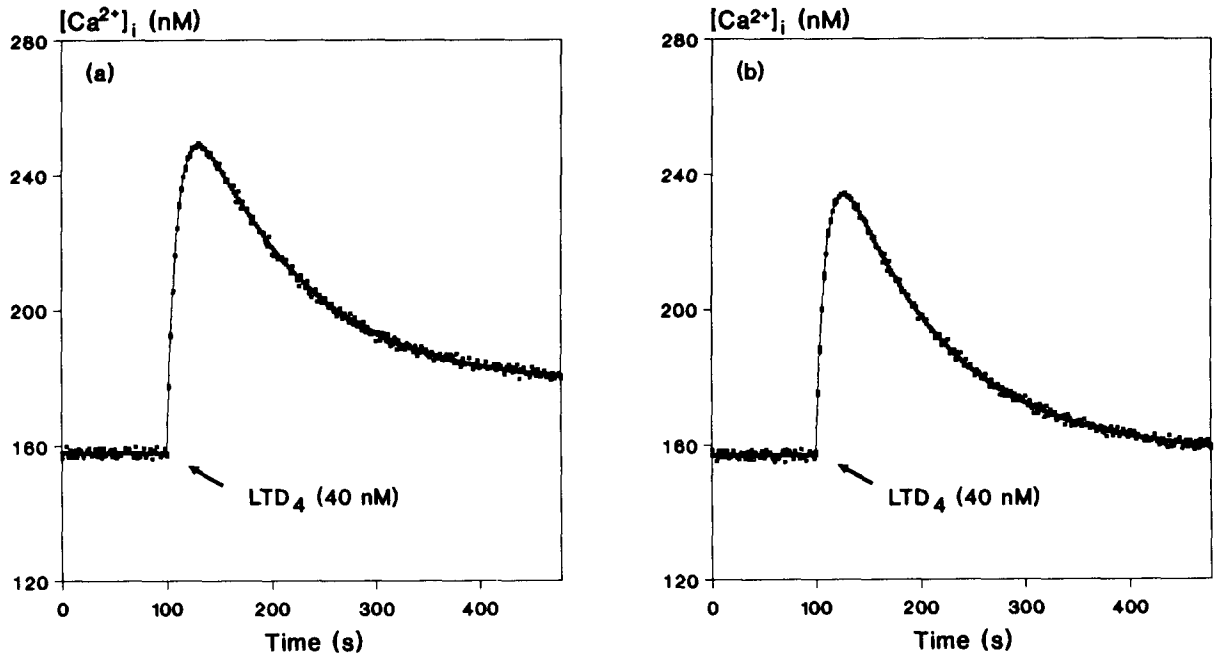

Figure 2. Ca^{2+} transients of mesangial cells loaded with fluo-3:AM after stimulation with 40 nM LTD_4 (*a*) in the presence of 1.3 mM $CaCl_2$ or (*b*) 1 mM EGTA in the buffer medium. Dotted points correspond to the experimentally observed data, whereas solid lines give the theoretically predicted timecourses by using the model function given in the text [equation (1)]. The associated fitting constants characterizing the Ca^{2+} transients in the presence and absence of 1.3 mM $CaCl_2$ are found in tables 1a and 1b, respectively.

and clearly suppressed in the presence of CGP 45715A. The basal Ca^{2+} concentration of mesangial cells was in reasonable agreement with earlier data measured in suspension [37], but significantly lower for those cells pretreated with CGP 45715A. In the control cell group the cytosolic Ca^{2+} level remained elevated over a long time period after stimulation with LTD_4. In contrast, the elimination of Ca^{2+} from the cytoplasm ($1/\tau_{el}$) was clearly enhanced in the presence of 50 nM CGP 45715A and the cytosolic Ca^{2+} concentration decreased approximately to the starting value (see fig. 4).

The timecourses in figures 3 and 4 and the fitting data in tables 1c and 1d indicate that the peak response and Ca^{2+} timecourse of cells pretreated with 50 nM CGP 45715A and stimulated with 40 nM LTD_4 closely matched the Ca^{2+} transient observed after stimulation of control cells with 25 nM LTD_4. However, the basal Ca^{2+} level and the response of the second Ca^{2+} channel were significantly lower following a long-term pretreatment with 50 nM CGP 45715A.

Figure 5 displays the peak response of the measured Ca^{2+} transients in terms of the LTD_4 concentration, whereas figures 6 and 7 demonstrate the effect of an increasing CGP 45715A dose on the maximal Ca^{2+} response and on the elimination time constant (τ_{el}) after stimulation of the cells with 40 nM LTD_4.

