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Role of Prostaglandins and Leukotrienes in Volume Regulation by Ehrlich Ascites Tumor Cells

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Summary. PGE_2 and LTC_4 syntheses in Ehrlich ascites cells were measured by radioimmunoassay. Hypotonic swelling results in stimulation of the leukotriene synthesis and a concomitant reduction in the prostaglandin synthesis. If the cells have access to sufficient arachidonic acid there is a parallel increase in the synthesis of both leukotrienes and prostaglandins following hypotonic exposure. PGE_2 significantly inhibits regulatory volume decrease (RVD) following hypotonic swelling in Na-containing medium but not in Na-free media, supporting the hypothesis that the effect of PGE_2 is on the Na permeability. PGE_2 also had no effect on RVD in Na-free media in the presence of the cation ionophore gramicidin. Since the Cl permeability becomes rate limiting for RVD in the presence of gramicidin, whereas the K permeability is rate limiting in its absence, it is concluded that PGE_2 neither affects Cl nor K permeability. Addition of LTD_4 accelerates RVD and since the K permeability is rate limiting for RVD this shows that LTD_4 stimulates the K permeability. Inhibition of the leukotriene synthesis by nordihydroguaiaretic acid inhibits RVD even when a high K conductance has been ensured by the presence of gramicidin. It is, therefore, proposed that an increase in leukotriene synthesis after hypotonic swelling is involved also in the activation of the Cl transport pathway.

Key Words volume regulation · regulatory volume decrease · arachidonic acid · leukotrienes · leukotriene C_4 · leukotriene D_4 · prostaglandins · prostaglandin E_2 · nordihydroguaiaretic acid · Cl permeability · Na permeability · Ehrlich mouse ascites tumor cells · eicosanoids · SRS-A

Introduction

The net loss of KCl observed in Ehrlich ascites cells during regulatory volume decrease (RVD) following hypotonic exposure involves activation of separate conductive K and Cl pathways (Hoffmann, 1978; Hoffmann, Lambert & Simonsen, 1984*a*). It was demonstrated that the Cl transport pathway as well as the K transport pathway is activated by Ca^{2+} and inhibited by anticalmodulin drugs (Hoffmann, Simonsen & Lambert, 1984*b*; Hoffmann, Lambert & Simonsen, 1986). The activation of the Cl transport pathway during regulatory volume decrease is transient and it has been suggested that this reflects a transient increase in cytosolic free Ca^{2+} (Hoffmann et al., 1986).

There is growing evidence that membrane phospholipids may participate in a number of membrane functions. Of particular interest for the present investigation is the role of calcium and calmodulin in the release of arachidonic acid (*see* Wong & Cheung, 1979, and Berridge, 1982) because arachidonic acid is the precursor of the biologically active prostaglandins and leukotrienes.

Arachidonic acid is a polyunsaturated fatty acid present in large amounts in phospholipids (PC, PE, PI)[1] in mammalian cell membranes. The concentration of free arachidonic acid within cells is extremely low. Therefore, the availability of free arachidonic acid as substrate for the enzymes that convert it to biologically active metabolites is very limited. However, hormonal and other stimuli including antigen challenge can induce rapid mobilization of arachidonic acid from lipid stores (Irvine, 1982; Feinstein & Sha'afi, 1983; Berridge, 1984). Released arachidonic acid is then (*see* Fig. 1): a) reacylated in the cell membrane, b) converted to prostaglandins, thromboxanes and prostacyclines through the action of the prostaglandin endoperox-

[1] *Abbreviations*: 5-HPETE, 5S-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid; LTA_4, 5,6-*trans*-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LTB_4, 5S,12R-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatraenoic acid; LTC_4, 5S-hydroxy-6R-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LTD_4, 5S-hydroxy-6R-S-cysteinyl, glycinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LTE_4, 5S-hydroxy-6R-S-cysteinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; DG, diacylglycerol; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

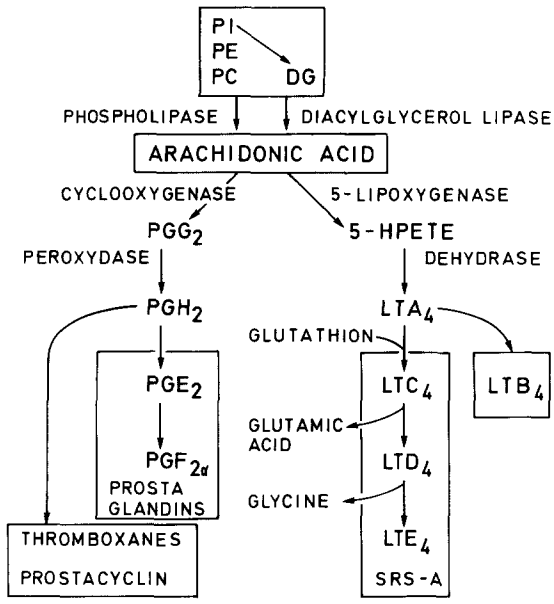

Fig. 1. Pathways of the arachidonic acid cascade which are relevant to the present investigation. Activation of the 5-lipoxygenase leads to formation of 5-HPETE which in turn is oxygenated to the unstable expoxide intermediate LTA_4. This epoxide is converted enzymatically by hydration to LTB_4, or by addition of gluthatione (α-glutamyl-cysteinyl-glycine) to LTC_4. Sequential elimination of a glutamic acid residue and a glycine residue from the peptide part of LTC_4 leads to the production of LTD_4 and LTE_4 (Hammerstrøm, Ørning & Bernström, 1985). Activation of the prostaglandin endoperoxide synthase (cyclooxygenase) pathway results in unstable cyclic prostaglandin endoperoxides PGG_2 and PGH_2. PGH_2 undergoes enzymatic transformation to prostacyclin (PGI_2) and to thromboxanes (TXA_2, TXB_2) or it undergoes isomerization into PGD_2 and PGE_2. $PGF_{2\alpha}$ is obtained by reduction of the keto group of PGE_2 (*see* Hansen, 1983). For abbreviations *see* footnote 1

