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5-Lipoxygenase Metabolites of Arachidonic Acid Regulate Volume Decrease by Mudpuppy Red Blood Cells

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Abstract. We examined whether metabolites of arachidonic acid (AA) regulate K^+ efflux during regulatory volume decrease (RVD) by mudpuppy red blood cells (RBCs). Volume regulation was inhibited by the phospholipase A_2 antagonists mepacrine (10 μM) and ONO-RS-082 (10 μM); the inhibitory effect of ONO-RS-082 was reversed by gramicidin (5 μM). Eicosatetraynoic acid (ETYA, 100 μM), a general antagonist of AA metabolism, also blocked RVD. In addition, volume regulation was inhibited by the lipoxygenase pathway antagonist nordihydroguaiaretic acid (NDGA, 10 μM), the 5 lipoxygenase antagonists AA-861 (5 μM) and curcumin (20 μM), and by the 5-lipoxygenase activating protein inhibitor L-655,298 (5 μM). Inhibition by all four of these agents was reversed with gramicidin. In contrast, the 12- and 15-lipoxygenase pathway inhibitor ethyl-3,4-dihydroxy-benzylidene-cyanoacetate (EDBCA, 1 μM) and the cytochrome P-450 monooxygenase pathway blocker ketoconazole (20 μM) had no effect. On the other hand, the cyclooxygenase pathway inhibitor aspirin (100 μM) slightly enhanced RVD. Consistent with these findings, a K^+-selective whole cell conductance responsible for K^+ efflux during cell swelling was inhibited by ONO-RS-082 (10 μM), NDGA (10 μM), AA-861 (5 μM), curcumin (20 μM), and L-655,298 (5 μM). In contrast, EDBCA (1 μM), ketoconazole (20 μM), and indomethacin (10 μM) did not block this whole cell conductance. These results indicate that a channel mediating K^+ loss during RVD is regulated by a 5-lipoxygenase metabolite of arachidonic acid.

Key words: Leukotriene — phospholipase A_2 — Patch clamp — Potassium channel — *Necturus maculosus*

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Introduction

Exposure of vertebrate cells to a hypotonic solution results in a rapid increase in cell volume followed by a slower, spontaneous regulatory volume decrease (RVD) close to the original size [10, 12, 21, 27]. Because water is in thermodynamic equilibrium across the plasma membrane, the intracellular solute content may be used to regulate cell volume. This is accomplished by selectively increasing permeability of the plasma membrane during cell swelling to allow for efflux of specific intracellular osmolytes, thereby decreasing the driving force for water influx [10, 12, 21, 27]. Most vertebrate cells lose K^+ and Cl^- during RVD [10, 21, 22, 24, 26]. This may occur by electroneutral ion transport pathways [3, 22, 24, 26] or by the separate activation of K^+ and Cl^- channels [9, 10, 11, 21, 28, 29, 32].

The cellular mechanisms that regulate ion channels during RVD are poorly understood. Calcium, however, appears to play a key role in this process for several cell types [12, 21, 27, 31]. Although it has been suggested that Ca^{2+} directly activates ion channels during RVD [11, 28], there also is evidence that several intracellular messengers are activated by Ca^{2+} during cell volume regulation [12, 21, 27]. One such messenger is arachidonic acid, a 20-carbon polyunsaturated fatty acid that is formed primarily from membrane glycerophospholipids by the action of phospholipase A_2, a Ca^{2+}-activated enzyme [13, 18, 23]. Minor amounts of arachidonic acid also may be produced from diacylglycerol and phosphatidic acid [13, 18, 20].

Arachidonic acid is quickly acylated back into the plasma membrane or converted into a number of biologically active oxygenated metabolites, called eicosanoids, by three major enzymatic pathways [13, 18]. The cyclooxygenase pathway catalyzes conversion of arachidonic acid to prostaglandin endoperoxidases, which are precursors to compounds such as thromboxane, prostacyclin,

and other prostaglandins [13, 18]. Lipoxygenases are a group of iron-containing dioxygenases [13, 18]. They exist in three distinct types 5-, 12-, and 15-lipoxygenase, and insert oxygen into arachidonic acid at carbon 5, 12, or 15, respectively [13, 18]. Leukotrienes are formed via the 5-lipoxygenase pathway [8, 13, 18]. For this to occur, after activation of 5-lipoxygenase by a rise in intracellular Ca^{2+}, the enzyme translocates from the cytosol to the plasma membrane where it binds to an integral protein called 5-lipoxygenase activating protein (FLAP) [8, 18]. The membrane association of 5-lipoxygenase requires Ca^{2+} and is a necessary step for efficient production of leukotrienes [18]. The enzyme also is thought to undergo suicide turnover-associated inactivation [18]. Finally, cytochrome P-450 epoxygenase, a monooxygenase, converts to arachidonic acid into a variety of epoxyeicosatrienoic acids by a NADPH-dependent mechanism [13, 18].

Several ion channels are regulated by arachidonic acid and eicosanoids. Arachidonic acid, for example, activates K^+ channels in cardiac cells [15] and in smooth muscle cells [25]. In addition, a 12-lipoxygenase metabolite of arachidonic acid directly stimulates the *Aplysia* S-K^+ channel [2]. In contrast, cytochrome P450 monooxygenase products activate a large conductance Ca^{2+}-activated K^+ channel in small renal arteries of the rat [34] and a K^+ channel in vascular smooth muscle of the pig [14]. On the other hand, leukotrienes, products of 5-lipoxygenase metabolism of arachidonic acid, stimulate a G-protein-gated cardiac K^+ channel [16] and a K^+ channel in Ehrlich ascites tumor cells [17–19]. Further, it has been shown that K^+ efflux during RVD by Ehrlich ascites tumor cells depends on eicosanoids [17–19]. Specifically, cell swelling results in stimulation of leukotriene production and a concomitant reduction in prostaglandin synthesis [18, 19]. In addition, the leukotriene LTD_4 accelerates an RVD response by stimulating a K^+ permeability in hypotonically swollen cells [17–19]. In contrast, the prostaglandin PGE_2 inhibits cell volume regulation [17–19]. This inhibition only occurs when Na^+ is present in the hypotonic medium, indicating that PGE_2 stimulates a Na^+ permeability [17–19].

Recent studies in our laboratory indicate that mudpuppy (*Necturus maculosus*) red blood cells (RBCs) have a remarkable ability to tolerate exposure to hypotonic solutions compared to other vertebrate cells [1]. Further, RVD by this cell type depends on a quinine-inhibitable K^+ conductance that is activated by a Ca^{2+}-calmodulin-dependent mechanism during cell swelling [1]. Whether calmodulin stimulates K^+ efflux directly by binding to the channel protein or indirectly by activating another intracellular messenger is not known. However, it has been shown by others that calmodulin can activate phospholipase A_2 [18, 30], and subsequent production of arachidonic acid and eicosanoids may modulate membrane permeability to ions [2, 14–19, 25, 34]. This study investigated whether arachidonic acid and its metabolites regulate a K^+ efflux during RVD by mudpuppy RBCs. This was accomplished by examining the limit of osmotic fragility from hemolysis experiments, estimating cell volume of osmotically stressed cells from the hematocrit, and studying membrane currents using the whole-cell patch clamp technique.

Materials and Methods

Animals

Mudpuppies (*Necturus maculosus*) were obtained from a local vendor (Lemberger Oshkosh, WI) and kept in well aerated, aged tap water at 5–10°C for no more than 6 days prior to use. They were anesthetized with 3-aminobenzoic acid ethyl ester (MS-222, 1%) and sacrificed by decapitation. Blood was obtained from a midventral incision and collected into heparin (10,000 units/ml) coated tubes. Immediately following esanguination, the blood was spun in a centrifuge (Hermel-Z230, National Labnet, Woodbridge, NJ) at 1,000 rpm for 1 min. The supernatant was aspirated and replaced with an equal volume of amphibian Ringer. This process of spinning and washing the cells was repeated twice.