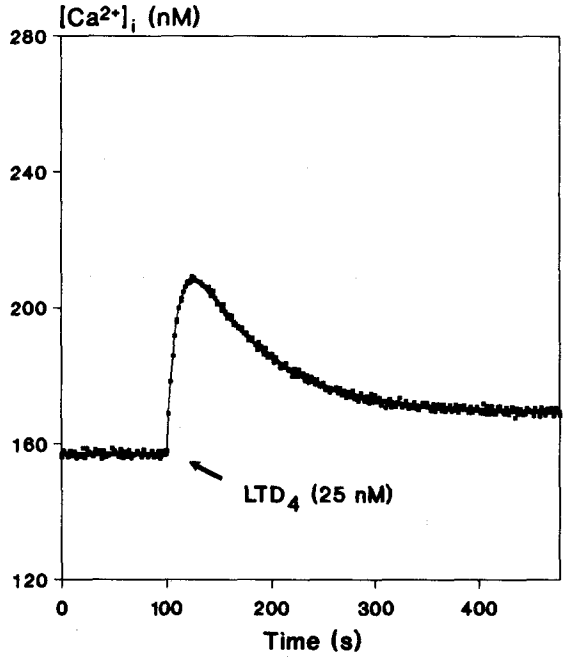

Figure 3. Ca^{2+} transient of mesangial cells after stimulation with 25 nM LTD_4. The fitting constants characterizing the Ca^{2+} transient are given in table 1c.

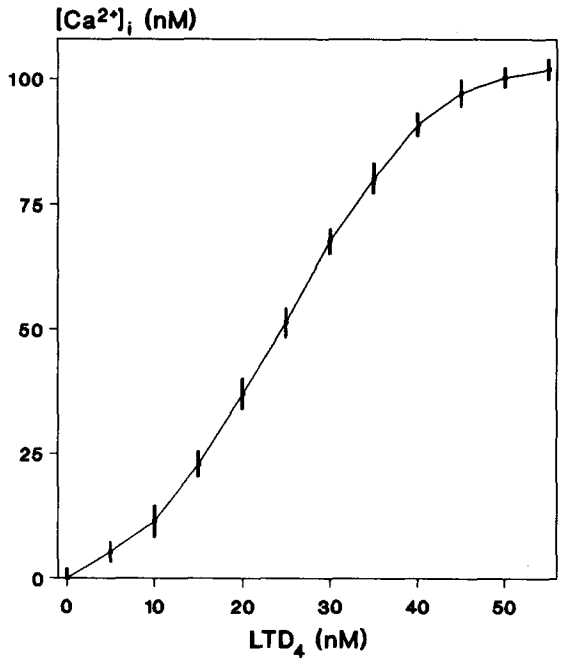

Figure 5. Ca^{2+} peak responses of mesangial cells stimulated with various concentrations of LTD_4. The bars give $\pm$ one standard deviation (σ) and were calculated from 8 independent measurements.

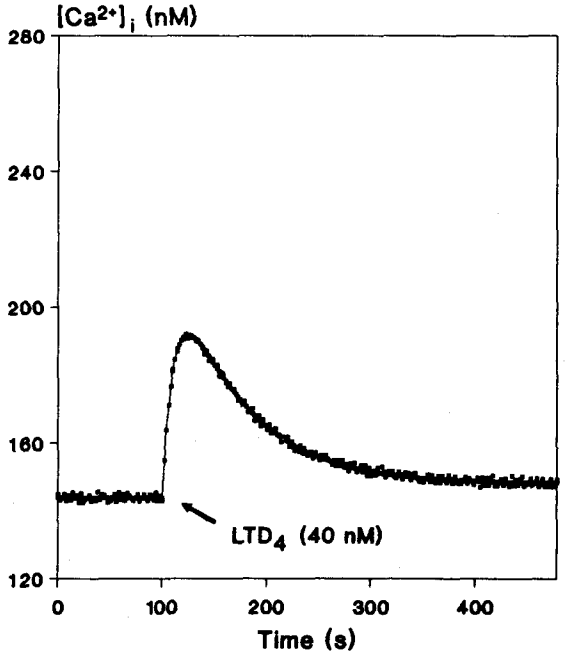

Figure 4. Ca^{2+} transient of mesangial cells pretreated with CGP 45715A (50 nM, 37 °C, 30 min) after stimulation with 40 nM LTD_4. The associated fitting constants are found in table 1d.