ide synthase system, or c) converted to hydroxy fatty acids and leukotrienes through the activation of 5-lipoxygenase enzymes (Needleman et al., 1986). The cysteinyl-containing leukotrienes LTC_4, LTD_4 and LTE_4 are released during immune and allergic reactions (Samuelsson, 1983).

Ca^{2+} does not seem to regulate the oxidation of arachidonic acid to prostaglandins, whereas Ca^{2+} promotes the production of leukotrienes through the 5-lipoxygenase pathway (Feinstein & Sha'afi, 1983).

The present investigation reports that Ehrlich ascites cells synthesize and release prostaglandins and leukotrienes and that addition of arachidonic acid stimulates the production of both compounds. During RVD the synthesis of leukotrienes is stimulated while concomitantly prostaglandin synthesis is reduced. Addition of LTD_4 accelerates RVD (which is rate limited by the K permeability), while addition of inhibitors of leukotriene synthesis blocks the volume response, even when a high K conductance has been ensured by addition of gramicidin. It is proposed that the activation of K and Cl transport pathways after hypotonic swelling (Hoffmann et al., 1986) involves an increase in leukotriene synthesis. Addition of PGE_2 inhibits RVD in hypotonic medium containing sodium but not in hypotonic sodium-free media, indicating that PGE_2 increases the passive sodium permeability in Ehrlich cells. The reduced prostaglandin synthesis during RVD could thus be responsible for the concomitant reduction in the passive permeability for sodium previously reported by Hoffmann (1978). Preliminary results of this study have previously been presented (Lambert, Hoffmann & Christensen, 1986).

Materials and Methods

Cells and Incubation Media

Ehrlich ascites tumor cells (hyperdiploid strain), maintained by weekly intraperitoneal transplantation in white Theiller mice were harvested 8 days after transplantation and suspended in standard incubation solution containing heparin (2.5 IU/ml). The cells were washed twice by centrifugation (700 × *g*; 45 sec) with standard incubation solution prior to preincubation for 40 min. The standard incubation medium (300 mOsm) had the following composition (mM): Na 150, K 5, Mg 1, Ca 1, Cl 150, sulfate 1, inorganic phosphate 1, MOPS (3-(N-morpholinopropane) sulfonic acid) 3.3, TES (N-Tris(hydroxymethyl)-methyl-2-aminoethane sulfonic acid) 3.3, and HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid) 5. The pH was adjusted to 7.4. In choline medium, choline chloride was substituted for NaCl in equimolar amounts. N-methyl-D-glucamine-medium, where N-methyl-D-glucammonium was substituted for Na, was prepared from a N-methyl-D-glucamine stock solution titrated with equimolar amounts of HCl. Hypotonic media (150 mOsm) were prepared by diluting the isotonic media with one volume distilled water containing buffer alone. The temperature was kept at 37°C under all conditions.

Reagents

All reagents were analytical grade. Arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid), gramicidin D, nordihydroguaiaretic acid (NDGA) and PGE_2 were obtained from Sigma, St. Louis, Mo., and added to the cell suspension from stock solutions in ethanol. LTC_4, LTD_4, and LTE_4, dissolved in distilled water, and LTB_4, dissolved in methanol, were kindly provided by Dr. J. Rokach (Merck Frosst Canada, Inc.).

Cell Volume Measurements

Cell volume distribution curves were obtained using a Coulter counter model Z with a Coulter channellyzer (C-1000). The orifice diameter was 100 μm. For measurements an aliquot of the cell

suspension was diluted 500-fold with filtered experimental solution (Millipore, pore size 0.45 μm) to give a final cell density of 85,000 to 95,000 cells per ml (equivalent to a cytocrit about 0.03%). The mean cell volume in arbitrary units was calculated as the median of the cell volume distribution curves. Absolute cell volumes were obtained using polystyrene latex beads (12.9 μm diameter) as standards.

Measurements of Prostaglandins and Leukotrienes

For determination of extracellular content of PGE_2, and $PGF_{2\alpha}$ and LTC_4, 1.0 ml cell suspension (cytocrit between 6 and 7%) was centrifuged (20,000 × *g*, 60 sec) in preweighed vials, the supernatant was stored on ice for determination of PGE_2, $PGF_{2\alpha}$ and LTC_4, while the cell pellet was dried (90°C, 48 hr) and reweighed for determination of the cell density of the cell suspension (g cell dry wt/ml). LTC_4 was measured with a leukotriene C_4 (^3H) radioimmunoassay kit (New England Nuclear) while samples for determination of PGE_2 and $PGF_{2\alpha}$ were prepared and measured by radioimmunoassay as previously described (Christensen & Leyssac, 1976; Leyssac & Christensen, 1981) and documented (Christensen, Gréen & Leyssac, 1983). The rate of synthesis was measured from points obtained within 3 to 5 min after transfer to the experimental solution.

