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Effect of Arachidonic Acid, Fatty Acids, Prostaglandins, and Leukotrienes on Volume Regulation in Ehrlich Ascites Tumor Cells

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Summary. Arachidonic acid inhibits the cell shrinkage observed in Ehrlich ascites tumor cells during regulatory volume decrease (RVD) or after addition of the Ca ionophore A23187 plus Ca. In Na-containing media, arachidonic acid increases cellular Na uptake under isotonic as well as under hypotonic conditions. Arachidonic acid also inhibits KCl and water loss following swelling in Na-free, hypotonic media even when a high K conductance has been ensured by addition of gramicidin. In isotonic, Na-free medium arachidonic acid inhibits A23187 + Ca-induced cell shrinkage in the absence but not in the presence of gramicidin. It is proposed that inhibition of RVD in hypotonic media by arachidonic acid is caused by reduction in the volume-induced Cl and K permeabilities as well as by an increase in Na permeability and that reduction in A23187 + Ca-induced cell shrinkage is due to a reduction in K permeability and an increase in Na permeability. The A23187 + Ca-activated Cl permeability in unaffected by arachidonic acid. PGE_2 inhibits RVD in Na-containing, hypotonic media but not in Na-free, hypotonic media, indicating a PGE_2-induced Na uptake. PGE_2 has no effect on the volume-activated K and Cl permeabilities. LTB_4, LTC_4 and LTE_4 inhibit RVD insignificantly in hypotonically swollen cells. LTD_4, moreover, induces cell shrinkage in steady-state cells and accelerates the RVD following hypotonic exposure. The effect of LTD_4 even reflects a stimulating effect on K and Cl transport pathways. Thus none of the leukotrienes show the inhibitory effect found for arachidonic acid on the K and Cl permeabilities. The RVD response in hypotonic, Na-free media is, on the other hand, also inhibited by addition of the unsaturated oleic, linoleic, linolenic and palmitoleic acid, even in the presence of the cationophor gramicidin. The saturated arachidic and stearic acid had no effect on RVD. It is, therefore, suggested that a minor part of the inhibitory effect of arachidonic acid on RVD in Na-containing media is via an increased synthesis of prostaglandins and that the major part of the arachidonic acid effect on RVD in Na-free media, and most probably also in Na-containing media, is due to the inhibition of the volume-induced K and Cl transport pathways, caused by a nonspecific detergent effect of an unsaturated fatty acid.

Key Words volume regulation · leukotrienes · prostaglandins · arachidonic acid · fatty acids

Introduction

In the animal cell membrane most of the fatty acids are found acylated to glycerol in phospholipids, constituting the basic structural elements of the lipid bilayer. Only small amounts of fatty acids exist as free acids in the membrane. Since a number of macromolecular transport carriers, embedded in the membrane lipid bilayer, seem to be regulated at least in part by the composition of the surrounding lipids, it is reasonable to expect some changes in their transport properties, following variation in the amount of free fatty acids. For example, it has been reported that changes in the fatty acid composition of the Ehrlich cell membrane are associated with changes in the temperature dependence of the α-aminoisobutyric acid (AIB) uptake system, in the activation energy for AIB uptake and in the affinity of the transport carrier for AIB (Kaduce et al., 1977).

Fatty acids, like arachidonic acid might also play an important role in regulation of transport carriers in the membrane via some of their metabolites. Arachidonic acid is a polyunsaturated fatty acid, stored in large amounts in phospholipids in mammalian cell membranes. Free arachidonic acid may be liberated after activation of phospholipases or diacylglycerol lipases by hormones, neurotransmitters, antigens or other stimuli (Irvine, 1982; Fienstein & Sha'afi, 1983; Berridge, 1984). Released or exogenous arachidonic acid can either be acylated into the cell membrane, be converted to prostaglandins, thromboxanes and prostacyclins through the action of the cyclooxygenase enzyme system (Hansen, 1983), or be converted to lipoxygenase products through the activation of lipoxygenase enzymes (Samuelsson, 1983; Hammerström, Örning & Berström, 1985). The activation of

the lipoxygenases leads to formation of an unstable epoxide intermediate, leukotriene A_4 (LTA_4), which can be converted enzymatically by hydration to leukotriene B_4 (LTB_4), or by addition of the tripeptide gluthatione (γ-glutamylcysteinyl-glycine) to leukotriene C_4 (LTC_4). Sequential loss of a glutamic acid residue and a glycine residue leads to the production of leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) (Hammerström et al., 1985). The cysteinyl-containing leukotrienes LTC_4, LTD_4 and LTE_4 are of particular interest since they collectively account for the biological activity known as slow-reacting substance of anaphylaxis, released during immune and allergic reaction (Samuelsson, 1983). They are potent bronchoconstrictors (Drazen et al., 1980) and mucus secretagogues (Marom et al., 1982).

We have recently shown that Ehrlich ascites tumor cells produce and release leukotrienes and prostaglandins to their surroundings (Lambert, Hoffmann & Christensen, 1986, 1987). Osmotic swelling of Ehrlich cells in hyptonic solutions results in an increased release of LTC_4 and a concommitant decrease in PGE_2 production (Lambert et al., 1986, 1987). Hypotonically swollen Ehrlich cells recover their volume by net loss of KCl, amino acids, taurine and osmotically obliged water (Hoffmann & Hendil, 1976). The loss of KCl is mainly via separate, Ca/calmodulin-activated K- and Cl-conductive pathways (Hoffmann, Simonsen & Lambert, 1984; Hoffmann, Lambert & Simonsen, 1986) while the loss of amino acids is due to an increase in passive permeabilities for these compounds (Hoffmann & Lambert, 1983; Lambert, 1984*a*). During RVD the Na permeability is reported to be concommitantly decreased (Hoffmann, 1978). A substantial increase of the conductive K and Cl permeabilities in Ehrlich cells and subsequent cell shrinkage can also be induced by addition of the Ca-ionophore A23187 (Lambert, 1984*b*; Hoffmann et al., 1986).

The present work was, therefore, initiated in order to examine the effect of exogenous added fatty acids, in special arachidonic acid and its metabolites, on volume regulation in Ehrlich ascites tumor cells.

Materials and Methods

Cells and Incubation Media

Ehrlich ascites cells (hyperdiploid strain), maintained and transplanted in white Theiller mice were isolated and prepared for experiments as described previously (Hoffmann, Simonsen & Sjöholm, 1979). The standard NaCl 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-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid) 5. The pH was adjusted to 7.4. In choline medium, cholineCl 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. Arachidic acid (eicosanoic acid), arachidonic acid (5,8,11,14-eicosatetraenoic acid), stearic acid (octadecanoic acid), oleic acid (*cis*-9-octadecenoic acid), elaidic acid (*trans*-9-octadecenoic acid), linoleic acid (*cis*-9-*cis*-12-octadecadienoic acid), linolenic acid (9,12,15-octadecatrienoic acid), palmitoleic acid (*cis*-9-hexadecenoic acid), A23187, gramicidin D, and PGE_2 were obtained from Sigma, St. Louis, Mo. A23187 and gramicidin were added to the cell suspension from stock solutions in ethanol. LTB_4, 5(S), 12(R)-dihydroxy-6,8,10,14-eicosatetraenoic acid; LTC_4, 5(S)-hydroxy, 6(R)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LTD_4 5(S)-hydroxy, 6(R)-S-cysteinyl glycinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, and LTE_4; 5(S)-hydroxy-6(R)-S-cysteinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid 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 Coulter channellyzer (C-1000) and recorder (HR 2000). 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 75,000 to 85,000 cells per ml (equivalent to a cytocrit about 0.008%). 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 Ion Content

The cellular content of Na, K and Cl were measured in cells from 1 ml cell suspension (cytocrit 7%), separated from the incubation medium by centrifugation (1 min, 20,000 × *g*), lysed in distilled water and deproteinized with perchloric acid as previously described in detail (Hoffmann et al., 1979). Na and K were measured by atomic absorption flamephotometry (Perkin Elmer atomic absorption spectrophotometer, model 2380). Cl content was obtained by coulometric titration (CMT chloride titrator, Radiometer, Denmark). All values were corrected for trapped incubation medium using ^{3}H-inulin (Amersham) as marker (Hoffmann et al., 1979).

All curves are, when not otherwise stated, representative of at least three equivalent experiments. Mean values given in text are accompanied by SEM with the number of independent experiments in brackets.