Osmotic Fragility

The limit of osmotic fragility in hypotonic solutions was determined by finding the lowest osmolality a suspension of RBCs could be bathed in without lysing. Cell lysis (hemolysis) was determined by observing a turbidity shift (cloudy to clear) that occurs when the integrity of the plasma membrane is compromised. This was detected with a spectrophotometer (Spectronic 20D, Milton Roy Co) 10, 15, or 20 min after blood (30 μl) was added to saline solutions (3 ml) of different osmolalities and compositions. Spectrophotometric experiments were conducted at 625 nm; this wavelength provided the greatest difference in optical density (OD) between intact and lysed cells [1]. Cell lysis also was confirmed microscopically (Nikon Diaphot, Hoffman differential interference contrast optics, 400×).

A hemolytic index (%) was determined using the formula: HI(%) = (OD of Test Compound—OD of Negative Control)/(OD of Positive control—OD of Negative Control) × 100. The positive control was blood in distilled water, the negative control referred to blood in diluted Ringer, and the test compound was blood in diluted Ringer containing a specific pharmacological agent. All reported hemolytic indices were calculated using the concentration of Ringer that provided the limit of osmotic fragility for the negative control.

Hematocrit

Hematocrit was used to estimate the volume of RBCs exposed to solutions of different osmolalities. We assumed that cells remained isomorphic and packed equally regardless of their volume. Blood was collected in heparinized capillary tubes (Labcraft Brand, Red-Tip) and spun for 60 sec by a microhematocrit centrifuge (model MB, International Equipment, Needham Hts., MA). Hematocrit was measured by a microcapillary reader (International Equipment, Needham Hts., MA). We also confirmed cell size and shape by observing individual cells with a microscope (Nikon Diaphot, Hoffman DIC optics, 400×).

Patch Clamp

Patch pipettes were fabricated from Kovar sealing glass (Corning model 7052, 1.50 mm OD, 1.10 mm ID; Garner Glass, Claremont, CA) using a two-pull method (Narishige PP-7). Pipette tips were fire polished (Narishige MF-9) to give a direct current resistance of approximately 5–8 MΩ in symmetrical 100 mM KCl solutions. All pipette solutions were filtered immediately before use with a 0.22 μm membrane filter (Millex-GS, Bedford, MA), and the pipettes were held in a polycarbonate holder (E.W. Wright, Guilford, CT). Membrane currents were measured with a 10^{10} Ω feedback resistor in a headstage (CV-201A, Axon Instruments, Foster City, CA) with a variable gain amplifier set at 1 mV/pA (Axopatch 200A, Axon Instruments, Foster City, CA). The current signals were filtered at 1 kHz through a 4-pole low-pass Bessel filter and digitized at 5 kHz with an IBM-486 computer.

Acquisition and analysis of data were conducted with P-Clamp® (version 6, Axon Instruments, Foster City, CA). Data were acquired during 100 msec voltage pulses and the command potential was set to −15 mV (close to the resting potential for RBCs) for 100 msec between each pulse. All voltage measurements refer to the cell interior.

RBCs, attached to glass coverslips (5 mm diam., Bellco Biotech., Vineland, NJ) with poly-D-lysine (150,000–300,000; 1 mg/ml), were placed in a specially designed open-style chamber (250 μl volume, Warner Instruments, Hamden, CT). The bath solution could be changed by a six-way rotary valve (Rheodyne, Cotati, CA). The whole-cell configuration was achieved following formation of a gigaohm seal (cell-attached configuration) by applying suction to disrupt the patch of membrane beneath the pipette or by applying a large voltage (>200 mV) to the patch. A sudden increase in the capacitance current transient accompanied disruption of the membrane.

Solutions

Amphibian Ringer solution consisted of (in mM): 110 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES (titrated to pH 7.4 with NaOH), and 5 glucose. A low Na^+ Ringer was prepared by substituting choline chloride for NaCl (used for all experiments with gramicidin), and a 0.5× Ringer was obtained by mixing equal volumes of Ringer and distilled water. A stock solution of gramicidin was dissolved in methanol and diluted 1000× to give a final concentration of 5 μM. Mepacrine (quinacrine, 6-chloro-9-[(4-diethylamino)-1-methylbutyl]-amino-2-methoxy-acridine), ONO-RS-082 (2-(p-amyl-cinnamoyl)-amino-p-chlorobenzoic acid), ETYA (5,8,11,14-eicosatetraynoic acid), AA-861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone), curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), EDBCA (ethyl-3,4-dihydroxybenzylidene-cyanoacetate), indomethacin, ketoconazole, aspirin (acetylsalicylic acid), arachidonic acid (cis-5,8,11,14-eicosatetraenoic acid), and L-655,238 (α-pentyl-4-(2-quinolinylmethoxy)-benzene-methanol) were dissolved in ethanol or dimethyl sulfoxide (DMSO), and then diluted to give a final working concentration.

Patch pipettes were filled with an intracellular Ringer solution containing (in mM): 95 KCl, 3.5 NaCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 2.0 EGTA, 5.0 HEPES (pH 7.4 with KOH), 5.0 glucose, 1.0 Mg^{2+}-ATP, and 0.5 GTP. During seal formation, the extracellular solution contained (in mM): 105 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 10.0 HEPES (pH 7.4), and 5 glucose. An isosmotic high K^+ bath contained (in mM): 105 KCl, 2.5 NaCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 10.0 HEPES (pH 7.4), and 5 glucose. A hypotonic (0.5×) high K^+ bath contained (in mM): 2.5 NaCl, 50 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES (pH 7.4), and 5 glucose.

For all experiments, cells were incubated with a pharmacological agents or its vehicle for 5–30 min prior to experimentation. The osmolality of solutions were measured with a vapor pressure osmometer (#5500, Wescor, Logan, UT). Chemicals were purchased from Sigma Chemical (St. Louis, MO), LC Laboratories (Woburn, MA), and ICN (Costa Mesa, CA). All experiments were conducted at room temperature (21–23°C).

Statistics

Data are reported as mean ± SE. The statistical significance of an experimental procedure was determined by a paired Student's *t*-test or least significant difference test with paired design of analysis of variance (ANOVA)/multivariate ANOVA (MANOVA), as appropriate (Data Desk® software, Ithaca, NY). A $P < 0.05$ was considered significant. Each animal served as its own control. For patch clamp studies, each cell served as its own control.

Results

Osmotic Fragility Studies

Although the limit of osmotic fragility (LOF) depends on several factors, we first established this limit as one assessment of a cell's ability to regulate volume in a hypotonic medium. The lowest osmolality RBCs could tolerate without lysing was 11.1 ± 0.9 mosmol/kg H_2O ($n = 15$). To determine whether this limit depended on arachidonic acid, we used the phospholipase A_2 antagonist mepacrine (10 μM) [23]. In this case, the lowest osmolality cells could tolerate without lysing increased by 65% to 18.3 ± 1.7 mosmol/kg H_2O ($n = 15$ experiments, $P < 0.001$, Fig. 1).

Consistent with these results, the phospholipase A_2 inhibitor ONO-RS-082 (10 μM) [23] increased LOF by 169%, from 13.3 ± 2.5 mosmol/kg H_2O to 35.8 ± 2.5 mosmol/kg H_2O ($n = 12$, $P < 0.001$, Fig. 1). This compound also gave a hemolytic index of 90% ($n = 12$, calculated at the limit of osmotic fragility for the negative control). As illustrated in Fig. 1, the cationic ionophore gramicidin (5 μM) reversed the inhibitory effect of ONO-RS-082 bringing the LOF to 16.7 ± 2.7 mOsm/kg H_2O ($n = 12$, $P < 0.01$), a value which was not significantly different from the control. In addition, osmotic fragility with arachidonic acid (1 μM) and ONO-RS-082 together was significantly lower than with ONO-RS-082 alone ($n = 8$, $P < 0.05$). Interestingly, arachidonic acid did not reverse the effect of ONO-RS-082 when Ca^{2+} was absent from the extracellular medium ($n = 6$) or if its concentration was higher than 5 μM ($n = 6$).