Statistical Evaluation

All values in text are expressed as the mean ± SEM with the number of experiments in brackets.

Results

Prostaglandin and Leukotriene Release During Regulatory Volume Decrease

Ehrlich ascites tumor cells swell in hypotonic media but subsequently regulate their volume, with 66% volume recovery after 10 min (Hoffmann et al., 1984*b*). Figure 2 shows that during the first 10 min after transfer to hypotonic media the release of LTC_4 is dramatically increased (upper frame), whereas the release of PGE_2 is decreased (lower frame). The increase in LTC_4 synthesis seems to be transient, ceasing after 5 to 10 min. Figure 3 shows that after addition of 0.05 mM arachidonic acid the PGE_2 synthesis is no longer reduced in hypotonic medium. Thus the decrease in PGE_2 synthesis during RVD (Fig. 2) is probably not a result of an actual inhibition of the prostaglandin endoperoxide synthase system (*see* Fig. 1) but rather caused by lack of arachidonic acid. From experiments like the ones in Figs. 2 and 3 it is found that addition of 0.05 mM arachidonic acid stimulates the prostaglandin synthesis from 6 ± 2.3 ng/g cell dry wt · min (n = 5) to 59 ± 11 ng/g cell dry wt · min (n = 4). Addition of

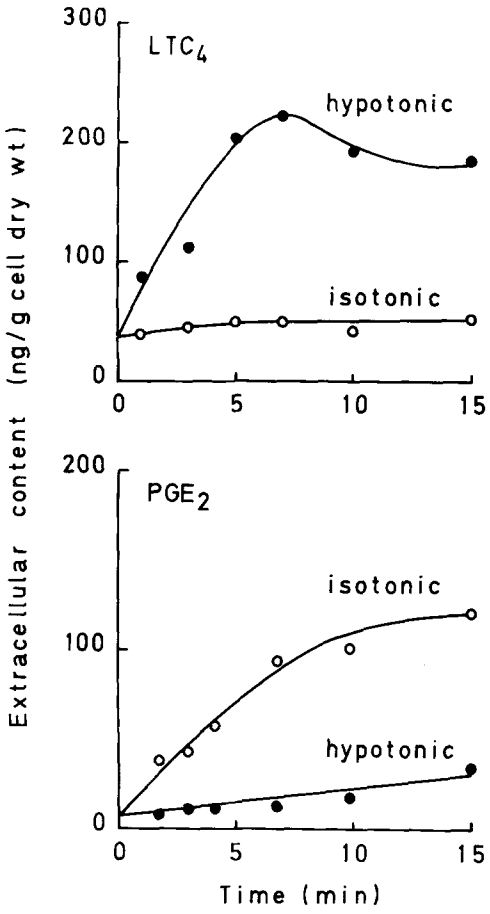

Fig. 2. LTC_4 and PGE_2 synthesis in Ehrlich cells suspended in standard incubation medium and in hypotonic incubation medium. Ehrlich cells preincubated in standard incubation medium were gently spun down and resuspended in either standard or hypotonic (150 mOsm) incubation medium. The cytocrit was adjusted to 6%. Release of LTC_4 and PGE_2 was followed with time by serially isolating cell-free medium by centrifugation and measuring the LTC_4 and PGE_2 concentration by radioimmunoassay. The extracellular content (ng/g cell dry wt) was calculated from the concentration in the medium (ng/ml) and the density of the cell suspension (g dry wt/ml). The Figure is representative of four independent experiments.

0.2 mM arachidonic acid increases PGE_2 synthesis to 308 ng/g cell dry wt · min (n = 2) with the corresponding LTC_4 synthesis increased from 0.4 ± 0.1 ng/g cell dry wt · min (n = 4) to 4 ng/g cell dry wt · min (n = 2). Table 1 summarizes the changes during RVD in the synthesis of PGE_2 and LTC_4 as well as the parallel changes in the synthesis of the PGE_2 metabolite $PGF_{2\alpha}$. After addition of arachidonic acid the synthesis of all three compounds are unchanged or stimulated during RVD, whereas PGE_2 and $PGF_{2\alpha}$ synthesis decreases during RVD when no extra arachidonic acid is added.

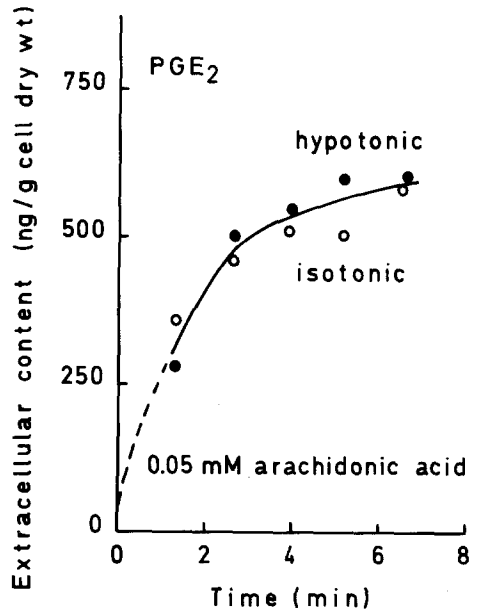

Fig. 3. PGE_2 synthesis in Ehrlich cells suspended in standard and hypotonic incubation media containing arachidonic acid. Experimental protocol as in Fig. 2, except that 0.05 mM arachidonic acid was added to the cell suspension within the first seconds following transfer to the experimental solution. The Figure is representative of two experiments at 0.05 mM arachidonic acid. Experiments at 0.1 and 0.2 mM arachidonic acid gave qualitatively similar results