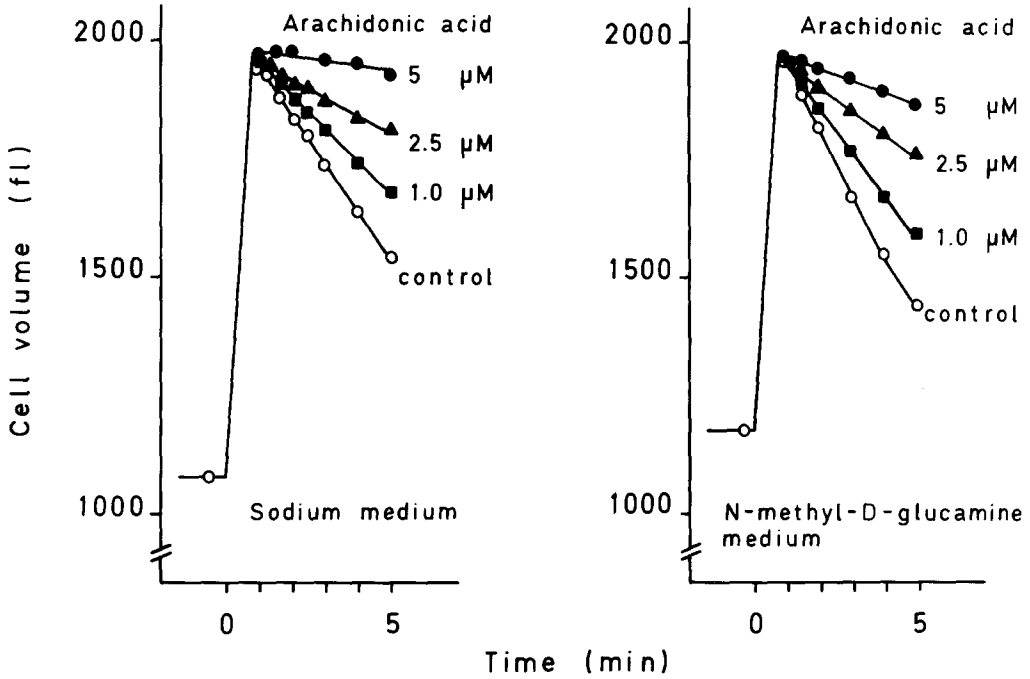

Fig. 1. Inhibition by exogenous arachidonic acid of regulatory volume decrease following hypotonic swelling in Na-containing (left panel) and in Na-free (right panel) media. Ehrlich ascites cells, preincubated in isotonic (300 mOsm) chloride medium for 35 min or more, were diluted 500-fold with hypotonic (150 mOsm) NaCl medium (left panel) or with N-methyl-D-glucamine medium (right panel) to a final cell density of 75,000 to 85,000 cells per ml. Cell volume was followed with time using a Coulter counter. In the experimental groups (closed symbols) arachidonic acid was added in the indicated concentrations, within seconds following hypotonic challenge. The curves are representative of four independent sets of experiments

Results

Arachidonic Acid Inhibits Regulatory Volume Decrease (RVD) Following Osmotic Swelling

Ehrlich ascites cells swell initially as nearly perfect osmometers upon transfer to hypotonic media, reaching a maximal degree of swelling within 0.9 min after change in osmolarity. Subsequently the cells regulate their volume (regulatory volume decrease, RVD) mainly by losing KCl via separate conductive pathways for K and Cl (Hoffmann et al., 1986). Figure 1 (left panel) shows a typical volume response in hypotonic NaCl medium and its inhibition by exogenous added arachidonic acid. The inhibitory effect of arachidonic acid on RVD is dose dependent, with 1 μM arachidonic acid giving 50% inhibition (Fig. 2, closed circles) and 5 μM arachidonic acid giving 93 ± 4% inhibition of the initial rate of volume recovery (n = 7).

Arachidonic acid also inhibits RVD in hypotonic, Na-free media. This is seen in Fig. 1 (right panel) where sodium has been substituted by the apparently inert cation N-methyl-D-glucammonium (Blackstock, Ellory & Stewart, 1985) and in Fig. 9 (left panel) where choline has been substituted for sodium. The inhibition in nominally sodium-free media, however, seems not to be as strong as the inhibition seen in hypotonic NaCl medium. 50% inhibition of the initial rate of volume recovery is obtained at 2 μM arachidonic acid in hypotonic choline and N-methyl-D-glucamine media (Fig. 2), and 5 μM arachidonic acid results only in 70% (n = 2) and 82 ± 4% (n = 7) inhibition of RVD in hypotonic N-methyl-D-glucamine and choline media, respectively. The difference in the inhibitory potency of arachidonic acid in Na-containing and in Na-free media could reflect a cationic effect on the solubility of the fatty acid and its critical micellar concentration (CMC).

The cellular Na content is increased in cells after swelling in hypotonic NaCl medium containing arachidonic acid. This is seen from Table 1, which shows changes in cell volume and cellular ion content, 10 min after transfer to hypotonic media containing arachidonic acid in the range from 0 to 0.4 mM. The net gain in Na is seen to increase with the concentration of arachidonic acid. The concentration of arachidonic acid used for the assessment of net electrolyte movement (0.1 to 0.4 mM) was significantly higher than the concentration used in the functional studies of volume regulation in Figs. 1

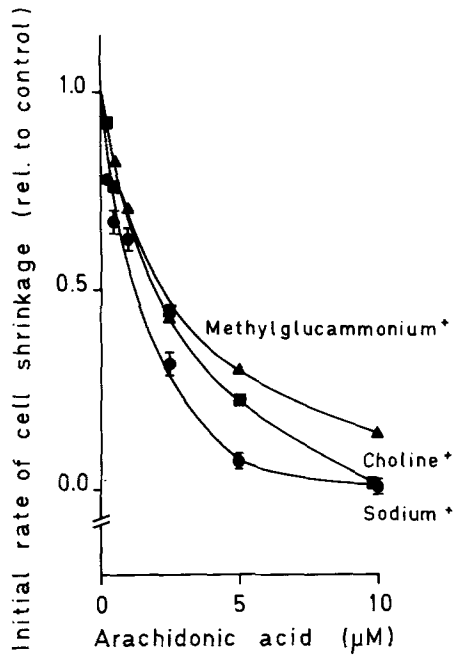

Fig. 2. Saturation curves for the inhibition by exogenous arachidonic acid of volume regulation in Ehrlich ascites cells after swelling in hypotonic media. Cells were preincubated in isotonic NaCl medium for 35 min or more before 500-fold dilution in hypotonic NaCl, cholineCl or N-methyl-D-glucamineCl media. The final cell density was 75,000 to 85,000 cells/ml. Arachidonic acid was added within the first seconds following reduction in osmolarity. The initial rate of volume recovery (fl/min) after swelling in hypotonic medium was read from curves similar to those in Fig. 1, using lines fitted to four to six absolute volume values taken between 1 and 4 min after change in osmolarity. The ordinate shows the initial rate of volume recovery at varying arachidonic acid concentration, given relative to controls with no arachidonic acid. In the case of NaCl (●), each point is the mean ± SEM of four independent experiments. In the case of cholineCl (■) and N-methyl-D-glucamineCl (▲) each point are mean of two independent experiments

and 2 (0 to 10 μM), because the cell density in ion experiments (7%) was about 800 times higher than in the Coulter counter experiments (0.008%). The Ehrlich cells are known to swell as perfect osmometers after transfer to hypotonic medium, with the perfect osmometer value, under the present conditions, estimated at 1.81 times the original cell volume (Hoffmann et al., 1984). Since the relative cell volume, taken after 10 min in 0.4 mM arachidonic acid, is equivalent to the value for the perfect osmometer (*see* Table 1) it is concluded that 0.4 mM arachidonic acid is needed to obtain complete inhibition when the cell density is about 7%.

The net loss of K, following hypotonic swelling, is not reduced in the presence of arachidonic acid while the concommitant net loss of Cl is significantly reduced to about 56% in the presence of 0.4 mM of arachidonic acid (Table 1; $P < 0.01$ in a students' *t*-test). On the basis of this, some of the inhibitory effect of arachidonic acid on RVD in hypotonic, Na-containing media (*see* Fig. 1) could be explained by an increased inflow of Na which balances the outflow of K and thereby reduces the net loss of cations and cell water.