We next examined whether osmotic fragility depended on arachidonic acid directly or alternatively whether eicosanoid metabolites were responsible for regulation of cell volume. This was accomplished by using ETYA (5,8,11,14-eicosatetraynoic acid), an acetylenic analogue of arachidonic acid that inhibits cyclooxygenase, lipoxygenase, and cytochrome P-450 monoox-

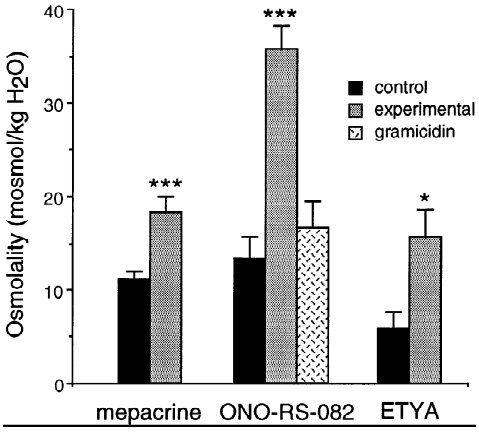

Fig. 1. Inhibition of arachidonic formation or its subsequent metabolism increased the osmotic fragility of mudpuppy RBCs. Cells were incubated for 10–30 min in isosmotic Ringer with mepacrine (10 μM, $n = 15$), ONO-RS-082 (10 μM, $n = 12$), or ETYA (100 μM, $n = 6$) before being diluted. The *control solution* was diluted amphibian (high Na^+) Ringer, the *experimental solution* was diluted Ringer with antagonist, and the *gramicidin solution* contained both the antagonist and ionophore (5 μM). Sodium was replaced with choline for experiments with gramicidin. Vehicle for gramicidin (methanol, diluted 1:1000, $n = 6$) or replacing Na^+ with choline ($n = 6$) had no effect on osmotic fragility. Data reported as mean ± SE. $*P < 0.05$, $***P < 0.001$.

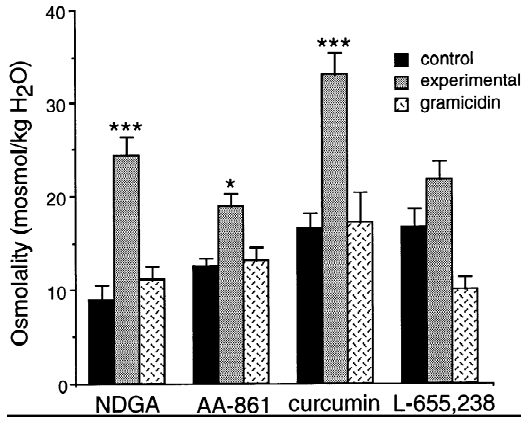

Fig. 2. Inhibition of 5-lipoxygenase increased the limit osmotic fragility. Cells were incubated for 10–30 min in isosmotic Ringer with NDGA (10 μM, $n = 17$), AA-861 (5 μM, $n = 10$), curcumin (30 μM, $n = 10$), or L-655,238 (5 μM, $n = 9$) before dilution. Vehicle for these agents (ethanol, diluted 500–1000×) and for gramicidin (methanol) had no effect on osmotic fragility ($n = 12$). Substitution of choline for Na^+ when using gramicidin also had no effect. The *control solution* was diluted amphibian (high Na^+) Ringer, the *experimental solution* was diluted Ringer with antagonist, and the *gramicidin solution* contained both the antagonist and ionophore (choline Ringer). Data reported as mean ± SE. $*P < 0.05$, $***P < 0.001$.

ygenase enzymes [25]. ETYA (100 μM) increased the LOF fragility by 172%, from 5.8 ± 0.8 mosmol/kg H_2O to 15.8 ± 4.1 mosmol/kg H_2O ($n = 6$, $P < 0.05$, Fig. 1). This indicated that the LOF depended on an eicosanoid product of arachidonic acid.

We then determined whether the limit of osmotic fragility depended on lipoxygenase metabolites of arachidonic acid. The general lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA, 10 μM) [15, 16, 33] increased the LOF by 174%, from 8.9 ± 1.6 mosmol/kg H_2O to 24.4 ± 2.0 mosmol/kg H_2O ($n = 17$, $P < 0.001$, Fig. 2). Further, the inhibitory effect of NDGA was reversed by gramicidin (5 μM, $n = 11$, $P < 0.01$, Fig. 2). With this cationophore, osmotic fragility was reduced to 11.1 ± 1.4 mosmol/kg H_2O.

To determine which lipoxygenase enzyme(s) was involved in this process, we used pharmacological agents specific for 5-, 12-, and 15-lipoxygenase. The 5-lipoxygenase inhibitor AA-861 (5 μM) [16, 33] increased the LOF by 52%, from 12.5 ± 0.9 mosmol/kg H_2O to 19.0 ± 1.2 mosmol/kg H_2O ($n = 10$, $P < 0.05$, Fig. 2). This compound gave a hemolytic index of 61% ($n = 10$). Further, the inhibitory effect of AA-861 was reversed with gramicidin, reducing osmotic fragility to 13.0 ± 1.5 mosmol/kg H_2O ($n = 10$, $P < 0.01$, Fig. 2), which was not significantly different from the control. Curcumin (20 μM), which inhibits 5-lipoxygenase and cyclooxygenase [7], had a similar effect. It increased the LOF by 100%, from 16.5 ± 1.6 mosmol/kg H_2O to 33.0 ± 2.3 mosmol/kg H_2O ($n = 10$, $P < 0.001$, Fig. 2), and gave a hemolytic index of 80%. Gramicidin also reversed the inhibitory effect of curcumin ($n = 8$, $P < 0.05$). In this case, the LOF decreased to 17.2 ± 3.2 mosmol/kg H_2O, a value that was not significantly different from the control. In addition, the 5-lipoxygenase activating protein (FLAP) inhibitor L-655,238 (5 μM) [6] increased the LOF by 30%, from 16.7 ± 2.0 mosmol/kg H_2O to 21.7 ± 2.0 mosmol/kg H_2O ($n = 9$, $P = 0.07$, Fig. 2). This antagonist gave a hemolytic index of 43% ($n = 9$). Gramicidin also reduced the LOF with L-655,238 to 10.0 ± 1.3 mosmol/kg H_2O ($n = 6$, $P < 0.001$).

In contrast to 5-lipoxygenase antagonists, the 12- and 15-lipoxygenase inhibitor ethyl 3,4-dihydroxybenzidene-cyanoacetate (EDBCA, 1 μM) [4] had no effect on osmotic fragility nor the hemolytic index ($n = 10$, Fig. 3). Further, the cytochrome P-450 monooxygenase blocker ketoconazole (20 μM) also had no effect on the LOF nor the hemolytic index ($n = 8$, Fig. 3). Interestingly, cyclooxygenase inhibitors had the opposite effect as 5-lipoxygenase antagonists. Aspirin (100 μM) decreased the LOF by 46%, from 11.1 ± 0.9 mosmol/kg H_2O to 7.4 ± 0.5 mosmol/kg H_2O ($n = 8$, $P < 0.01$, Fig. 3) whereas indomethacin (10 μM, ref. 15) changed osmotic fragility by 19%, from 20.0 ± 4.5 mosmol/kg H_2O to 16.2 ± 5.5 mosmol/kg H_2O ($n = 5$, $P = 0.06$, Fig. 3).