Table 1. Changes in the synthesis of PGE_2, $PGF_{2\alpha}$ and LTC_4 in Ehrlich cells during regulatory volume decrease after swelling in hypotonic media[a]

Arachidonic acid added (mM)	Eicosanoid synthesis in hypotonic media (relative to values in a parallel, isotonic control)		
	PGE_2	$PGF_{2\alpha}$	LTC_4
None	0.44 ± 0.11 (n = 5)	0.49 ± 0.1 (n = 3)	18 ± 5 (n = 3)[b]
0.05	1.2 (n = 2)	3.2 (n = 2)	16 (n = 2)
0.10	1.4 (n = 2)	1.4 (n = 2)	—
0.20	1.3 (n = 2)	1.8 (n = 2)	4 (n = 2)

[a] Experimental protocol as described in the legend to Figs. 2 and 3. The rate of synthesis in hypotonic incubation medium is given relative to the rate of synthesis in a parallel cell culture in standard incubation medium. The values without arachidonic acid are given as mean ± SEM with the number (n) of independent experiments in brackets while values with arachidonic acid added for each concentration are given as the mean of 2 independent experiments. In all experiments where arachidonic acid is added the decreases in PGE_2 and $PGF_{2\alpha}$ (seen when no arachidonic acid is added) were turned into a small increase.
[b] One additional experiment showed no change in LTC_4 synthesis.

Table 2. Effect of added PGE_2 on the initial rate of volume recovery following hypotonic swelling in Na-containing media and Na-free media[a]

Hypotonic medium	PGE_2 (μM)	Initial rate of volume recovery		
		fl/min	relative to control	P
NaCl	0	97 ± 17		
	5	66 ± 16	0.65 ± 0.01	<0.001
N-methyl-D glucamineCl	0	135 ± 12		
	5	126 ± 10	0.94 ± 0.02	NS
CholineCl	0	111 ± 9		
	5	109 ± 4	0.99 ± 0.08	NS
CholineCl + gramicidin	0	702 ± 28		
	5	719 ± 61	1.02 ± 0.07	NS

[a] Gramicidin was added in order to impose a high cation permeability. Experimental protocol as in Fig. 4. In experiments without gramicidin the initial rate of RVD was obtained from lines fitted to 4 to 6 values taken within 1 and 4 min after the shift in osmolarity (*see* Fig. 4). In experiments with gramicidin the initial rate of RVD was estimated from values taken within the first min after addition of gramicidin (*see* Fig. 4, right panel). The rate is given in absolute values (fl/min) and also relative to controls without addition of PGE_2. Values are mean ± SEM of three independent sets of experiments. P is the level of significance in a Students' t-test where the relative values are tested against the hypothetical value 1. NS is nonsignificant at a 1% level.

Effect of Added PGE_2 on Membrane Permeabilities in Osmotically Swollen Cells

Figure 4 shows that PGE_2 inhibits the regulatory volume decrease (RVD) following hypotonic swelling in Na-containing medium (left frame) but not in Na-free choline medium (middle frame). It is, therefore, proposed that the inhibition in Na-containing media (left frame) is caused by an increased Na permeability resulting in an increased uptake of Na. In the experiment shown in the right frame, gramicidin was added to impose a high cation permeability. Since the cells were suspended in Na-free choline medium the addition of gramicidin will result in a net cation and Cl loss, the rate of which will be limited by the Cl permeability (Grinstein et al., 1982; Hoffmann et al., 1986). It can be seen that PGE_2 does not inhibit the Cl permeability (Fig. 4, right frame). Table 2 summarizes results similar to the ones in Fig. 4. It is seen that the initial rate of volume recovery in Na-containing medium is significantly reduced by 35 ± 1% after addition of 5 μM PGE_2, whereas no change is seen in the Na-free media where Na was replaced by the impermeable cations choline or N-methyl-D-glucammonium