Addition of arachidonic acid to cells suspended in isotonic NaCl medium also leads to an increase in cellular content of Na and a concommitant loss of K (Table 1). These changes in ion content in isotonic media, although smaller than the changes in ion content seen in hypotonic NaCl medium, reveal that arachidonic acid induces Na uptake and K loss regardless of the initial cell volume.

The Na uptake seen after swelling in hypotonic NaCl medium containing arachidonic acid is not reduced by the presence of the NaCl cotransport inhibitor bumetanide (*data not shown*), arguing against entrance of Na via an electroneutral NaCl cotransport system. Neither is the increase in Na a result of inhibition of the Na/K pump, since inhibition of the pump by 10 mM ouabain results only in an increase of cellular Na of 2.7 μmol/g dry wt · min. In 10 min, pump inhibition would lead to an increase of cellular Na content of about 27 μmol dry wt. This is equivalent to about 15 and 20% of the sodium uptake seen in hypotonic and isotonic NaCl media, respectively, after addition of 0.4 mM arachidonic acid (Table 1). Since tryphan exclusion in cells treated for 10 min with 0.4 mM arachidonic acid (cell density 7%) revealed that less than 5% of the cells were stained, it is concluded that the reported changes in ion content (Table 1) are not a result of loss of cell viability. The Na uptake seen after addition of arachidonic acid, therefore, appears to be the result of either an increased conductance for Na or an increased Na uptake via an electroneutral Na/H exchange system.

Swelling in hypotonic, Na-free choline medium also results in a net loss of K and Cl. This is seen in Table 1, which demonstrates changes in cell content of K and Cl in Ehrlich cells 10 min after wash from isotonic NaCl medium to hypotonic choline medium. The net loss of K and Cl, which is somewhat larger than the net loss seen after wash to hypotonic NaCl medium (Table 1), is significantly reduced to about 60% ($P < 0.005$ in a paired students' *t*-test) in the presence of 0.4 mM arachidonic acid. This indicates that the inhibitory effect of arachidonic acid on RVD seen in Fig. 1 is not entirely due to an increased influx of sodium, but that some additional effects are involved. Provided that the inhibition of RVD, in media where the apparent impermeant cations choline and N-methyl-D-glucammonium have been substituted for Na, is not due to an increased

Table 1. Net movements of water, K, Na and Cl across the membrane of Ehrlich ascites cells following addition of arachidonic acid[a]

Medium	Arachidonic acid (mM)	Volume (relative values)	Net movement		
			K	Na	Cl
			(μequiv/g dry wt per 10 min)		
Hypotonic NaCl					
	0.0 (5)	1.32 ± 0.03	−155 ± 11	14 ± 5	−75 ± 9
	0.1 (2)	1.32	−163	33	−90
	0.2 (3)	1.67 ± 0.09	−150 ± 17	65 ± 14	−87 ± 6
	0.4 (5)	1.82 ± 0.08	−185 ± 25	177 ± 17	−42 ± 6
Isotonic NaCl					
	0.4 (3)	0.96 ± 0.01	−142 ± 20	85 ± 13	−49 ± 14
Hypotonic CholineCl					
	0.0 (3)	1.21 ± 0.02	−208 ± 17	—	−93 ± 8
	0.4 (4)	1.67 ± 0.02	−125 ± 8	—	−51 ± 2

[a] Cells, incubated in isotonic NaCl medium, were washed to either hypotonic NaCl medium (150 mOsm), hypotonic cholineCl medium (150 mOsm), or isotonic NaCl medium (300 mOsm), giving a final cytocrit of 7%. Arachidonic acid in the concentration range 0 to 0.4 mM was added within a few seconds after change in medium. Samples for determination of electrolytes were taken 10 min after change in osmolarity. Net movements of ions were calculated on the basis of ion content of untreated cells in isotonic NaCl medium. Na was not assessed in Na-free media. Values are given as mean ± SEM with the number of independent experiments in brackets. Cell volumes are given relative to volumes of untreated control cells in isotonic medium.

inflow of cations, then a reduced RVD could be explained by an inhibition of the volume-activated K and/or Cl channels.

Effect of Arachidonic Acid on the Conductive Pathways Which are Activated by Hypotonic Swelling

In the swollen cells the Cl permeability exceeds that for K, so that the rate-limiting step in RVD is the K permeability (Hoffmann et al., 1986). The effect of addition of valinomycin or gramicidin to osmotic swollen cells is, therefore, an acceleration of RVD (Hoffmann et al., 1984, 1986). After addition of sufficient gramicidin the cation permeability exceeds that for Cl, so that the Cl permeability now becomes the rate-limiting step in RVD. It is thus assumed that any drug inhibiting volume changes, after initial osmotic swelling in the presence of gramicidin, acts by blocking the anion pathway. On the other hand, any drug that inhibits volume changes in the absence but not in the presence of gramicidin can be classified as a specific inhibitor of the volume-induced K pathway.

The rate of RVD seen after addition of gramicidin to cells swollen in hypotonic choline medium is reduced after addition of arachidonic acid. This is seen from Fig. 3, where 0.5 μM gramicidin was added to cells at the point of maximal swelling after transfer to hypotonic choline medium. The inhibition is more pronounced at 10 μM arachidonic acid than at 5 μM (Fig. 3). Since arachidonic acid inhibits RVD in the absence (Figs. 1 & 2) and in the presence of gramicidin (Fig. 3) it seems reasonable to conclude that arachidonic acid inhibits the Cl conductance in Ehrlich cells, activated by swelling in hypotonic media. From the fact that RVD after addition of 5 μM arachidonic acid is faster in the presence of gramicidin (Fig. 3) than in its absence (Figs. 2 & 9) it is concluded that arachidonic acid inhibits the volume-induced K conductance as well as the volume-induced Cl conductance.

Arachidonic Acid Inhibits A23187 + Ca-Induced Cell Shrinkage

We have previously shown that addition of the Ca ionophore A23187 to osmotic swollen Ehrlich cells accelerates the regulatory volume decrease (Hoffmann et al., 1984; Lambert, Simonsen & Hoffmann, 1984), and that addition of the ionophore to cells in steady state in isotonic media results in cell shrink-

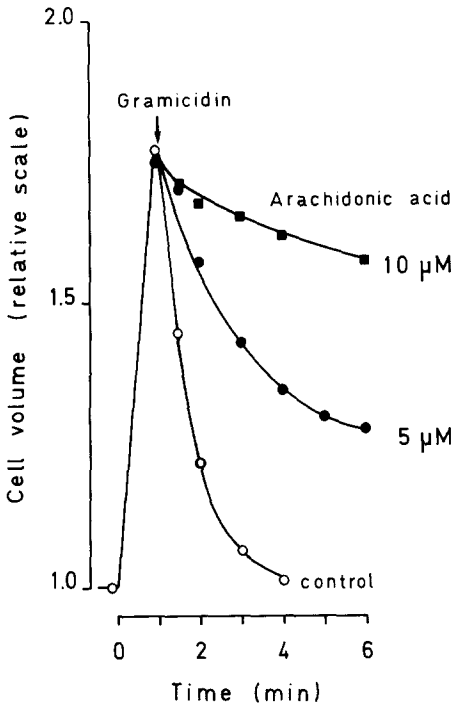

Fig. 3. Inhibition by arachidonic acid of net Cl permeability induced by cell swelling. Gramicidin was added in order to impose a high cation permeability. Ehrlich cells were preincubated in standard incubation medium and diluted 500 times at time zero to a final cell density of 80,000 cells per ml with hypotonic cholineCl medium. Cell volume was followed with time using a Coulter counter. 0.5 μM gramicidin was added 0.95 sec after change in osmolarity, at which time the cells had reached their maximal degree of swelling. 5 μM (●) or 10 μM (■) arachidonic acid was added to the cell suspension within seconds following dilution of the medium. Control cells (○) contained no arachidonic acid. Cell volumes are given relative to initial cell volumes, measured by dilution of a parallel sample of the cell suspension in isotonic incubation medium

age (Hoffmann et al., 1984; Lambert, 1984*b*). In both cases the effect of A23187 was explained by activation of Ca-sensitive K and Cl pathways, leading to a net loss of K, Cl and osmotic obliged cell water (Hoffmann et al., 1986). The effect of A23187 on cell volume is confirmed in Fig. 4, which shows RVD in hypotonic choline medium after addition of A23187 at the point of maximal cell swelling (left panel), and cell shrinkage following addition of A23187 to cells suspended in isotonic choline medium (right panel). Figure 4 also demonstrates that exogenous arachidonic acid inhibits the cells shrinkage induced by the Ca ionophore A23187 and that this is the case whether the ionophore is added to osmotic swollen cells (left panel) or to cells in isotonic media (right panel).