Hematocrit Studies

The hematocrit of whole blood was 20.4 ± 4.7 ($n = 40$), a value close to that previously reported for this species

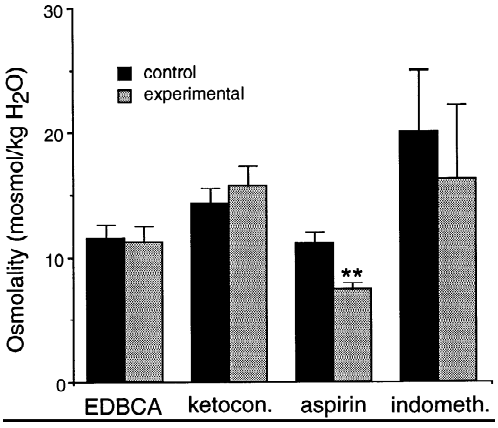

Fig. 3. Inhibition of 12- and 15-lipoxygenase, monooxygenase and cyclooxygenase did not increase osmotic fragility. Cells were incubated for 10–30 min in isosmotic Ringer with EDBCA (1 μM, $n = 10$), ketoconazole (10 μM, $n = 8$), aspirin (100 μM, $n = 8$), or indomethacin (10 μM, $n = 5$) before being diluted. The *control solution* was diluted amphibian (high Na^+) Ringer, the *experimental solution* was diluted Ringer with antagonist, and the *gramicidin solution* contained both the antagonist and ionophore (choline Ringer). Vehicle for these agents (ethanol) had no effect on osmotic fragility. Data reported as mean ± SE. $^{**}P < 0.01$.

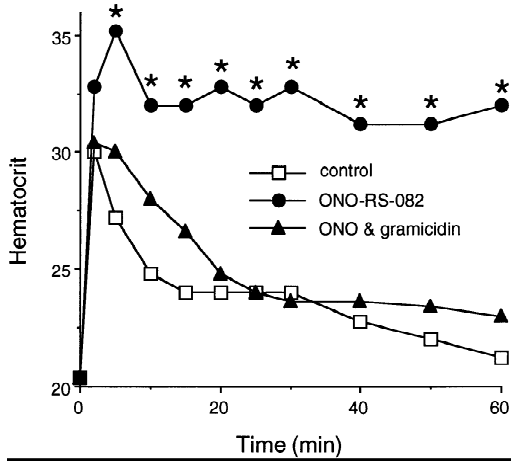

Fig. 4. Inhibition of phospholipase A_2 blocked RVD. The *control solution* was diluted (0.5×) high Na^+ Ringer, the *experimental solution* was 0.5× Ringer with ONO-RS-082 (10 μM, $n = 10$), and the *gramicidin solution* contained both antagonist and ionophore (5 μM). Cells were incubated for 10–30 min in isosmotic Ringer with the antagonist before being diluted, and ONO-RS-082 and gramicidin also were present in the bath solution (choline chloride Ringer) before addition of RBCs. At time 0, cells were abruptly exposed to hypotonic Ringer. Vehicle (ethanol or methanol) and choline substitution for Na^+ had no effect. $^{*}P < 0.05$. Because of the variability in hematocrit (20.4 ± 4.7, $n = 40$) and in the rate and amplitude of an RVD response, only a single representative experiment is illustrated. However, the effect of each experimental procedure was consistent, and statistical analysis was based on replicate experiments. Significant differences between means at the same time point were calculated using the least significant difference (LSD) test of analysis of variance (ANOVA)/multivariate ANOVA (MANOVA).

[5]. When RBCs were placed in a hypotonic (0.5×) Na^+ Ringer solution, they quickly swelled (Fig. 4).[1] This was followed by a slower, spontaneous decrease in volume (Fig. 4). Consistent with the LOF studies, mepacrine (10 μM) inhibited RVD ($n = 13$, $P < 0.05$ after 10 min of incubation). ONO-RS-082 (10 μM) had a similar effect ($n = 16$, $P < 0.05$ at ⩾5 min, Fig. 4). Also consistent with the hemolysis studies, gramicidin (5 μM) reversed the inhibitory effect of ONO-RS-082 ($n = 16$, $P < 0.05$ at ⩾15 min, Fig. 4). Mean values for hematocrit with both ONO-RS-082 (1 μM) and gramicidin (5 μM) in the extracellular medium were not significantly different from control values at any time ($n = 16$, Fig. 4).

To determine whether arachidonic acid metabolism was important for an RVD response, we measured hematocrit in the presence of ETYA (100 μM). This solution inhibited RVD ($n = 7$, $P < 0.05$ at ⩾10 min, Fig. 5). We next examined the effect on RVD of inhibiting the lipoxygenase pathway. NDGA (10 μM) consistently and significantly inhibited an RVD response ($n = 17$, $P < 0.05$ at ⩾10 min, Fig. 6). This effect was reversed by gramicidin ($n = 6$, $P < 0.05$ at ⩾10 min, Fig. 6), and mean values for hematocrit with both NDGA (10 μM) and gramicidin (5 μM) in the extracellular medium were not significantly different from control values at any time ($n = 6$, Fig. 6). In addition, the 5-lipoxygenase antagonist curcumin (20 μM) inhibited cell volume recovery ($n = 10$, $P < 0.05$ at ⩾30 min).

In contrast to 5-lipoxygenase antagonists, the 12- and 15-lipoxygenase inhibitor EDBCA (1 μM) had no effect on RVD ($n = 9$). In addition the cytochrome P-450 monooxygenase antagonist ketoconazole (20 μM, $n = 5$) and the cyclooxygenase inhibitor indomethacin (10 μM, $n = 5$) did not alter cell volume recovery. Although not statistically significant, aspirin (100 μM) slightly, but consistently, enhanced the rate of an RVD response ($n = 5$).

Patch Clamp Studies

The membrane of mudpuppy RBCs formed gigaohm seals without enzymatic treatment (~25% success rate). After a gigaohm seal was formed (cell-attached patch), negative pressure or voltage was used to form the whole-cell configuration (in approximately 20% of the patches,

[1] Cells behaving as perfect osmometers should double their size in this solution. However, we did not observe this amount of swelling. One possible explanation for this phenomenon is that there was a relatively large volume of cells compared to the extracellular volume. This could have resulted in a significant shift of water from the bath into the cells during hypotonic shock (sufficient enough to increase the extracellular osmolality), thereby decreasing the expected level of swelling.

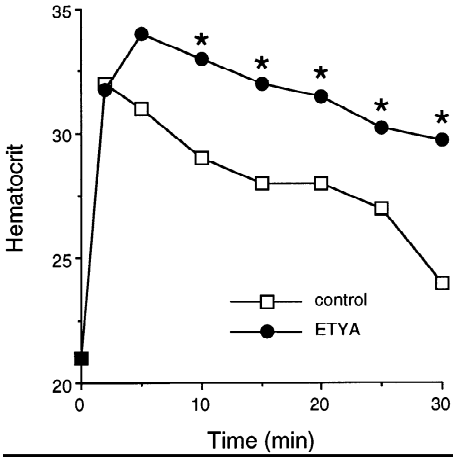

Fig. 5. Inhibition of eicosanoid formation blocked RVD. The *control solution* was diluted (0.5×) high Na^+ Ringer and the *experimental solution* was 0.5× Ringer with ETYA (100 μM, $n = 7$). Cells were incubated for 10–30 min in isosmotic Ringer with the antagonist before being diluted, and ETYA also was present in the bath solution before addition of RBCs. At time 0, cells were abruptly exposed to hypotonic Ringer. Vehicle (ethanol) had no effect. *$P < 0.05$. Only a single representative experiment is illustrated. Significant differences between means at the same time point were calculated using the least significant difference (LSD) test of ANOVA/MANOVA.