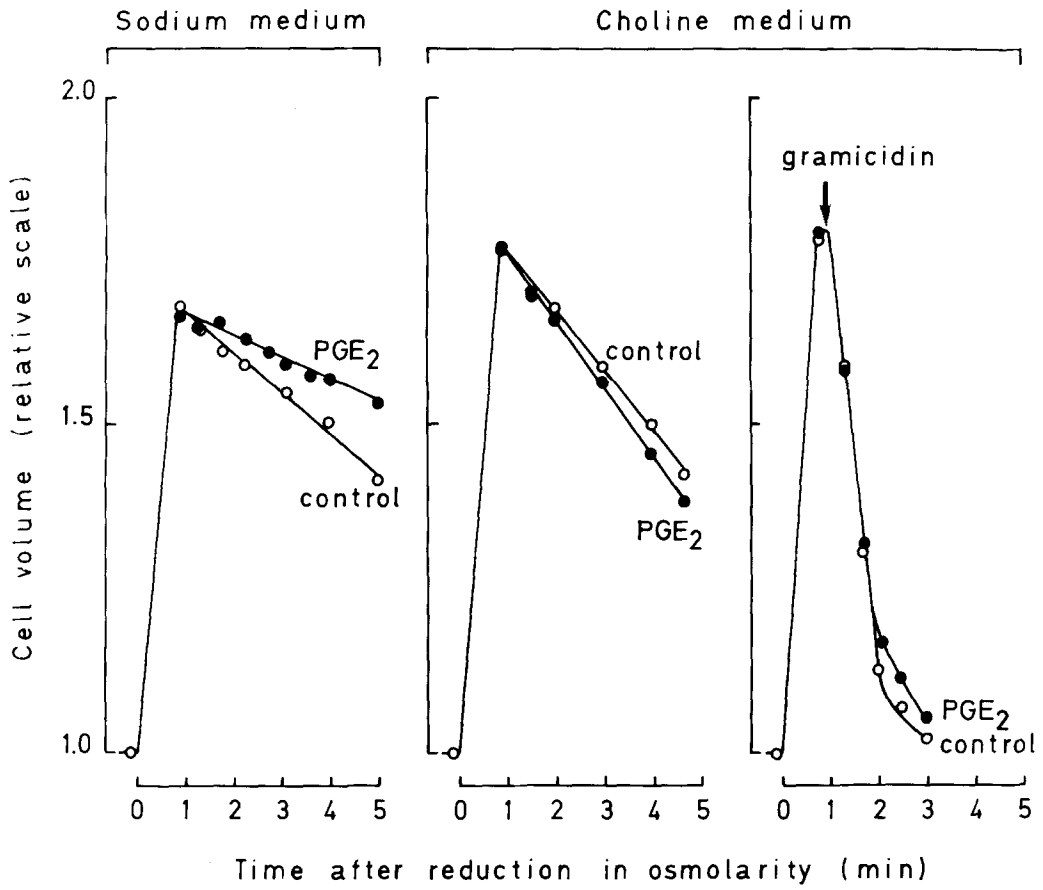

Fig. 4. Effect of PGE_2 on regulatory volume decrease in hypotonic sodium medium (hypotonic standard medium) and in hypotonic choline medium (choline chloride substituted for NaCl) in the absence and presence of gramicidin. Gramicidin was added in order to ensure a high cation permeability. Ehrlich cells were preincubated in standard incubation medium and at time zero diluted 500-fold in either hypotonic standard medium (left panel) or in hypotonic choline chloride medium (middle and right panel). Cell density was 90,000 cells/ml. 5 μM PGE_2 was added to the cells within the first seconds after the reduction in osmolarity, and 0.5 μm gramicidin (right panel) was added at the time of maximal swelling as indicated by the arrow. The cell volume was followed with time and given relative to the initial cell volume. The curves are representative of three independent experiments (included in Table 2).

(Blackstock, Ellory & Stewart, 1985). The results after addition of gramicidin confirm the conclusion from Fig. 4 that PGE_2 has no significant effect ($P > 0.10$) on the swelling-induced Cl permeability. This supports the hypothesis that the only effect of PGE_2 is an increase in the Na permeability.

Effect of Leukotrienes and of a Leukotriene Synthesis Inhibitor on Membrane Permeabilities in Osmotically Swollen Cells

Figure 5 shows that one of the leukotrienes, LTD_4 accelerates the hypotonic volume response dramatically, whereas LTB_4, LTC_4 and LTE_4 seem to have little effect. The results of three to four experiments similar to the ones shown in Fig. 4 are summarized in Table 3, from which it can be seen that addition of 60 nM LTD_4 increases the initial rate of volume recovery significantly ($P > 0.001$) by a factor of 5.9 ± 0.15. Addition of only 10 nM LTD_4 more than doubles the initial rate of volume recovery (Table 3). None of the other leukotrienes had more than marginally significant effects at a concentration of 300 nM. The increase in LTC_4 synthesis during RVD (Fig. 2 and Table 1) is thus not likely to be directly responsible for the permeability changes observed during RVD but rather seems to reflect a simultaneous increase in the synthesis of the active metabolite LTD_4 (*see* Fig. 1). We have not been able to measure LTD_4 synthesis.

If LTD_4 is important for the permeability changes during RVD, an inhibitor of leukotriene synthesis (i.e. LTD_4 synthesis) should inhibit RVD. Figure 6 shows that the 5-lipoxygenase inhibitor NDGA (Cashman, 1985) inhibits the RVD re-