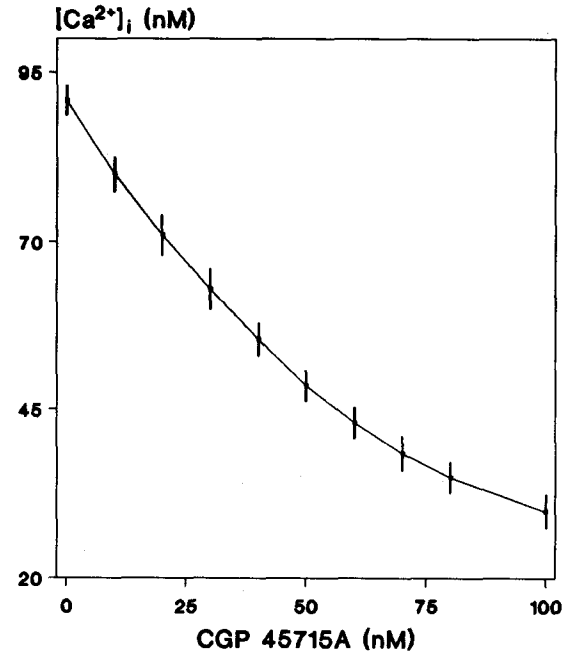

Figure 6. Ca^{2+} peak responses of cells pretreated with various concentrations of CGP 45715A (37 °C, 30 min) after stimulation with 40 nM LTD_4. The bars give $\pm$ one standard deviation (σ) and were calculated from 7 independent measurements.

Discussion

Cultivated mesangial cells contract in response to hormonal stimulation with LTD_4 ($EC_{50} = 10^{-8}$ M) in a dose- and time-dependent fashion, resulting in a significant reduction of the microscopically observed mean surface area of the cells [43]. As expected from theoretical considerations and supported by experimental data [37], the timecourse of $[Ca^{2+}]_i$ is the main factor determining the contractile response of mesangial cells.

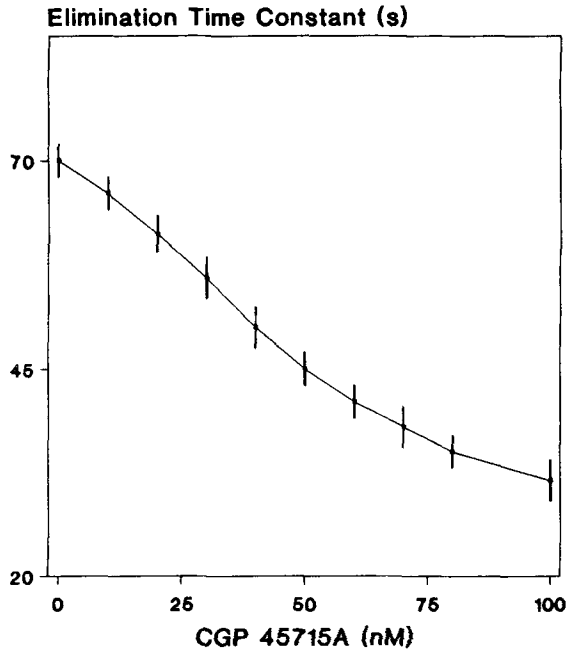

Figure 7. Elimination time constants [τ_{el}(s)] of cells pretreated with various concentrations of CGP 45715A (37 °C, 30 min) after stimulation with 40 nM LTD_4. The bars give ± one standard deviation (σ) and were calculated from 7 independent measurements.

Analogous to its function in smooth muscle cells, LTD_4 would be expected to impair the activity of adenylate cyclase in mesangial cells through an inhibitory guanyl nucleotide binding protein (G_i or a variant thereof) [10–17]. The down-regulating effect of LTD_4 on the cAMP concentration is supported by the experimental result (data not shown) that pertussis toxin (preincubation: 15 h; 100 ng/ml) at least partially abolished the LTD_4-induced Ca^{2+} response in mesangial cells. As outlined above, a drop in cAMP concentration acts to decrease the sequestration of Ca^{2+} and to augment the net influx of Ca^{2+} through the plasma membrane. Due to the inhibitory effect of cAMP on the phospholipase C-mediated phosphoinositide hydrolysis, the generation of $InsP_3$ and DAG and subsequently the intracellular release of Ca^{2+} were clearly enhanced in the control cell group. However, whereas earlier observations suggested that the LTD_4 response is mainly coupled to the generation of cAMP [10–17], recent results strongly suggest that in mesangial cells the phospholipase C-mediated phosphoinositide hydrolysis and the subsequent mobilization of $InsP_3$, DAG and Ca^{2+} constitute the main transduction mechanism [44]. Related to its steady-state level, the intracellular $InsP_3$ concentration of mesangial cells prelabeled with myo-[^{3}H]inositol (4 μCi/ml) was elevated by 26 ± 16%, 47 ± 14% and 93 ± 8% 15 s after a challenge with 10^{-9}, 10^{-8} and 5×10^{-8} M LTD_4, respectively [44]. Consequently, the LTD_4-induced increase in the cytosolic Ca^{2+} concentration is primarily caused by an $InsP_3$-mediated release of Ca^{2+} from intracellular stores.