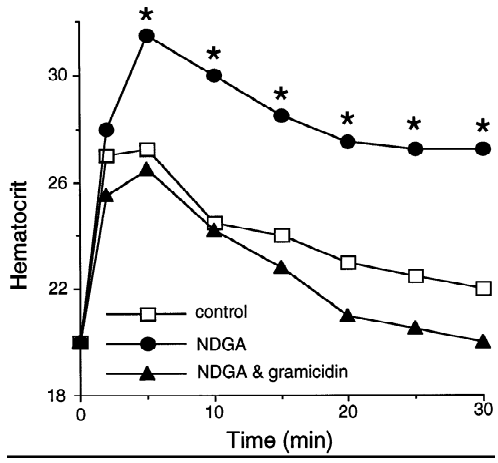

Fig. 6. Inhibition of lipoxygenase enzymes blocked RVD. The *control solution* was diluted (0.5×) high Na^+ Ringer, the *experimental solution* was 0.5× Ringer with NDGA (10 μM, $n = 17$), and the *gramicidin solution* contained both antagonist and ionophore (5 μM). Cells were incubated for 10–30 min in isosmotic Ringer with the antagonist before being diluted, and NDGA and gramicidin also were present in the bath solution (choline chloride Ringer) before addition of RBCs. At time 0, cells were abruptly exposed to hypotonic Ringer. Vehicle (ethanol or methanol) and choline substitution for Na^+ had no effect. *$P < 0.05$. Significant differences between means at the same time point were calculated using the LSD test of ANOVA/MANOVA.

a whole-cell configuration formed spontaneously shortly after obtaining a cell-attached patch). Previously, we described a whole-cell K^+ conductance that was inhibited by quinine and activated by a Ca^{2+}-calmodulin dependent mechanism during cell swelling [1]. Below we describe the role of arachidonic acid and eicosanoids in regulating this channel.

We first examined whether activation of whole-cell currents during swelling required arachidonic acid. The PLA_2 antagonist ONO-RS-082 (10 μM) consistently inhibited a whole-cell conductance when cells were bathed in a 0.5× high K^+ Ringer (Fig. 7). After initial addition of ONO-RS-082 to the extracellular bath, the conductance gradually decreased until a maximum inhibition occurred by approximately 5 min. No decrease in current was observed in the control cells over a similar time period. The whole-cell conductance was reduced by 52% with ONO-RS-082, from 39.3 ± 5.4 ± nS to 18.9 ± 3.0 nS ($n = 6$, $P < 0.01$). Further, this antagonist shifted the reversal potential (E_{rev}) from −9.1 ± 1.4 mV to −4.0 ± 1.7 ($n = 6$, $P < 0.01$, Fig. 7). The only major ions in these solutions were K^+ and Cl^-, and the equilibrium potentials for perfectly cation- and anion-selective conductances were −16.2 mV and +14.7 mV, respectively. The shift in E_{rev} by ONO-RS-082 was therefore towards E_{Cl}. From E_{rev} and the Goldman-Hodgkin-Katz equation, we calculated a change in the K^+ permeability-to-Cl^- permeability (P_K:P_{Cl}) ratio from 3.9:1 to 1.7:1. This indicated that inhibition of phospholipase A_2 by ONO-RS-082 blocked a K^+ conductance. Although not shown, mepacrine (10 μM) had a similar effect ($n = 3$).

We next examined the effect of the lipoxygenase inhibitor NDGA (10 μM) on whole-cell currents for cells in a hypotonic bath. Application of NDGA to the extracellular bath caused a similar decrease in conductance as ONO-RS-082. No decrease in current was observed in the control cells over a similar time period. NDGA decreased whole-cell conductance by 51%, from 49.2 ± 5.5 nS to 23.9 ± 6.0 nS ($n = 11$, $P < 0.001$, Fig. 8), a value that was not different from the conductance in an isosmotic bath (23.8 ± 2.1 nS). Further, NDGA shifted E_{rev} closer to E_{Cl}, from −8.8 ± 2.2 mV to 0.7 ± 2.6 mV ($n = 11$, $P < 0.01$, Fig. 8). This represented a change in the P_K:P_{Cl} ratio from 3.7:1 to 0.9:1, a value that was not different for swollen cells exposed to quinine [1].

The 5-lipoxygenase antagonist AA-861 (5 μM) decreased whole-cell conductance in a manner similar to ONO-RS-082 and NDGA. It reduced conductance by 25%, from 23.6 ± 3.3 nS to 17.8 ± 3.9 nS ($n = 6$, $P < 0.05$). AA-861 also changed E_{rev} closer to E_{Cl}, from −5.1 ± 0.7 mV to 0.5 ± 1.7 mV ($n = 6$, $P < 0.01$). This resulted in a shift in the P_K:P_{Cl} ratio from 2.1:1 to 0.9:1, indicating that a K^+ conductance was inhibited.

Curcumin (20 μM) had a similar effect as AA-861, and reduced whole-cell currents by 44%, from 27.3 ± 3.9 nS to 15.4 ± 3.1 nS ($n = 9$, $P < 0.001$, Fig. 9). Curcumin also changed E_{rev} closer to E_{Cl}, from −5.9 ± 1.1 mV to 1.4 ± 1.5 mV ($n = 9$, $P < 0.01$, Fig. 9). With curcumin,

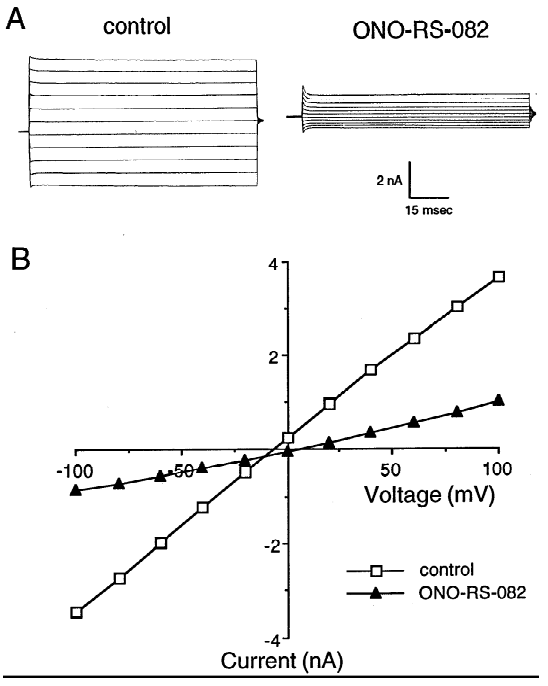

Fig. 7. Inhibition of phospholipase A_2 blocked a K^+ conductance. Cell was maintained at a holding potential of −15 mV and stepped to potentials between −100 to +100 mV in 20 mV intervals. (*A*) whole-cell currents for a RBC exposed to a hypotonic (0.5×) high K^+ bath and inhibition of these currents by adding ONO-RS-082 (10 μM) to the bath. (*B*) corresponding current-voltage (*I–V*) relationship. The conductance decreased by 52% ($n = 6$) with ONO-RS-082 and the P_K:P_{Cl} shifted from 3.9:1 to 1.7:1 ($n = 6$, calculated from the reversal potential and the Goldman-Hodgkin-Katz equation). Although only a single representative experiment is illustrated, statistical analysis was based on replicate experiments.