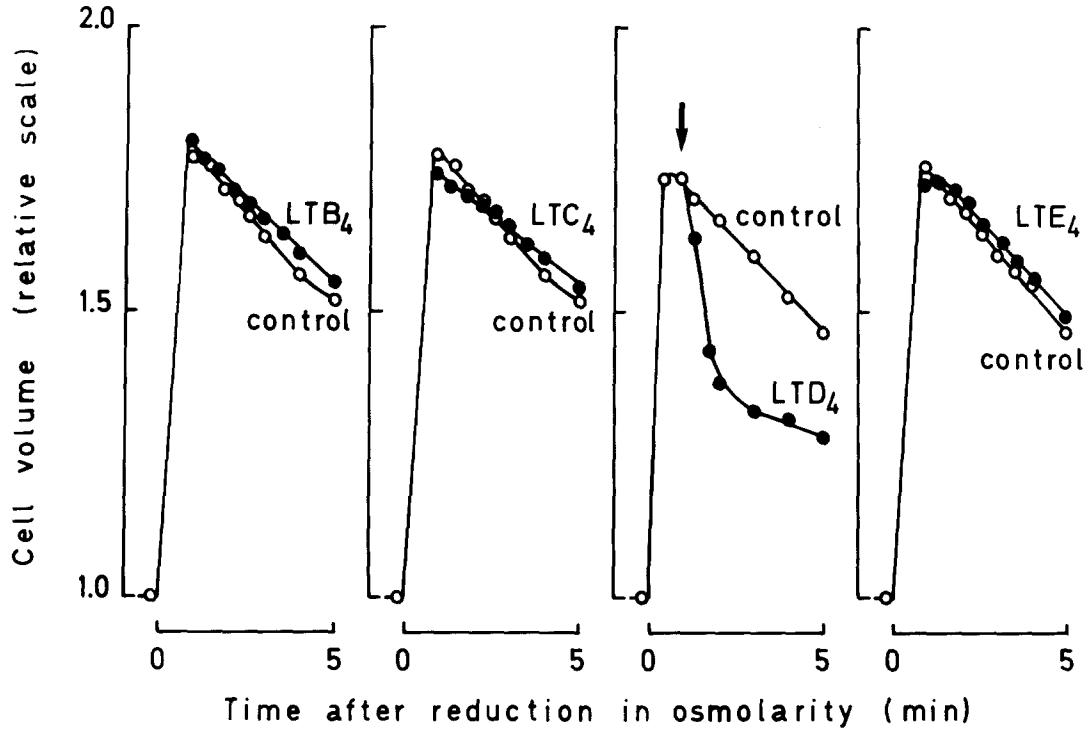

Fig. 5. Effect of added leukotrienes on regulatory volume decrease in Ehrlich cells after swelling in hypotonic standard incubation medium. Cells were preincubated in standard incubation medium for 40 min or more and at zero time diluted 500-fold with hypotonic standard incubation medium. 300 nM LTB_4, LTC_4, or LTE_4 were added within seconds after reduction in osmolarity, while 60 nM LTD_4 was added at the time of maximal swelling, i.e. 0.9 min after hypotonic challenge (arrow). Cell volume was followed with time and given relative to the initial cell volume in standard incubation medium. The curves are representative of three independent experiments (included in Table 3).

sponse. In the experiments in Fig. 6 the cells were preincubated for 3 min with the inhibitor (100 μM). The initial rate of volume recovery was inhibited 76 $\pm$ 1% (n = 4) after addition of 50 μM NDGA at time zero. Omission of the preincubation with NDGA reduced the inhibition to 63 $\pm$ 4% (n = 5). Figure 6 (lower frame) shows that NDGA inhibits the hypotonic volume response also when a high K permeability is ensured by the presence of gramicidin, demonstrating the the Cl transport pathway, activated by cell swelling, is inhibited by NDGA. It is, therefore, proposed that LTD_4 is involved also in the activation of the anion transport pathway.

Discussion

Activation of Prostaglandin and Leukotriene Synthesis by Cell Swelling. Role of Internal Ca^{2+}

The release and the subsequent metabolism of polyunsaturated fatty acids to biologically active substances constitutes a new and fascinating area of bioorganic chemical research. Biotransformation of arachidonic acid gives rise to a family of very important mediators such as the prostaglandins, prostacyclin, thromboxanes and leukotrienes (*see* Fig. 1). There is very little free arachidonic acid in cells because most of the arachidonic acid is attached at the 2-position of the phospholipids such as PC, PE and PI. The first enzyme in the release of arachidonic acid and in the biosynthesis of the leukotrienes and prostaglandins is phospholipase A_2, although other lipases may also be important in arachidonic acid turnover. Phospholipase A_2 is associated with the inner cellular membrane and has an absolute requirement for Ca^{2+} (Van den Bosch, 1980). Free arachidonic acid is converted to 5-HPETE by a 5-lipoxygenase which is also membrane bound and requires Ca^{2+}, ATP and other stimulatory factors (Rouzer & Samuelsson, 1985). An increase in free cytoplasmic Ca^{2+}, therefore, results in an increase in the synthesis of the leukotrienes. This is demonstrated by addition of the Ca^{2+} ionophore A23187 in rat brain slices (Dembinska-Kiec et al., 1984), in human leukocytes (Braquet et al., 1984), in rat tumor and testicular Leydig cells (Sullivan & Cooke, 1985), and in rabbit lungs (Schulz & Seeger, 1986). In some cases addition of A23187 is also found to induce release of pro-

Table 3. Effect of added LTB_4, LTC_4, LTD_4 and LTE_4 on the initial rate of volume recovery following hypotonic swelling[a]

Compound	Concentration (nM)	Initial rate of volume recovery			
		(fl/min)	Relative to control	P	n
LTB_4	0	88 ± 7			
	300	82 ± 9	0.92 ± 0.03	NS	3
LTC_4	0	83 ± 3			
	300	57 ± 9	0.69 ± 0.1	NS	3
LTD_4	0	83 ± 6			
	10	185	2.9		2
	60	527 ± 41	5.9 ± 0.15	<0.001	4
LTE_4	0	89 ± 4			
	300	71 ± 5	0.80 ± 0.05	NS	4

[a] Experimental protocol as in Fig. 5. The initial rate of volume recovery was obtained as described in Table 2. In the case of LTD_4 where RVD was accelerated dramatically only values within the first minute after addition of LTD_4 were used for calculation of the initial rate of volume recovery. For other details *see* Table 3.

staglandins in frog skin (Erlij, Gersten & Sterba, 1981) and in macrophages (Brune & Peskar, 1985).