The hormone-induced rapid rise in cytosolic Ca^{2+} was in all cell fractions primarily transient. Sequestration of Ca^{2+} back to intracellular storage sites and Ca^{2+} efflux via the membrane-bound Ca^{2+} ATPase and the Na^+/Ca^{2+} exchanger are certainly the key elements responsible for the transient nature of the Ca^{2+} signal [30]. After pretreatment of the cells with 50 nM CGP 45715A, the LTD_4 (40 nM)-induced Ca^{2+} response was much smaller, the elimination of Ca^{2+} from the cytoplasm ($1/\tau_{el}$) enhanced and $[Ca^{2+}]_i$ decreased approximately to the starting value (see figs 2a and 4). Since all these effects were strictly dose-dependent (figs 5 and 6), they are most likely caused by a displacement of LTD_4 from the receptor sites, i.e. the higher the CGP 45715A dose the smaller the Ca^{2+} response, the faster the intracellular sequestration of Ca^{2+} and the lower the final Ca^{2+} level. The Ca^{2+} transients in figures 2a and 4 also revealed that in the control cell groups the cytosolic Ca^{2+} level remained elevated over a long time period after stimulation with LTD_4. The sustained influx of Ca^{2+} through the plasma membrane in control cells is in reasonable harmony with an increased LTD_4-mediated generation of $InsP_3$, a species known to act synergistically with its phosphorylated product, Ins(1,3,4,5)P_4, in enhancing the permeability of the plasma membrane towards calcium ions [45]. In analogy to the results obtained with differentiated HL-60 cells, LTD_4-gated, voltage-independent Ca^{2+} channels may additionally contribute to enhance the influx of Ca^{2+} through the plasma membrane [46].

As far as airway smooth muscle and mesangial cells are concerned, there is ample, convincing evidence that receptor-operated (ROCC) and voltage-operated (VOCC) Ca^{2+} channels of the L-type are present [47, 48]. Since a pretreatment with nifedipine had no effect on the Ca^{2+} transients, Ca^{2+} does not enter mesangial cells through VOCCs. In contrast, the LTD_4-induced Ca^{2+} influx was almost completely inhibited by the LTD_4 receptor antagonist CGP 45715A (see fig. 4), indicating that Ca^{2+} enters mesangial cells primarily through ROCCs. Interestingly, drugs which block Ca^{2+} entry through VOCCs, such as nifedipine, verapamil and diltiazem, have not proved effective in treating asthma [49].

The peak response and Ca^{2+} timecourse of cells pretreated with 50 nM CGP 45715A and stimulated with 40 nM LTD_4 closely matched the Ca^{2+} transient observed after stimulation of control cells with 25 nM LTD_4. Comparing these results, the basal Ca^{2+} level and the response of the second Ca^{2+} channel were significantly lower after a pretreatment with 50 nM CGP 45715A. Recently, the cAMP content of mesangial cells has been determined using a commercially available radioimmunoassay ([cAMP] = 20.4 ± 1.2 pmol per

mg of protein; n = 9; New England Nuclear) [50]. The cellular cAMP concentration increased by 5.8 ± 1.0 pmol per mg of protein (n = 7) after incubation of the cells with CGP 45715A (50 nM, 37 °C, 30 min; Ochsner, unpubl. results, 1994). The observation that the basal Ca^{2+} level and Ca^{2+} influx were higher in the control cell group is highly consistent with a CGP 45715A-mediated increase in the steady-state level of cAMP. The cAMP-related inhibition of Ca^{2+} influx, the faster intracellular sequestration and catalyzed extrusion of Ca^{2+} via Ca^{2+} ATPase convincingly explain the lower basal Ca^{2+} level in CGP 45715A (50 nM)-pretreated cells. In addition, cAMP activates membrane-bound Ca^{2+} ATPase and catalyzes the extrusion of Ca^{2+} via the Na^+/Ca^{2+} exchanger to compensate for the higher Na^+ gradient caused by an increased activity of Na^+/K^+-ATPase [30, 51, 52]. Nevertheless, whether the reduction in the basal Ca^{2+} concentration is primarily caused by an enhanced generation of cAMP or by an inhibition of LTD_4-gated Ca^{2+} channels remains to be elucidated [46].