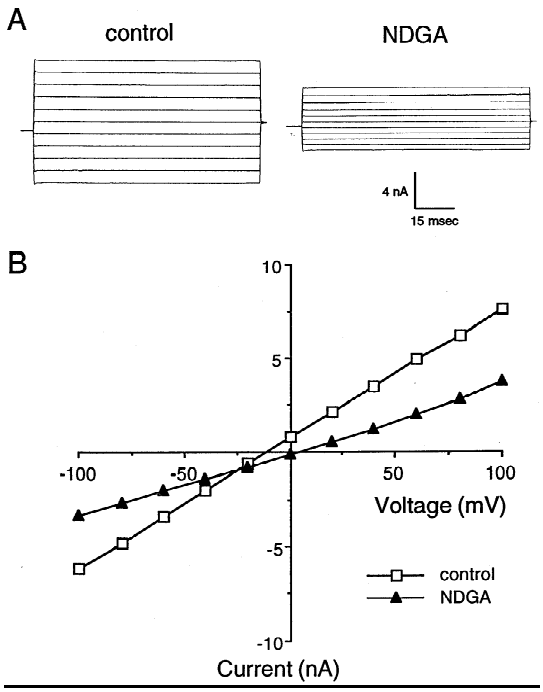

Fig. 8. Inhibition of lipoxygenase enzymes blocked a K^+ conductance. Cell was maintained at a holding potential of −15 mV and stepped to potentials between −100 to +100 mV in 20 mV intervals. (*A*) whole-cell currents for a RBC exposed to a hypotonic (0.5×) high K^+ bath and inhibition of these currents by adding NDGA (10 μM) to the bath. (*B*) corresponding current-voltage (*I–V*) relationship. The conductance decreased by 51% ($n = 11$) with NDGA and the P_K:P_{Cl} shifted from 3.7:1 to 0.9:1 ($n = 11$). A single experiment is illustrated, however, statistical analysis was based on replicate experiments.

the K^+ permeability-to-Cl^- permeability ratio was reduced from 2.3:1 to 0.8:1, revealing that a K^+ current was blocked. Although not quite statistically significant, the 5-lipoxygenase activating protein (FLAP) inhibitor L-655,238 (5 μM) had a similar effect and reduced the conductance by 49% ($n = 3$) and changed P_K:P_{Cl} to 1.0 ($n = 3$).

In contrast to inhibitors of 5-lipoxygenase, the 12- and 15-lipoxygenase inhibitor EDBCA (1 μM) had no effect on whole cell conductance ($n = 6$) nor E_{rev} ($n = 6$). The cyclooxygenase antagonist indomethacin (10 μM) also did not inhibit the K^+ conductance ($n = 6$). In fact, whole conductance increased slightly with indomethacin, from 34.5 ± 2.6 nS to 46.4 ± 2.6 nS ($n = 6$, $P < 0.05$). Arachidonic acid (1 μM) also had the opposite effect on whole-cell currents as compared to 5-lipoxygenase antagonists. For cells in an isosmotic high K^+ Ringer, arachidonic acid increased whole-cell conductance by 66%, from 19.0 ± 3.4 nS to 31.6 ± 3.8 nS ($n = 7$, $P < 0.01$, Fig. 10). The amplitude of this increase was similar to that observed for cells exposed to a hypotonic bath without arachidonic acid. Further, the increase in conductance with arachidonic acid was reversed by curcumin (10 μM, $n = 5$, Fig. 10) and by NDGA ($n = 3$). Although not statistically significant, arachidonic acid slightly, but consistently, increased the conductance for cells in hypotonic medium ($n = 3$).

Discussion

Recent studies in our laboratory have shown that RVD by mudpuppy RBCs depends on a Ca^{2+}-calmodulin dependent mechanism [1]. The major finding of this study was that regulatory volume decrease by these cells also depends upon a 5-lipoxygenase metabolite of arachidonic acid. A dependence of cell volume regulation on 5-lipoxygenase was demonstrated by bathing cells with various and specific inhibitors of this enzyme. We chose antagonists that blocked the action of 5-lipoxygenase by different mechanisms. Direct inhibitors, such as AA-861 and curcumin, block the 5-lipoxygenase enzyme by competitive inhibition [4, 7, 8, 33]. In contrast, indirect inhibitors, such as the quinoline L-655,238, are poor antagonists of soluble 5-lipoxygenase and block leukotri-

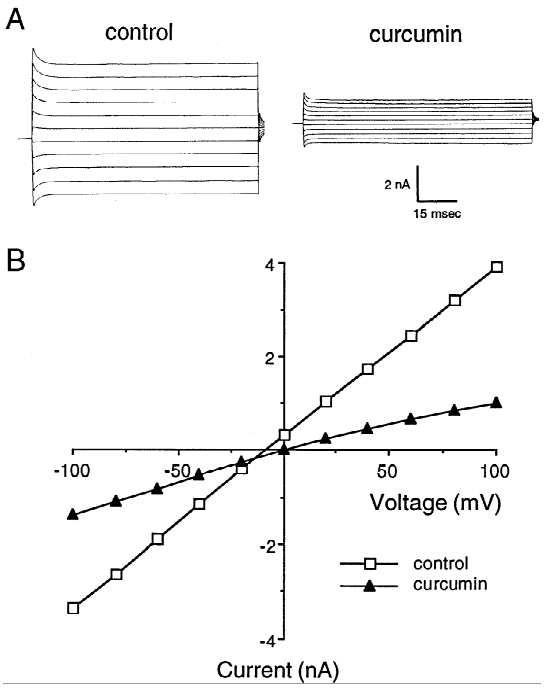

Fig. 9. Inhibition of 5-lipoxygenase enzyme blocked a K^+ conductance. Cell was maintained at a holding potential of −15 mV and stepped to potentials between −100 to +100 mV in 20 mV intervals. (*A*) whole-cell currents for a RBC exposed to a hypotonic (0.5×) high K^+ bath and inhibition of these currents by adding curcumin (30 μM) to the bath. (*B*) corresponding current-voltage (*I–V*) relationship. The conductance decreased by 44% ($n = 9$) with curcumin and the P_K:P_{Cl} shifted from 2.3:1 to 0.8:1 ($n = 9$). A single experiment is illustrated, however, statistical analysis was based on replicate experiments.

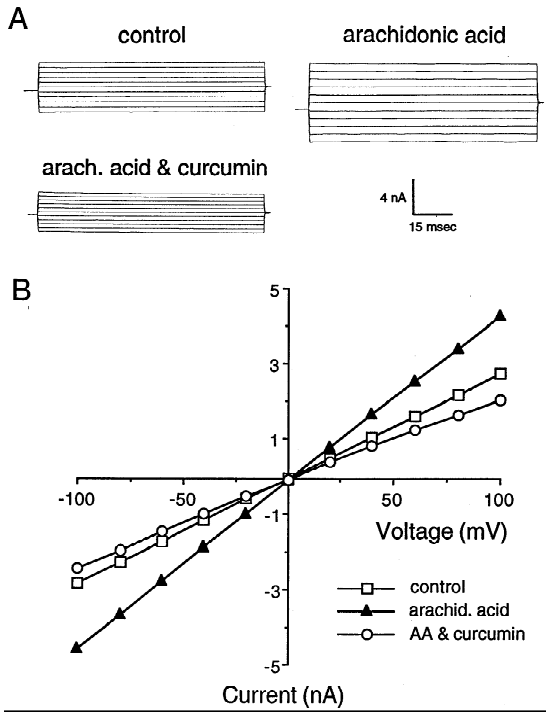

Fig. 10. Addition of arachidonic acid activated a whole-cell conductance. Cell was maintained at a holding potential of −15 mV and stepped to potentials between −100 to +100 mV in 20 mV intervals. (*A*) whole-cell currents for a RBC exposed to an isosmotic high K^+ bath. Activation of these currents by adding arachidonic acid (1 μM) to the bath and subsequent inhibition by adding curcumin (30 μM) to the bath containing arachidonic acid. (*B*) corresponding current-voltage (*I–V*) relationship. Arachidonic acid increased the conductance by 66% ($n = 7$) whereas it decreased by 40% with curcumin ($n = 4$). A single experiment is illustrated, however, statistical analysis was based on replicate experiments.

ene production by interfering with 5-lipoxygenase activating protein (FLAP). FLAP is a membrane-bound 18 kDa protein. After 5-lipoxygenase is activated by a significant rise in intracellular Ca^{2+}, it translocates to the FLAP [6, 8]. The formation of a FLAP-enzyme complex regulates the interaction of 5-lipoxygenase with its substrate, arachidonic acid, resulting in leukotriene synthesis [6, 8].

We found both types of antagonists (direct and indirect) consistently increased the limit of osmotic fragility and reduced the degree of cell volume recovery following hypotonic shock. This indicated that RVD by mudpuppy RBCs is associated with a 5-lipoxygenase metabolite. A similar phenomenon was reported for Ehrlich ascites tumor cells [17–19]. In this case, hypotonic shock stimulated production of leukotrienes and simultaneously inhibited synthesis of prostaglandins. However, if these cells had access to sufficient arachidonic acid there was no decrease in the synthesis of prostaglandins. It was concluded that in the absence of exogenous arachidonic acid, the 5-lipoxygenase pathway is favored during cell swelling at the expense of metabolism by the cyclooxygenase pathway [18, 19].