We have previously demonstrated that addition of the ionophore A23187 plus Ca^{2+} activates separate K and Cl transport pathways in Ehrlich cells (Hoffmann et al., 1984*a*; 1986). Ca^{2+} also seems to play a key role in the loss of KCl during regulatory volume decrease in Ehrlich cells (Hoffmann et al., 1984*b*), suggesting that Ca^{2+} is involved in the volume-induced activation of K and Cl channels. It was suggested that a transient increase in free cytosolic Ca^{2+} may account for the transient activation of the Cl channel during RVD (Hoffmann et al., 1986). A concomitant volume-induced increase in the release of leukotrienes is demonstrated in Fig. 2 and in Table 1. The rate of synthesis of leukotrienes (i.e. LTC_4) is very low in standard incubation medium but increases dramatically (18-fold) immediately after transfer to hypotonic solutions. If arachidonic acid is added to the cell suspension there is a parallel increase in the synthesis of prostaglandins (Fig. 3 and Table 1). Under standard conditions, without addition of arachidonic acid, there is a decrease in the prostaglandin synthesis parallel to the increase in leukotriene synthesis (Fig. 2). The increase in leukotriene synthesis, about 6 ng/g cell dry wt · min, is roughly similar to the decrease in prostaglandin synthesis, which is about 4 ng/g cell dry wt · min. This finding suggests that arachidonic acid metabolism via the 5-lipoxygenase pathway (*see* Fig. 1) is favored during regulatory volume decrease at the expense of synthesis via the cyclooxygenase pathway.

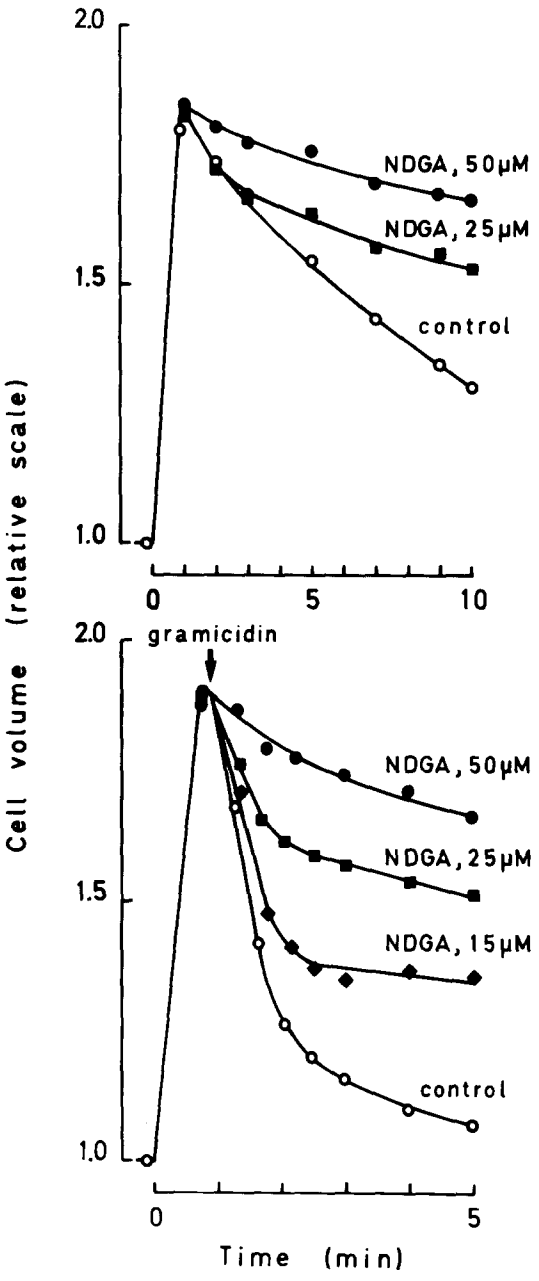

Fig. 6. Inhibition by the leukotriene synthesis inhibitor nordihydroguaiaretic acid (NDGA) of the increase in Cl net permeability induced by cell swelling. Gramicidin was added in order to ensure a high K permeability. Ehrlich cells were preincubated in standard incubation medium (cell density 4%) for 40 min or more. 100 μM NDGA was added to the cells 3 min before reduction in osmolarity, in order to obtain maximal inhibitory effect of NDGA. The concentration of the inhibitor was higher in the preincubation medium because the cell density was about 500 times higher during preincubation than in the Coulter counter. At time zero the cell suspension was diluted 500-fold in hypotonic choline chloride containing NDGA in the concentrations indicated on the Figure, and the cell volume was followed with time. Control cells contained about 0.2 μM NDGA (estimated from the 500-fold dilution). 0.5 μM gramicidin (lower panel) was added at the time of maximal swelling as indicated by the arrow. The curves are representative of three independent sets of experiments.