Effect of Arachidonic Acid on the Conductive Pathways Which are Activated by Addition of A23187 Plus Ca

Rate of cell shrinkage seen after addition of A23187 to cells in isotonic choline medium treated with gramicidin is unaffected by arachidonic acid. This is seen from Fig. 5, which shows A23187 + Ca-induced cell shrinkage in isotonic choline medium containing gramicidin in the absence (open symbols) and in the presence (filled symbols) of 10 μM arachidonic acid. Gramicidin was added to ensure a high cation permeability. The conductive Cl permeability of the cell membrane in steady-state Ehrlich cells is low (Hoffmann et al., 1979, 1984) and consequently the cell volume changes only slowly when a high K permeability is imposed by addition of gramicidin (Fig. 5). This is also the case when a high K permeability is provided by addition of valinomycin (Hoffmann et al., 1984). Since arachidonic acid inhibits A23187 + Ca-induced cell shrinkage in the absence (Fig. 4, right panel) but not in the presence of gramicidin (Fig. 5, filled symbols) it seems reasonable to conclude that arachidonic acid inhibits the K conductance, activated by the Ca ionophore A23187. On the other hand, since arachidonic acid does not inhibit the A23187 + Ca-induced cell shrinkage in the presence of gramicidin it is concluded that the fatty acid has no effect on the Ca-activated Cl pathway under isotonic conditions. This is in contrast to the inhibitory effect of arachidonic acid on the volume-induced Cl transport pathway.

Effect of Arachidonic Acid Metabolites on RVD Following Hypotonic Swelling

To see whether the effect of arachidonic acid was caused by some of its metabolites, experiments like the ones shown in Figs. 6–8 were designed. In such experiments the effect of PGE_2, LTB_4, LTC_4, LTD_4 and LTE_4 was examined.

Figure 6 shows that PGE_2, a cyclooxygenase product of arachidonic acid, inhibits RVD in hypotonic NaCl medium (left panel) but not in hypotonic N-methyl-D-glucamineCl medium (right panel). The initial rate of RVD in hypotonic NaCl medium containing 5 μM PGE_2 was reduced to 65 ± 1% (n = 3) of the rate found in controls with no prostaglandin. In introductory experiments (*not shown*) 5 μM PGE_2 was found to give maximal inhibition of RVD. PGE_2 is previously shown to stimulate the transepithelial Na-transport in the frog skin by an increase in the Na permeability of the apical membrane (Nielsen & Bjerregaard, 1984). The inhibitory

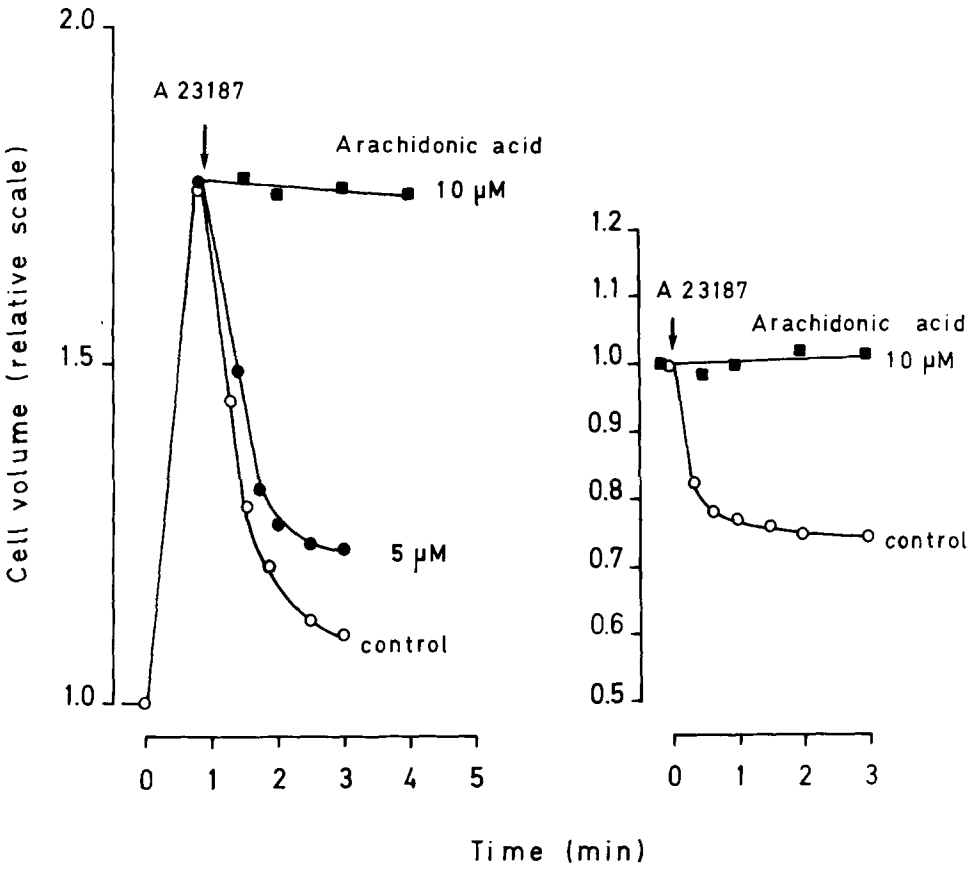

Fig. 4. Inhibition by arachidonic acid of cell shrinkage induced by addition of A23187 to osmotic swollen cells (left panel) or to cells suspended in isotonic medium (right panel). Ehrlich cells were preincubated in standard incubation medium and diluted 500-fold at time zero to a final cell density of 75,000 to 85,000 cells per ml with either hypotonic (150 mOsm) cholineCl medium containing 0.15 mM Ca plus 0.15 mM Mg (left panel) or isotonic (300 mOsm) cholineCl medium containing 0.15 mM Ca plus 0.15 mM Mg (right panel). The cell volume was followed with time using a Coulter counter. 2 μM A23187 were added as indicated by the arrows. Arachidonic acid (5 μM, ●) or (10 μM, ■) was added within the first seconds following dilution of the cell suspension. Control cells (○) contained no arachidonic acid. The cell volumes are given relative to the initial cell volume

effect of PGE_2 on volume regulation in hypotonic NaCl medium might, therefore, be explained by an increased uptake of Na. Taking into account that addition of exogenous arachidonic acid stimulates the synthesis of PGE_2 in Ehrlich ascites cells (Lambert, Hoffmann & Christensen, 1987) it may be suggested, that the stimulating effect of arachidonic acid on sodium uptake (*see* Table 1) could be via an increased synthesis of prostaglandins such as PGE_2.

In order to see whether PGE_2 could affect the Cl permeability in Ehrlich cells, PGE_2 was added to gramicidin-treated cells in isotonic, Na-free choline medium. Addition of gramicidin results in cell shrinkage (*see* Fig. 5) due to a net loss of cations and Cl, the rate of which is limited by the Cl permeability. In three sets of Coulter counter experiments it was found that cells shrank 29 ± 4 fl/min in the presence of gramicidin, and that this rate of water loss was reduced to 26 ± 4 fl/min following addition of 5 μM PGE_2. This reduction was not significant ($P \gg 0.1$, students' *t*-test), indicating that PGE_2 does not affect cell volume, i.e., the Cl permeability in isotonic cells. PGE_2 was also without effect on the rate of RVD in hypotonic, Na-free choline medium when a high K permeability had been ensured by addition of the cationophore gramicidin (*not shown*), indicating that volume-induced Cl transport pathway is not affected by the presence of PGE_2. It is, therefore, suggested that PGE_2 has no significant effect on the Cl permeability in Ehrlich ascites cells. Since the K permeability is rate-limiting for RVD and PGE_2 does not inhibit RVD in Na-free media (Fig. 6, right panel) an effect of PGE_2 on the K permeability is ruled out as well. Thus the effect of arachidonic acid on Na permeability can be caused by the metabolite PGE_2, whereas the effect on K and Cl channels is not effected via PGE_2.