It also was recently shown in our laboratory that RVD by mudpuppy RBCs depends on K^+ efflux through a conductance that is activated during cell swelling by a Ca^{2+}-calmodulin dependent process [1]. The time course for activation of this K^+ current during cell swelling coincided with the time course for volume recovery from hypotonic shock [1]. In addition, the rate of RVD was accelerated in the presence of gramicidin, indicating that the K^+ permeability was a rate-limiting process and that Cl^- permeability was high in swollen cells [1]. This cell type also displayed a high Cl^- permeability in isosmotic conditions [1].

In this study we demonstrated both pharmacologically and electrophysiologically that the K^+ conductance is modulated by 5-lipoxygenase metabolites. Firstly, a dependence of K^+ efflux on 5-lipoxygenase was shown using the cationophore gramicidin in a low Na^+, choline Ringer solution. In this case, the only two permeable ions of significance were K^+ and Cl^-. Gramicidin consistently reversed the effect of 5-lipoxygenase antagonists so that the limit of osmotic fragility and cell volume recovery with the ionophore and inhibitor together were

not different from control values. Secondly, using the whole cell patch clamp technique, we found that 5-lipoxygenase antagonists blocked a K^+ conductance that is activated by cell swelling in a hypotonic Ringer. In fact, with 5-lipoxygenase inhibitors the K^+ conductance in a hypotonic bath was reduced to levels found for control cells in isosmotic conditions. Further, the inhibition of both RVD and the K^+ conductance with 5-lipoxygenase antagonists was indistinguishable from the inhibition we previously reported with quinine, a specific inhibitor of K^+ channels [1]. For example, the decrease in whole-cell P_K:P_{Cl} with 5-lipoxygenase antagonists was identical to the decrease we previously observed with quinine [1]. Taken together, these results indicate that 5-lipoxygenase metabolites stimulate K^+ efflux during RVD by opening K^+ channels. This is consistent with other reports that have shown K^+ channels are regulated by leukotrienes (LT), products of the 5-lipoxygenase pathway [18]. For example, LTD_4 stimulates K^+ channels in Ehrlich ascites tumor cells [17, 19], whereas the pertussis toxin-sensitive G protein-gated cardiac K^+ channel is activated by LTA_4 and LTC_4 [16].

Although all 5-lipoxygenase antagonists used in this study increased the LOF and reduced cell volume recovery, the effect of curcumin was greater than AA-861. This finding, however, is consistent with the observation that diarylheptanoids, like curcumin, are more potent inhibitors of 5-lipoxygenase than AA-861 [7]. It also should be mentioned that curcumin has been shown to block cyclooxygenase as well as 5-lipoxygenase [7]. Nevertheless, there are several lines of evidence to support the conclusion that the effect of curcumin we observed on mudpuppy RBCs can best be explained by its inhibition of 5-lipoxygenase. For instance, curcumin consistently increased osmotic fragility and reduced cell volume recovery whereas, indomethacin and aspirin had the opposite effect and actually enhanced RVD. We also found that curcumin significantly inhibited a K^+ conductance in swollen cells. In contrast, specific cyclooxygenase antagonists slightly increased whole cell currents. Further, we used a concentration of curcumin that was 2.5 times the reported IC_{50} for 5-lipoxygenase (8.0 μM) but less than half the IC_{50} for cyclooxygenase (52.0 μM) [7]. Lastly, we also utilized specific inhibitors of 5-lipoxygenase that do not block cyclooxygenase (e.g., AA-861 and L-655,238) [6, 33], and these agents had the identical effect as curcumin on osmotic fragility, cell volume recovery, and whole cell currents. Thus, inhibition of RVD by curcumin can best be explained by its antagonism of 5-lipoxygenase.

Our results not only indicate that activation of a K^+ conductance and RVD by mudpuppy RBCs depend on 5-lipoxygenase, but that these processes do not rely on other lipoxygenase enzymes. This was supported by using EDBCA, which inhibits 12- and 15-lipoxygenases. EDBCA had no effect on osmotic fragility, cell volume recovery, nor whole cell currents. The lack of an effect by EDBCA in our study also indicates that inhibition of RVD and K^+ currents by NDGA, a general lipoxygenase antagonist [15, 33], can best be explained by its blocking 5-lipoxygenase. Our observation that a K^+ conductance was specifically modulated by the 5-lipoxygenase pathway is consistent with studies concerning regulation of the G protein-gated K^+ channel in cardiac cells [16]. The cardiac K^+ channel also was inhibited by NDGA and AA-861 but not by indomethacin nor by baicalein, an inhibitor of 12-lipoxygenase [16]. Further, the cardiac channel was specifically stimulated by 5-lipoxygenase metabolites (e.g., 5-HPETE, LTA_4, and LTD_4) and was not effected by products of 12-lipoxygenase (e.g., 12-HPETE).

On the other hand, our finding that 12-lipoxygenase metabolites had no effect on the K^+ conductance in mudpuppy RBCs is different from what was reported for some K^+ channels in other cell types. For example, the S-K^+ channel in *Aplysia* is modulated by 12-lipoxygenase products of arachidonic acid [2]. In this case, probability of S-K^+ channel opening increases with application of 12-HPETE to cell-free membrane patches [2]. It is therefore apparent that different K^+ channels are modulated by different eicosanoids. This view is further supported by our use of inhibitors of cytochrome P450 monooxygenase. For instance, we found ketoconazole, a monooxygenase antagonist, had no effect on whole cell currents. In contrast, K^+ channels in coronary arteries of the pig [14] and in small renal arteries of the rat [34] are activated by cytochrome P450 metabolites. In the latter case, 11, 12-epoxyeicosatrienoic acid activates a large conductance Ca^{2+}-sensitive K^+ channel [34]. Further, K^+ channels in cardiac cells [15] and in smooth muscle cells [25] are activated by arachidonic acid. However, unlike mudpuppy RBCs, in these two cases arachidonic acid activates channels without being metabolized by cyclooxygenase, lipoxygenase, and cytochrome P450.

We found that application of low concentrations of arachidonic acid (0.5–1 μM) reversed the effect of phospholipase A_2 antagonists on osmotic fragility and cell volume recovery, and also increased the whole cell conductance in an isosmotic Ringer solution. In fact, the conductance with arachidonic acid in an isosmotic bath increased to the same level for control cells exposed to a hypotonic bath. This indicated that normally quiescent channels were activated by arachidonic acid, and that the resulting increase in membrane permeability mimicked the change that occurred during cell swelling.

Interestingly, compared to lower levels of arachidonic acid, higher concentrations (>5 μM) of this compound gave inconsistent and variable results that ranged from no effect at all to causing the opposite effect on cell

volume regulation. This concentration dependence for the action of arachidonic acid may have been due to the reported nonspecific detergent effect of an unsaturated fatty acid on membrane permeability [17]. For example, although LTD_4 accelerates RVD and activates K^+ efflux in Ehrlich ascites tumor cells, arachidonic acid in the 5–10 μM range inhibited RVD by these same cells. It was concluded that the inhibitory effect of arachidonic acid was due to its inhibition of the volume-induced K^+ and Cl^- transport pathways by nonspecific detergent effects [17].

We also found that arachidonic acid was only effective at enhancing RVD when Ca^{2+} was present in the extracellular medium, and it had no effect on RVD when Ca^{2+} was absent. This observation is consistent with our previous finding that RVD by mudpuppy RBCs depends on Ca^{2+} influx through a stretch-activated channel during cell swelling [1]. It also is consistent with the results of our present study that 5-lipoxygenase metabolites are necessary to activate the K^+ conductance, and that phospholipase A_2 and 5-lipoxygenase are Ca^{2+}-sensitive enzymes [8, 13, 18, 23]. Further, the finding that arachidonic acid stimulated a whole-cell conductance in an isosmotic Ringer suggests that basal Ca^{2+} levels were sufficient for 5-lipoxygenase activity.