The increase in the leukotriene synthesis seems not to reflect a general increase in metabolic rate in hypotonic media. On the contrary, we have previously shown that dilution of the medium is followed by a reduction in the protein synthesis, the O_2 consumption and the CO_2 production, indicating a general decrease in the metabolic activity in hypotonic media (Lambert & Hoffmann, 1982).

Effect of Prostaglandins on the Na Permeability

Arachidonic acid and PGE_2 have been shown to stimulate the active transepithelial Na transport in frog skin (Hall et al., 1976), presumably by an increase in the Na permeability of the apical membrane (Nielsen & Bjerregård, 1984). In the Ehrlich cells there is evidence that the Na permeability decreases during RVD (Hoffmann, 1978). Since the synthesis of prostaglandins is strongly inhibited during RVD (Fig. 2 and Table 1) it is tempting to suggest that the decrease in the Na permeability is secondary to the decrease in prostaglandin synthesis. This would agree with the observation that addition of arachidonic acid to cells in either hypotonic or isotonic media stimulates the prostaglandin synthesis (Fig. 2 and Table 1) and induces a considerable uptake of Na (Lambert, 1987). An increase in Na permeability following addition of PGE_2 to hypotonic swollen cells results in an inhibition of volume regulatory decrease in Na-containing media, because the net loss of cell K is largely balanced mainly by a net gain of Na. Figure 4 and Table 2 demonstrate that PGE_2 significantly inhibits RVD in Na-containing medium but not in Na-free media, supporting the hypothesis that the effect of PGE_2 is on the Na permeability.

This is also in agreement with the observation that addition of PGE_2 (3.3 μM) induces a substantial depolarization of the cell membrane (Lambert, Jørgensen & Hoffmann, *unpublished observations*). In Na-free media PGE_2 had no effect on the volume regulation in the presence or in the absence of the cationophore gramicidin (Fig. 4, right and middle frame; Table 2). Since the Cl permeability is rate limiting for RVD in the presence of gramicidin, whereas the K permeability is rate limiting in its absence (*see* Hoffmann et al., 1986) it can be concluded that PGE_2 neither affects Cl nor K permeability.

Effect of Leukotrienes on the K and Cl Transport Pathways

The experiments described in this paper show that Ehrlich cells have the capability to form LTC_4 and that this synthesis is stimulated when the cells are incubated with arachidonic acid. Our radioimmunoassay does not permit determination of the metabolites of LTC_4, i.e. LTD_4 and LTE_4 (Fig. 1), but it seems likely that these metabolites are also synthesized in Ehrlich cells. The experiments also demonstrate that there is a dramatic increase in LTC_4 synthesis during RVD (Fig. 2 and Table 1) and it is likely that the synthesis of LTD_4 and LTE_4 is also increased. The experiments in Fig. 5 and Table 3 demonstrate that LTC_4 and LTE_4 slightly inhibit, whereas LTD_4 strongly accelerates RVD in Ehrlich cells when added at a concentration of 10 nM to the medium. This concentration does not appear to be unphysiologically high since the medium concentration of LTC_4 10 min after transfer to hypotonic medium was measured at 0.5 to 0.8 nM without addition of arachidonic acid. Since the K permeability is known to be rate limiting for RVD the results show that LTD_4 stimulates the K transport pathway.

It has previously been shown (Lambert, 1987) that addition of LTD_4 to Ehrlich cells in isotonic NaCl medium results in a rapid loss of KCl followed by cell shrinkage. Since the Cl permeability of Ehrlich cells is low (Hoffmann et al., 1979) it has been proposed that LTD_4 increases the Cl permeability in isotonic medium (Lambert, 1987). Provided the increased leukotriene synthesis during RVD is responsible for the increase in either K or Cl permeabilities an inhibition of the 5-lipoxygenase (*see* Fig. 1) should inhibit RVD. This is found to be the case for the lipoxygenase inhibitor NDGA. Since NDGA was found to inhibit RVD also when a high K permeability was ensured by addition of gramicidin (*see* Fig. 6) it is concluded that leukotrienes are responsible also for the increase in Cl permeability during RVD. In conclusion, LTD_4 is a highly potent modulator of both K and Cl permeabilities and activation of the lipoxygenase pathway during RVD is necessary for the associated activation of the K and Cl transport pathways. LTC_4 has been reported to stimulate chloride transport across the isolated frog cornea (Schaeffer & Zadunaisky, 1986) and across canine tracheal epithelium (Leikauf et al., 1986) probably via activation of the cyclooxygenase pathway, resulting in enhanced PGE_2 synthesis. The stimulation of the chloride transport pathway by LTD_4 demonstrated in the present report cannot be mediated via an enhanced PGE_2 synthesis since LTD_4 and PGE_2 have opposite effects on the regulatory volume response (compare Figs. 4 and 5).

We have previously shown that Ca^{2+} and calmodulin seem to be involved in the activation of the K and Cl transport pathways. Since numerous studies (*see* e.g. Van den Bosch, 1980; Craven &

DeRubertis, 1983) have emphasized the importance of Ca^{2+} and calmodulin in the mobilization of arachidonic acid from membrane lipids it could be suggested that the role for Ca and calmodulin in activation of at least the Cl transport pathway is accounted for by their role in arachidonic acid release and leukotriene synthesis.

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