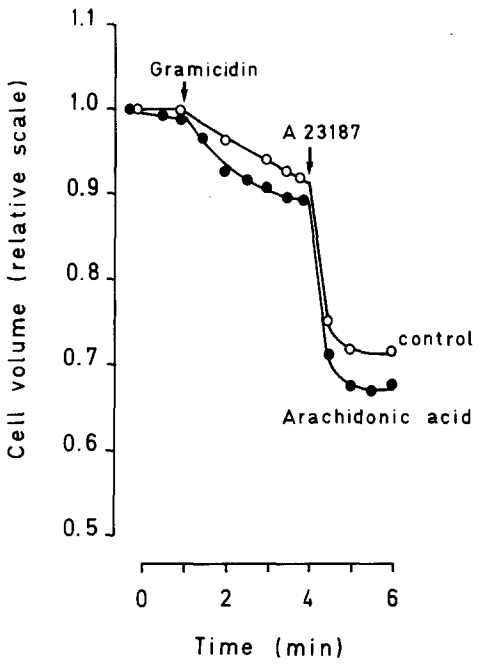

Fig. 5. Effect of arachidonic acid on the net Cl permeability induced by A23187 plus Ca. A high K permeability was imposed by addition of gramicidin. The cell volume was followed after transfer of Ehrlich cells, preincubated in standard NaCl medium, to isotonic cholineCl medium containing 0.15 mM Ca plus 0.15 mM Mg. 10 μM arachidonic acid (●) was added within seconds after dilution. 0.5 μM gramicidin and 2 μM A23187 were added as indicated by arrows. Control cells (○) contained no arachidonic acid. Cell volumes were followed with time and given relative to the initial cell values

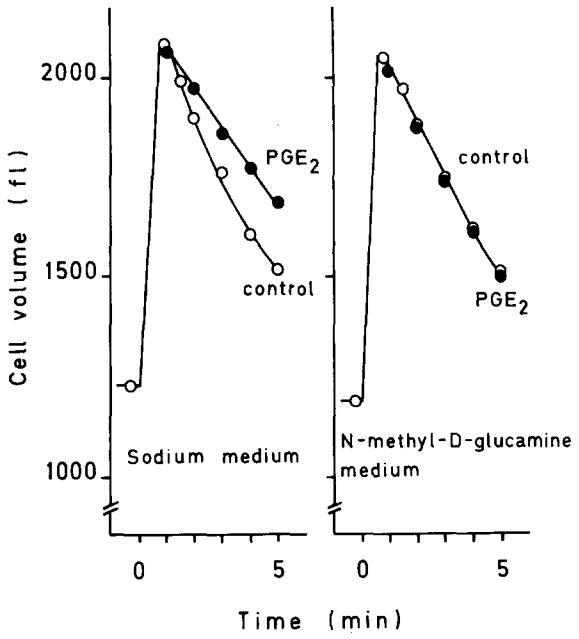

Fig. 6. Effect of PGE_2 on regulatory volume decrease in Ehrlich cells in NaCl medium (left panel) and in Na-free, N-methyl-D-glucamineCl medium (right panel). Cells were preincubated in standard medium for 40 min or more and at time zero diluted 500-fold (final cell density about 75,000 cells/ml) with either hypotonic NaCl medium (left panel) or hypotonic N-methyl-D-glucamineCl medium (right panel). 5 μM PGE_2 was added (●) within seconds after change in osmolarity. Control cells (○) were not treated with PGE_2. Cell volumes are given relative to cell volumes in isotonic NaCl medium measured in parallel

The influence of the lipoxygenase products of arachidonic acid on cell shrinkage after hypotonic swelling was tested by addition of LTB_4, LTC_4, LTD_4 or LTE_4. It was found that addition of 60 to 300 nM LTB_4, LTC_4 or LTE_4 (*data not shown*) maximally reduced the initial rate of volume recovery in hypotonic NaCl medium to about 92, 69, and 80%, respectively, of the rats found in the absence of leukotrienes. None of these changes were significant at a 1% level (students' *t*-test, where the relative values of RVD were tested against the hypothetical value 1). On the basis of these data it is proposed that only an insignificant part of the inhibitory effect of arachidonic acid on RVD in Na-containing media could be by means of an increased synthesis of LTB_4, LTC_4 or LTE_4.

In contrast to the other leukotrienes, LTD_4 accelerates RVD in hypotonic NaCl medium dramatically. This is seen from Fig. 7, where LTD_4 in the range from 20 to 60 nM was added near the point of maximal swelling, i.e. 0.95 min after change in osmolarity. Addition of LTD_4 at the time of exposure to the hypotonic medium (*not shown*) reduced the initial swelling considerably. Taking into account, that K movement is rate-limiting for the KCl and water loss during the volume regulation after hypoosmotic swelling (Hoffmann et al., 1986), it seems reasonable to propose that LTD_4 increases K conductance in Ehrlich cells. In four experiments it was found that 60 nM LTD_4 increased the initial rate of volume recovery, i.e., water loss (fl/min) in the first minute following addition of LTD_4, 5.9 ± 0.15 times in hypotonic NaCl media. The corresponding initial rates of volume recovery of untreated cells were 80 ± 7 fl/min.

Addition of LTD_4 to suspensions of Ehrlich cells in isotonic NaCl medium results in cell shrinkage. This is seen in Fig. 8 (left panel), which shows changes in cell volume with time after addition of 60 nM LTD_4. The cell shrinkage induced by LTD_4 is transient and the cells regain their volume within about 5 min (*see* Fig. 8, left panel). The cell volume at the point of maximal shrinkage, which is attained about 0.9 min after addition of LTD_4 was 92 ± 1% (n = 3) of the initial cell volume. Addition of 60 nM LTD_4 to cells suspended in isotonic choline medium

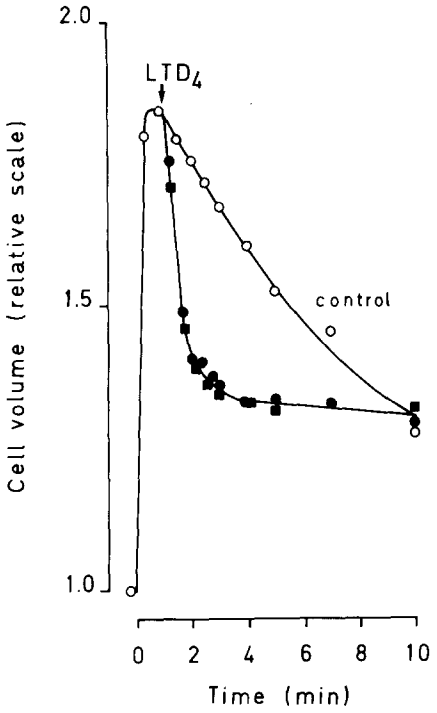

Fig. 7. LTD_4-induced cell shrinkage in Ehrlich ascites cells after swelling in hypotonic NaCl medium. Cells preincubated for about 35 min in standard incubation medium were diluted 500-fold in hypotonic NaCl medium and cell volume was followed with time using a Coulter counter. 20 nM (■) or 60 nM (●) LTD_4 was added to the hypotonic media 0.95 min after change in osmolarity. Control cells (○) contained no leukotriene. Cell volumes are given relative to cell volume of untreated cells in isotonic incubation medium

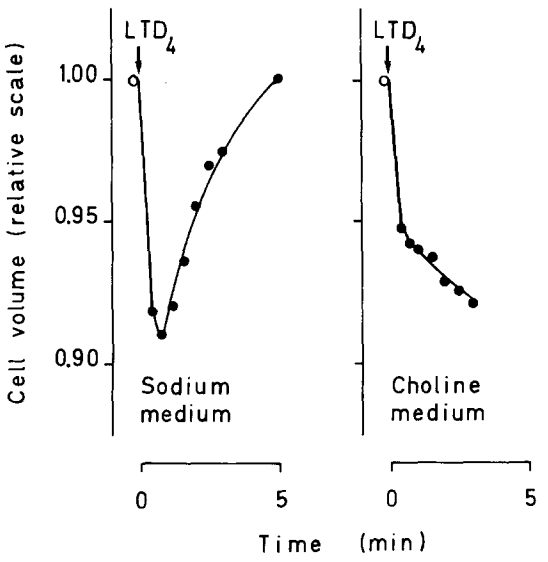

Fig. 8. LTD_4-induced cell shrinkage in Ehrlich ascites cells under steady-state conditions in isotonic media. Cells were preincubated for 35 min or more in isotonic standard NaCl medium. The cells were diluted 500-fold in isotonic NaCl medium (left panel) or isotonic cholineCl medium (right panel). 60 nM LTD_4 (●) was added at time zero and cell volume was measured with time. The cell volumes are given relative to cell volumes measured immediately before addition of LTD_4

(Fig. 8, right panel) also results in cell shrinkage of similar magnitude.