In conclusion, RVD by mudpuppy RBCs was mediated by K^+ efflux through a channel that was activated during cell swelling. Both RVD and this channel were inhibited by antagonists of 5-lipoxygenase metabolites of arachidonic acid. In contrast, neither RVD nor the K^+ channel were blocked by inhibitors of other eicosanoids. We therefore propose that leukotrienes stimulate recovery of cell volume in a hypotonic solution by activating a K^+ channel.

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References

1. Bergeron, L.J., Stever, A.J., Light, D.B. 1996. Potassium conductance activated during regulatory volume decrease by mudpuppy red blood cells. *Am. J. Physiol.* **270:** R801–R810
2. Buttner, N., Siegelbaum, S.A., Volterra, A. 1989. Direct modulation of *Aplysia* S-K^+ channels by 12-lipoxygenase metabolite of arachidonic acid. *Nature* **342:**553–555
3. Cala, P.M. 1983. Volume regulation by red blood cells: mechanisms of ion transport. *Mol. Physiol.* **4:**33–52
4. Cho, H., Ueda, M., Tamaoka, M., Hamaguchi, M., Aisaka, K., Kiso, Y., Inoue, T., Ogino, R., Tatsuoka, T., Ishihara, T., Noguchi, T., Morita, I., Murota, S. 1991. Novel caffeic acid derivatives: extremely potent inhibitors of 12-lipoxygenase. *J. Med. Chem.* **34:**1503–1505
5. Duellman, W.E., Trueb, L. 1986. Biology of Amphibians, p. 220. McGraw-Hill, New York
6. Evans, J.F., Leveille, C., Mancini, J.A., Prasit, P., Therien, M., Zamboni, R., Gauthier, J.Y., Fortin, R., Charleson, P., MacIntyre, D.E., Luell, S., Bach, T.J., Meurer, R., Guay, J., Vickers, P.J., Rouzer, C.A., Gillard, J.W., Miller, D.K. 1991. 5-Lipoxygenase-activating protein is the target of a quinoline class of leukotriene synthesis inhibitors. *Molecular Pharmacol.* **40:**22–27
7. Flynn, D.L., Rafferty, M.F. 1986. Inhibition of 5-hydroxy-eicosatetrainoic acid (5-HETE) formation in intact human neutrophils by naturally-occurring diarylheptanoids: inhibitory activities of curcuminoids and yakuchinones. *Prostaglandins, Leukotrienes, and Medicine* **22:**357–360
8. Ford-Hutchinson, A.W. 1991. Inhibition of leukotriene biosynthesis. *Ann. N.Y. Acad. Sci.* **629:**133–142
9. Furlong, T.J., Spring, K.R. 1990. Mechanisms underlying volume regulatory decrease by *Necturus* gallbladder epithelium. *Am. J. Physiol.* **258:**C1016–C1024
10. Grinstein, S., Foskett, J.K. 1990. Ionic mechanisms of cell volume regulation in leukocytes. *Annu. Rev. Physiol.* **52:**399–414
11. Hazama, A., Okada, Y. 1988. Ca^{2+} sensitivity of volume-regulatory K^+ and Cl^- channels in cultured human epithelial cells. *J. Physiol.* **402:**687–702
12. Hoffman, E.K., Simonsen, L.O. 1989. Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* **69:**315–382
13. Holtzman, M.J. 1991. State of the Art: Arachidonic acid metabolism. *Am. Rev. Respir. Dis.* **143:**188–203
14. Hu, S., Kim, H. 1993. Activation of K^+ channels in vascular smooth muscle by cytochrome P450 metabolites of arachidonic acid. *Eur. J. Pharmacol.* **230:**215–221
15. Kim, D., Clapham, D.E. 1989. Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. *Science* **244:**1174–1176
16. Kurachi, Y., Ito, H., Sugimoto, T., Shimizu, T., Miki, I., Ui, M. 1989. Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel. *Nature* **337:**555–557
17. Lambert, I.H. 1987. Effect of Arachidonic acid, fatty acids, prostaglandins, and leukotrienes on volume regulation in Ehrlich ascites tumor cells. *J. Membrane Biol.* **98:**207–221
18. Lambert, I.H. 1994. Eicosanoids and cell volume regulation. *In:* Cellular Molecular Physiology of Cell Volume Regulation, K. Strange, editor. Chapter 16. CRC Press, Boca Raton
19. Lambert, I.H., Hoffman, E.K., Christensen, P. 1987. Role of prostaglandins and leukotrienes in volume regulation of Ehrlich ascites tumor cells. *J. Membrane Biol.* **98:**247–256
20. Mahadevappa, V.G. Holub, B.J. 1985. Diacylglycerol lipase pathway is a minor source of released arachidonic acid in thrombin-stimulated human platelets. *Biochem. Biophys. Res. Comm.* **134**(3): 1327–1333
21. McCarty, N.A., O'Neil, R.G. 1992. Calcium signaling in cell volume regulation. *Physiol. Rev.* **72:**1037–1061
22. McManus, T.J., Haas, M., Starke, L.C., Lytle, C.Y. 1985. The duck red cell model of volume-sensitive chloride-dependent cation transport. *Ann. N.Y. Acad. Sci.* **456:**183–186
23. Nakashima, S., Suganuma, A., Matsui, A., Hattori, H., Sato, M., Takenaka, A., Nozawa, Y. 1989. Primary role of calcium ions in arachidonic acid release from rat platelet membranes. *Biochem. J.* **259:**139–144
24. O'Neill, W.C. 1989. Cl-dependent K transport in a pure population of volume-regulating human erythrocytes. *Am. J. Physiol.* **256** :C858–C864

25. Ordway, R.W., Walsh, J.W., Jr., Singer, J.J. 1989. Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science* **244:**1176–1179
26. Parker, J.C. 1983. Hemolytic action of potassium salts on dog red blood cells: Anion effects. *Am. J. Physiol.* **244:**C313–C317
27. Pierce, S.K., Politis, A.D. 1990. Ca^{2+}-activated cell volume recovery mechanisms. *Annu. Rev. Physiol.* **52:**27–42
28. Ulb, J., Murer, H., Kolb, H.A. 1988. Hypotonic shock evokes opening of Ca^{2+}-activated K^+ channels in opossum kidney cells. *Pfluegers Arch.* **412:**551–553
29. Welling, P.A., O'Neil, R.G. 1990. Cell swelling activates basolateral membrane Cl and K conductances in rabbit proximal tubule. *Am. J. Physiol.* **258:**F951–F962
30. Wong, P.Y.-K., Cheung, W.Y. 1979. Calmodulin stimulates human platelet phospholipase A_2. *Biochem. Biophys. Res. Comm.* **90:**473–480
31. Wong, S.M.E., Debell, M.C., Chase, H.S., Jr. 1990. Cell swelling increases intracellular free [Ca] in cultured toad bladder cells. *Am. J. Physiol.* **258:**F292–F296
32. Worrell, R.T., Butt, A.G., Cliff, W.H., Frizzell, R.A. 1989. A volume-sensitive chloride conductance in human colonic cell line T84. *Am. J. Physiol.* **256:**C1111–C1119
33. Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, S., Maki, Y., Ashida, Y., Terao, S., Shiraishi, M. 1982. 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow-reacting substance of anaphylaxis. *Biochimica et Biophysica Acta* **713:**470–473
34. Zou, A.-P., Fleming, J.T., Flack, J.R., Jacobs, E.R., Gebremedhin, D., Harder, D.R., Roman, R.J. 1996. Stereospecific effects of epoxyeicosatrienoic acids on renal vascular tone and K^+-channel activity. *Am. J. Physiol.* **270:**F822–F832