Altogether the available data demonstrate that in mesangial cells LTD_4 primarily stimulates the turnover of phosphoinositides through an allosteric activation of phospholipase C. The mechanism of the LTD_4-induced contraction is thus proposed to involve both an $InsP_3$-mediated intracellular release of Ca^{2+} and an enhanced influx of Ca^{2+} through the plasma membrane. Moreover, our results suggest that LTD_4 possesses a down-regulating effect on adenylate cyclase, a statement which, however, requires further verification. As illustrated in figure 8, the effects of CGP 45715A on mesangial cells are convincingly explained by a displacement of LTD_4 molecules from their receptor site(s).

Throughout the study mesangial cells were used as models for human pulmonary smooth muscle cells, since they are known to respond adequately to a wide variety of smooth muscle agonists [30]. We therefore expect that similar pathophysiological processes as those discussed above and presented in figure 8 are responsible for causing the LTD_4-induced bronchospasm in airway smooth muscle. GTP-regulated binding sites for LTD_4 have been clearly demonstrated in human lung preparations [10, 53]. In addition, at least one subtype of LTD_4 receptors exists in lung membranes which is coupled via a stimulatory guanine nucleotide binding protein to phospholipase C [22, 24]. Competition between LTD_4 and CGP 45715A at the same receptor site(s) is in reasonable agreement with the results of Bray and co-workers [28, 54]. CGP 45715A has been shown to be a potent and specific LTD_4 antagonist in guinea-pig smooth muscle [28]. The displacement of LTD_4 from their own receptor sites is not unanticipated, since CGP 45715A is a close structural analog of LTD_4. The carboxylic group in the eicosanoid chain of LTD_4 has been replaced by a trifluoromethyl group and the dipeptide sulphur side chain of LTD_4 by a chromone carboxylic acid. Both chemicals have the

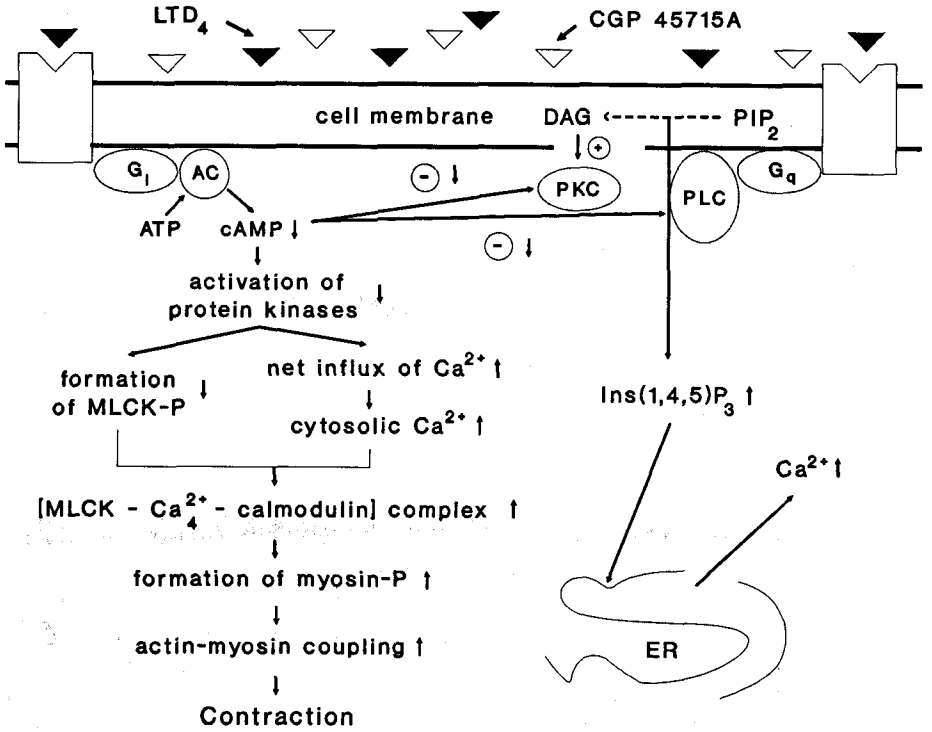

Figure 8. Cellular processes involved in the LTD_4-induced contraction of mesangial cells. A detailed discussion of the biochemical processes and all abbreviations are given in the text. The graph illustrates the competition of LTD_4 and CGP 45715A at the receptor sites coupled to the phosphoinositide hydrolysis and to the formation of cAMP.

same chain length of 20 atoms, whereas the 1*R*,2*S* stereochemistry at the CGP 45715A skeleton is a precondition for a high LTD_4 antagonizing potency [28].

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