The observed cell shrinkage in Fig. 8 results from a net loss of K and Cl from the cells followed by water. This is seen from Table 2 which shows that a definite net loss of KCl and water has taken place from cells in isotonic NaCl medium after treatment with LTD_4. Samples were taken 0.9 min after addition of LTD_4. Of a total K loss, which is about 15% of the original cellular K content, 23% is balanced by Na uptake and 44% by Cl loss (Table 2). The conductive Cl permeability of the Ehrlich cells is normally low (Hoffmann et al., 1979), and cell shrinkage, resulting from increased K permeability, can only occur if the Cl permeability is simultaneously increased. It is, therefore, concluded that LTD_4 also increases the Cl permeability in Ehrlich cells. The fact that LTD_4 increases the volume-activated K and Cl permeabilities while arachidonic acid reduces them (Figs. 1–3) excludes that the effect of arachidonic acid on the volume-activated K and Cl transport pathways is via an increased LTD_4 synthesis.

Table 2. Net movement of water, K, Na and Cl across the cell membrane of Ehrlich ascites cells following addition of LTD_4[a]

	Cell water (ml/g dry wt)	K	Na	Cl
		(μmol/g dry wt)		
Control	3.96 ± 0.02	707 ± 10	33 ± 5	260 ± 6
LTD_4: 4 μM	3.59 ± 0.04	598 ± 8	55 ± 2	215 ± 4
Net movement	0.37 ± 0.04	−105 ± 6	24 ± 4	−46 ± 5

[a] Ehrlich cells were incubated in isotonic standard NaCl medium for 40 min. At zero time 4 μM LTD_4 were added. The concentration of LTD_4 used for the assessment of net electrolyte movement (4 μM) was higher than the concentration in volume regulation experiments (20 to 60 nM, *see* Figs. 7 & 8), because the cell density in ion determinations (7%) was about 800 times higher than in the Coulter counter experiments (0.008%). Cell water and electrolytes were measured before (control) and 0.9 min after addition of LTD_4. Control and experimental values are given as mean ± SEM of three independent experiments. Net movement values are from paired experiments.

It may be noted that the transient, LTD_4-induced cell shrinkage in NaCl medium (Fig. 8, left panel) points to the activation of a previously described Na,Cl-cotransport system which leads to restoration of the cell volume (Hoffmann et al., 1984, 1986; Lambert, 1984*b*). The persistent cell shrinkage in cholineCl medium (Fig. 8, right panel) favors this hypothesis.

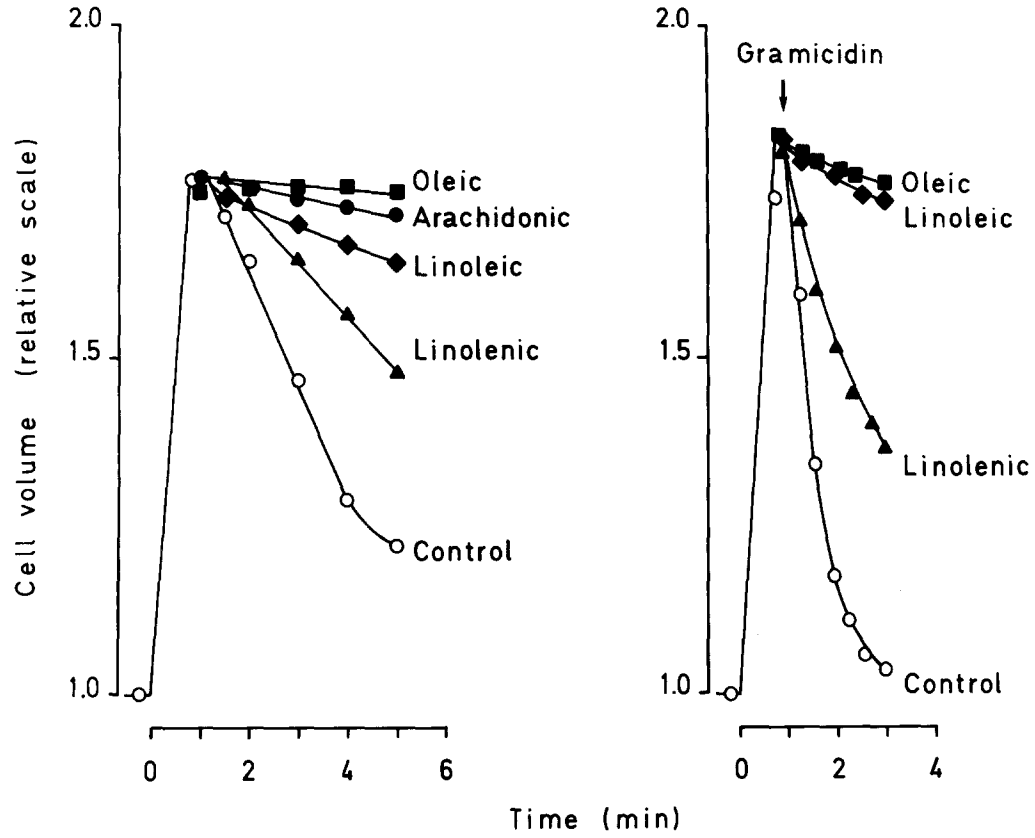

Fig. 9. Effect of fatty acids on regulatory volume decrease (left panel) and on the net Cl permeability activated by cell swelling (right panel). Cells were incubated in isotonic NaCl standard medium and at time zero transferred to hypotonic cholineCl medium. Cell density was 90,000 cells/ml. 5 μM (left panel) or 10 μM (right panel) of the indicated fatty acid was added within the first seconds following reduction in osmolarity. 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. Control cells (open symbols) contained no arachidonic acid. The curves are representative of at least three independent experiments (included in Table 3)

Effect of Unsaturated Fatty Acids on RVD

Since the ability of arachidonic acid to inhibit K and Cl channels is not related to either leukotrienes or prostaglandins it was tested whether the effect of arachidonic acid on RVD could be mimicked by other fatty acids, some of which were not precursors of arachidonic acid. From Fig. 9 (left panel) and Table 3 it is seen that 5 μM of the unsaturated arachidonic, linoleic, linolenic, oleic and palmitoleic acid inhibit significantly the RVD following swelling in hypotonic choline medium, while the saturated arachidic and stearic acid have no effect. This indicates that unsaturation could be a key factor related to the inhibitory potency. From Fig. 9 (right panel) and Table 3 it is furthermore seen that oleic, linoleic, linolenic and palmitoleic just like arachidonic acid (Fig. 3) inhibit RVD significantly in the presence of gramicidin, reflecting an inhibition of the Cl permeability in the hypotonically swollen cells. In contrast to the inhibitory effect of oleic acid it is noted that its *trans* isomer elaidic acid has no significant effect on RVD (Table 3). Increasing pH to 8.2, which increases the solubility of the fatty acid gave similar results. This could reveal that the *cis* double bond in oleic acid, linoleic, and linolenic acid is needed for inhibition of RVD, i.e., for inhibition of the Cl permeability, activated by hypotonic swelling.

From the presented data it is concluded that arachidonic acid and other unsaturated fatty acids inhibit RVD in hypotonically swollen Ehrlich cells. The effect of arachidonic acid is seen in the presence and in the absence of Na in the medium and it is independent of the initial cell volume. A part of the inhibition by arachidonic acid in Na-containing media could be due to an increased production of PGE_2 and probably other prostaglandins. The lipoxygenase products of arachidonic acid seem not to play any role in the inhibition by arachidonic acid, since both LTB_4, LTC_4 and LTE_4 had only weak inhibitory effect on RVD and since LTD_4 and

Table 3. Effect of fatty acids on the initial rate of volume recovery following hypotonic swelling in Na-free media. Gramicidin was added in order to impose a high cation permeability[a]

Fatty acid	Symbol	Initial rate of volume recovery (relative to control)	
		No gramicidin	Plus gramicidin
Arachidonic	20:4 Δ5,8,11, 14	0.18 ± 0.03 (7) ($P < 0.001$)	0.06 ± 0.01 (3) ($P < 0.001$)
Arachidic	20:0	0.97 ± 0.04 (3) NS	not tested
Linolenic	18:3 Δ9,12,15	0.44 ± 0.04 (4) ($P < 0.001$)	0.59 ± 0.09 (3) ($P < 0.025$)
Linoleic	18:2 Δ9,12	0.20 ± 0.03 (4) ($P < 0.001$)	0.13 ± 0.07 (3) ($P < 0.005$)
Oleic	18:1 Δ9, *cis*	0.004 ± 0.001 (3) ($P < 0.001$)	0.12 ± 0.08 (3) ($P < 0.005$)
Elaidic	18:1 Δ9, *trans*	0.98 ± 0.01 (4) NS	not tested
Stearic	18:0	0.96 ± 0.03 (3) NS	not tested
Palmitoleic	16:1 Δ9, *cis*	0.40 ± 0.01 (3) ($P < 0.005$)	0.42 ± 0.04 (3) ($P < 0.005$)

[a] Experimental protocol as in Fig. 9. In experiments without gramicidin the initial rate of RVD (fl/min) was obtained from lines fitted to four to six values taken within 1 and 4 min after shift in osmolarity (*see* Fig. 9, left panel). In experiments with gramicidin the initial rate of RVD was estimated from values taken within the first min after addition of gramicidin (*see* Fig. 9, right panel). The rate is given relative to controls without addition of fatty acids. Values are mean ± SEM with the number (n) of independent sets of experiments in brackets. P is the level of significance in a students' t-test where the relative values are tested against the hypothetical value 1. NS is not significant at a 1% level. Symbol, is a shorthand notation that designates the length of the carbon chain, the number and the position of double bonds.

arachidonic acid had opposing effects. The current hypothesis is that there is a direct effect of arachidonic acid and other unsaturated fatty acids on membrane structure and composition.

Discussion

Polyunsaturated fatty acids are known to have important roles maintaining cell membrane structure and fluidity, which in turn influence membrane functions. A change in viscosity by exogenously added long-chain fatty acids would therefore be expected to affect the cell membrane and the transport processes taking place across it. The present work was carried out in order to examine the effect of various fatty acids, in particular arachidonic acid, on the ion-transporting systems which are responsible for volume regulation in Ehrlich ascites tumor cells.

Arachidonic Acid Inhibits the Increase in K and Cl Permeabilities, Induced by Hypotonic Swelling

We have recently shown that the net loss of KCl observed in Ehrlich ascites cells during regulatory volume decrease (RVD), following hypotonic swelling, involves activation of separate, Ca/calmodulin-activated conductive K and Cl transport pathways (Hoffmann et al., 1984; 1986). The rate of RVD is increased when a parallel K transport pathway is provided by addition of gramicidin, indicating that the K conductance is rate-limiting during RVD (Hoffmann et al., 1986).

Arachidonic acid blocks the loss of cell water induced by cell swelling in hypotonic NaCl medium (Fig. 1). That is also the case when sodium has been substituted by the apparent inert cations N-methyl-D-glucammonium (Fig. 1, right panel, and Fig. 2) or choline (Fig. 9, left panel, and Fig. 2). Ehrlich cells treated with arachidonic acid do not volume regulate in hypotonic Na-containing media because they take up Na which counterbalances the net loss of cations (Table 1). On the other hand, ion data revealed that the impaired ability to volume regulate also in hypotonic, Na-free choline medium was caused by a reduction in net loss of both K and Cl (Table 1).

Figure 3 shows that arachidonic acid blocks RVD in Na-free media also when a high K permeability is ensured by the presence of gramicidin, demonstrating that the Cl transport pathway, activated by osmotic swelling, is inhibited by arachidonic acid. A reduced effect of gramicidin in the presence of arachidonic acid is ruled out since no reduction of the potency of gramicidin is seen in isotonic media containing arachidonic acid (*see* Fig. 5). It, therefore, seems reasonable to conclude that arachidonic acid reduces or inhibits the increase in the Cl permeability, following hypotonic swelling. Since the RVD in hypotonic choline medium after addition of 5 μM arachidonic acid is faster in the presence of gramicidin (Fig. 3) than in its absence (Fig. 9, left panel) it is concluded that arachidonic acid also inhibits the volume-induced K permeability.

If arachidonic acid inhibits the increase in Cl conductance during RVD more than the increase in K conductance then the swelling-induced increase in K conductance would result in hyperpolarization of the cell membrane potential. In NaCl media this is equivalent to an increased driving force for sodium uptake. This could explain the increase in cel-

lular content of sodium in Ehrlich ascites cells after swelling in hypotonic NaCl medium containing arachidonic acid (Table 1). In media where sodium has been substituted by an impermeant cation, a reduction in Cl permeability leads to reduction in net loss of Cl, K and cell water, i.e., an inhibition of cell shrinkage (Table 1). Thus all the effects of arachidonic acid during RVD could be explained by an inhibition of the volume-activated K and Cl conductance, although a simultaneous increase the Na permeability could not be ruled out.

Addition of arachidonic acid to Ehrlich cells in isotonic standard medium leads to net loss of K and net uptake of Na (Table 1). This could either mean that arachidonic acid in steady-state Ehrlich cells increases the passive permeability to K (and Na) or that it inhibits the Na,K pump. As previously shown, increase in the permeability to K, e.g. by addition of the K ionophore valinomycin to Ehrlich cells in isotonic NaCl medium, causes similarly a net loss of K which is balanced by a net uptake of Na (Hoffman et al., 1984). This is due to a low conductive permeability to Cl in Ehrlich ascites cells (Hoffmann et al., 1979). Experiments with the pump inhibitor ouabain revealed that only about 15 to 20% of the Na uptake, induced by arachidonic acid could be accounted for pump inhibition. Provided that 10 mM ouabain gave maximal inhibition it seems reasonable to propose that arachidonic acid also increases the passive permeability to cations in Ehrlich cells. In cardiac sarcolemmal vesicles, arachidonic acid had no significant effect on Na,K-ATPase activity (Philipson & Ward, 1985). On the other hand, 21 μM arachidonic acid gave 50% inhibition of beef brain Na,K-ATPase activity (Swann, 1984).

Arachidonic Acid Inhibits A23187 + Ca-Induced Cell Shrinkage Under Isosmotic and Hypoosmotic Conditions

It is well documented that both osmotic swelling and increase in the cytosolic Ca level, furnished by addition of the Ca ionophore A23187, in Ehrlich ascites cells result in activation of Ca-sensitive, conductive Cl and K channels and in cell shrinkage (Hoffmann et al., 1986). Since the Cl conductance after osmotic swelling significantly exceeds the K conductance, while addition of A23187 under isotonic conditions increases the K conductance more than the Cl conductance (Hoffmann et al., 1986), it might be suggested that modulation of the Ca sensitivity of the Ca-dependent Cl channel could also take place during RVD.

Under hypotonic conditions, addition of A23187 to Ehrlich cells at the point of maximal swelling accelerates volume recovery. This is the case in hypotonic choline medium (*compare* left panel controls in Figs. 4 & 9) and in hypotonic NaCl medium (Hoffmann et al., 1984; Lambert et al., 1984). Arachidonic acid inhibits the A23187 + Ca-induced cell shrinkage under isotonic (Fig. 4, right frame) as well as under hypotonic conditions (Fig. 4, left frame). In contrast, arachidonic acid has no inhibitory effect on the A23187 + Ca-induced cell shrinkage in isotonic choline medium when a high cation permeability has been ensured by addition of gramicidin (Fig. 5). Since arachidonic acid inhibits A23187 + Ca-induced cell shrinkage in choline medium in the absence but not in the presence of gramicidin (*compare* Fig. 4, right frame with Fig. 5) it is concluded that arachidonic acid inhibits the K permeability, activated by A23187. Thus arachidonic acid inhibits the K permeability induced by hypotonic swelling and by increase in free cytosolic Ca.

However, the fact that Ehrlich cells, treated with arachidonic acid and gramicidin, still shrink upon addition of A23187 (Fig. 5) indicates that 10 μM arachidonic acid has no or only weak effect on the A23187 + Ca-induced increase in Cl permeability. This is in contrast to the inhibitory effect of arachidonic acid on the swelling-induced Cl permeability (Fig. 3). A possible explanation for this discrepancy could be that modulation of the Ca sensitivity of the Cl channels takes place after cell swelling and that this modulation is affected by arachidonic acid. Measurements of cellular pH, free cytosolic Ca and membrane potential are in progress to elucidate the possibility of variation in the Ca sensitivity of the conductive channels during RVD and after treatment with A23187.

PGE_2 Inhibits Cell Shrinkage Following Osmotic Swelling

It is known that under resting conditions most animal cells contain little if any free arachidonic acid and that free arachidonic acid is a limiting factor in leukotriene and prostaglandin production (Feinstein & Sha'afi, 1983). Increased cytosolic levels of arachidonic acid, released from internal stores by addition of the synthetic peptide: formyl-methionyl-leucyl-phenylalanine (Bormann et al., 1984; Takenawa, Ishitoya & Nagai, 1986) or by addition of the fatty acid to the external compartment, would be reflected in increased prostaglandin and leukotriene synthesis (Feinstein & Sha'afi, 1983).

Ehrlich cells, which synthesize and release both prostaglandins and leukotrienes to their surround-

ings (Lambert et al., 1986, 1987), increase the production and the release of PGE_2 and $PGF_{2\alpha}$ after addition of arachidonic acid (Lambert et al., 1987). Addition of PGE_2 to osmotic swollen Ehrlich cells reduces the rate of regulatory volume decrease in hypotonic NaCl medium but not in hypotonic, Na-free N-methyl-D-glucamine medium (Fig. 6). Provided that the inhibition by PGE_2 seen in hypotonic NaCl medium represents an increased Na uptake, then the inhibitory effect of arachidonic acid seen in hypotonic NaCl medium could partly be explained by an increased PGE_2 synthesis following addition of arachidonic acid. PGE_2-induced inhibition of RVD due to an inhibition of either the K or the Cl transport pathway, activated after hypotonic swelling seems not to be the case because PGE_2 has no inhibitory effect on RVD in Na-free media (Fig. 6, right frame) and because PGE_2 does not reduce cell shrinkage in gramicidin-treated cells (*see* Results). Nielsen and Bjerregaard (1984) correspondingly found that PGE_2 increases the passive, amiloride-sensitive Na permeability in the apical membrane of the frog skin. Consistent with the hypothesis of a PGE_2-regulated Na permeability, it is emphasized that both PGE_2 synthesis (Lambert et al., 1986, 1987) and the conductive Na permeability are reduced under hypotonic condition (Hoffmann, 1978).

LTD_4 Induces Cell Shrinkage Under Isosmotic and Hypoosmotic Conditions

Leukotrienes, which are lipoxygenase metabolites of arachidonic acid, are presumed to be important mediators in allergic and anaphylactic reactions and in inflammation (Samuelsson, 1982, 1983). It is also well established that the activity of the allergic mediator slow-reacting substance of anaphylaxis (SRS-A) is almost entirely attributable to the leukotrienes C_4, D_4 and E_4 (Stjernschantz, 1984).

LTD_4 accelerates RVD (Fig. 7) in hypotonically swollen Ehrlich cells and produces cell shrinkage in cells suspended in isotonic media (Fig. 8). This is in contrast to the precursor of LTD_4, i.e., LTC_4 and its metabolite, LTE_4 which had weak inhibitory effect on RVD in hypotonic media and no effect on cell volume in isotonic media (*not shown*). Taking into account that K movement is rate limiting for the KCl and water loss during RVD, it is concluded that LTD_4 increases K conductance in osmotic swollen Ehrlich cells. The initial rate of volume recovery in hypotonic NaCl medium was increased about 5.9 times following addition of 60 nM LTD_4. If K movement is still rate limiting for KCl and water loss, following hypotonic swelling, even in the presence of 60 nM LTD_4, this is equivalent to sixfold increase in K conductance. Thus a sixfold increase in K conductance by LTD_4 is a minimum estimate.

The LTD_4-induced cell shrinkage under isotonic conditions is a result of a definite net loss of KCl (Table 2). Since the Cl permeability in Ehrlich cells under standard conditions is low this means that LTD_4 also increases Cl permeability in Ehrlich cells in isotonic media. The nature of the ion transport system(s) activated by LTD_4, is under investigation and will be dealt with in a later paper. Thus the effect of arachidonic acid on RVD seen in Figs. 1 and 2 cannot be via an increased production of LTB_4, LTC_4 or LTE_4 since they all have insignificant inhibitory effects. An effect of arachidonic acid via an increased synthesis of LTD_4 is also ruled out since they have opposing effects, i.e., the K and Cl permeabilities and RVD are stimulated by LTD_4 (Figs. 7, 8 and Table 2) but inhibited by arachidonic acid (Figs. 1, 2, 3, 9).

On the basis of experiments carried out with leukotrienes and prostaglandins it seems reasonable to propose that, although exogenously added arachidonic acid is metabolized by both cyclooxygenase and lipoxygenase pathways in Ehrlich ascites cells (Lambert et al., 1987), only a minor fraction of the inhibitory effect of arachidonic acid on RVD in hypotonic, Na-containing media can be by means of an increased synthesis of prostaglandins and leukotrienes. The increase in Na permeability seen after addition of arachidonic acid is probably an effect of an increased synthesis of prostaglandins, whereas the decrease in K and Cl permeabilities seems to be a direct effect of arachidonic acid itself.

Fatty Acid Inhibits RVD Following Osmotic Swelling

Mammals contain four distinct families of polyenoic acids which are named from their precursor fatty acids, i.e., palmitoleic, oleic, linoleic and linolenic acids. The linoleic acid family ((n-6)-fatty acids) acts as precursor for monoenoic prostaglandins, arachidonic acid and dieonic prostaglandins while the linolenic acid family ((n-3)-fatty acids) acts as precursor for trienoic acids. Plants synthesize linoleic acid and linolenic acid from oleic acid via aerobic desaturation reactions while linoleic and linolenic acid cannot be synthesized by mammals and must be provided from plant sources (essential fatty acids).

Since arachidonic acid seems to have a direct effect on K and Cl permeability, the ability of some other fatty acids to inhibit RVD has been tested. As seen in Fig. 9 and Table 3 arachidonic, oleic, lino-

leic, linolenic and palmitoleic acid reduce the rate of regulatory volume decrease, following cell swelling in hypotonic choline medium in the presence and in the absence of gramicidin. This suggests that inhibition of RVD by fatty acids is due to an inhibition of the Cl transport pathway activated by the hypotonic swelling. Since the Ehrlich cells belong to a mammalian cell line, it is presumed that they are not capable of transforming oleic acid into linoleic acid and arachidonic acid, eliminating an effect of oleic acid via synthesis of arachidonic acid. The lack of inhibitory effect of the saturated acids arachidic, stearic acid and the unsaturated *trans* isomer of oleic acid, elaidic acid suggest that the inhibitory potence is connected to the presence of double bonds with *cis* configuration. It is known that *cis* double bonds create kinks in the fatty acid chains, and that it lowers the transition temperature of the membrane, thereby increasing the fluidity and the disorder within the membrane. An increased disorder within the membrane, following addition of fatty acids, could well explain the inhibition/malfunction of the K and Cl transport pathways activated by cell swelling.

In human neutrophiles it is shown that the ability of long-chain fatty acids to activate protein kinase C to some degree paralleled the number of *cis* double bonds (McPhail, Clayton & Snyderman, 1984). Modulation of protein kinase C activity by diacylglycerol has been demonstrated and an effect of arachidonic acid on volume-regulating transport processes in Ehrlich ascites cells involving the protein kinase C, therefore, becomes puzzling.

It has been found in cardiac sarcolemma vesicles that fatty acids increase passive Ca permeability and stimulate Na/Ca exchange, although the potency depends on both chain length and degree of unsaturation (Philipson & Ward, 1985). Stimulating Na/Ca exchange could protect the Ehrlich cells against Ca overload or could reduce the level of available free Ca in the cytosol for activation of the Ca-sensitive K and Cl channels. In beef brain tissue, fatty acids inhibit Na,K-ATPase activity and the inhibition increased as the number of double bonds increased (Swann, 1984). Na,K-pump inhibition could take place in the Ehrlich cells, although it seems not to be quantitatively sufficient to explain the Na^+ uptake, following arachidonic acid treatment.

From the experiments with fatty acids it is, therefore, proposed that the inhibitory effect of arachidonic acid on volume regulation in Ehrlich cells suspended in Na-free media is due to an inhibition of the volume-induced K and Cl pathways, caused by a nonspecific detergent effect of an unsaturated fatty acid containing *cis* double bonds.

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