Inhibition by polyunsaturated fatty acids of cell volume regulation and osmolyte fluxes in astrocytes

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Sánchez-Olea, R., M. Morales-Mulia, J. Morán, and H. Pasantes-Morales. Inhibition by polyunsaturated fatty acids of cell volume regulation and osmolyte fluxes in astrocytes. *Am. J. Physiol.* 269 (*Cell Physiol.* 38): C96–C102, 1995.—The polyunsaturated fatty acids, arachidonic, linoleic, and linolenic acids, were potent blockers of regulatory volume decrease (RVD) and of the swelling-activated efflux of [^{3}H]taurine, D-[^{3}H]aspartate, [^{3}H]inositol, and ^{125}I (used as marker of Cl) from rat cerebellar astrocytes in culture. The monounsaturated oleic and ricinoleic acids and saturated fatty acids were ineffective. The amino acid and ^{125}I fluxes were similarly inhibited by fatty acids, whereas inositol release was less sensitive. Polyunsaturated fatty acids appear to directly affect RVD in trypsinized astrocytes as the inhibition was immediate and fully reversible. Blockers of the arachidonic acid metabolic pathways, indomethacin (cyclooxygenase), esculetin (lipoxygenases), and metyrapone (*P*-450 monooxygenases), did not prevent the effect of arachidonic acid, suggesting that further metabolism is not required for displaying the effects of arachidonic acid on RVD and osmolyte fluxes. Some blockers of arachidonic acid metabolic pathways, such as nordihydroguaiaretic acid (lipoxygenases) and naphthoflavone (*P*-450 monooxygenases), also exhibited marked inhibitory effects on RVD and on osmolyte fluxes. The predominant arachidonic acid metabolite in astrocytes, 12-hydroxyeicosatetraenoic acid, did not affect RVD or osmolyte fluxes. These results suggest that arachidonic acid and other polyunsaturated fatty acids directly inhibit the permeability pathways correcting cell volume after swelling in cultured astrocytes.

hyposmolarity; taurine; D-aspartate; iodide; arachidonic acid; regulatory volume decrease

THE REGULATORY MECHANISM by which astrocytes adjust volume in response to osmotic swelling involves the activation of transmembrane pathways extruding intracellular solutes and osmotically obliged water. Osmolytes associated to this regulatory volume decrease (RVD) include the inorganic ions K^+ and Cl^- and a variety of organic molecules such as amino acids, polyamines, and polyalcohols (13, 23, 31). Recent evidence indicates that the translocation of K^+ and Cl^- associated to cell swelling in astrocytes occurs via separate conductive channels (10, 24, 28). The efflux of amino acids and polyalcohols also occurs through a diffusional pathway different from the energy-dependent carriers in charge of the uptake of these compounds (25, 29, 31). Moreover, available evidence suggests that the efflux of amino acids and polyalcohols during RVD occurs through an anionic channel that also translocates Cl^- (10, 30). An increase in the permeability of this channel is the most remarkable feature of the regulatory response of astrocytes after swelling (24). Therefore, it is of interest to characterize this pathway and know about its possible activation mechanisms. To contribute to this purpose, in the present work we examined the effect of arachidonic acid and other unsaturated and saturated fatty acids on RVD and on the volume-associated efflux of taurine, D-aspartate, inositol, and ^{125}I (as tracer for Cl^-). Arachidonic acid is known to modulate directly the activity of a variety of ion channels (22) and to affect glucose (33) and amino acid transport (2, 34) in astrocytes. In addition, components of the arachidonic acid cascade are known to function as transduction signals in a number of systems (7, 19, 27), including possibly RVD (6, 12, 15, 17). The function of second-messenger cascades in RVD is poorly understood in astrocytes (3, 4), and the possibility exists that arachidonic acid cascade is part of the signal system for activation of the osmolyte fluxes in these cells.

MATERIALS AND METHODS

Materials and solutions. All radiolabeled chemicals were obtained from New England Nuclear. ^{125}I (as NaI, 370 mCi/ml), [^{3}H]inositol (23.45 Ci/mmol), D-[^{3}H]aspartate (12.8 Ci/mmol), or [^{3}H]taurine (21.9 Ci/mmol) were used for the experiments. To prevent oxidation of fatty acids, original solutions were dissolved in dimethyl sulfoxide, fractionated into aliquots for daily experiments, and stored under nitrogen. The culture medium consisted of basal Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. Krebs-*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) medium contained (in mM) 135 NaCl, 5 KCl, 0.6 $MgSO_4$, 1 $CaCl_2$, 10 glucose, 10 HEPES (pH 7.4); 300 mosmol/kg medium. Hyposmotic solutions were always prepared by reducing the concentration of NaCl. For experiments with gramicidin, NaCl was replaced by choline chloride.

Cell cultures and experimental procedures. Primary cultures of cerebellar astrocytes were obtained as previously described (23, 24). Briefly, the dissociated cell suspensions from cerebella of 8-day-old rats were plated at a density of 210×10^3 cells/cm^2 in 60-mm diameter plastic dishes. The culture dishes were incubated at 37°C in humidified 5% CO_2-95% air atmosphere. The enrichment of cultures in astrocytes and the proportion of other cells were assessed by immunocytochemical techniques as previously described (24).

For volume measurements (24, 25), cells cultured for 2–3 wk were detached by treatment for 5 min with phosphate-buffered saline without calcium and containing 1 mM EDTA and 0.01% trypsin. The detached cells were mixed with the same volume of serum-containing medium and then centrifuged and resuspended in isosmotic Krebs-HEPES medium. A sample of the cell suspension was diluted approximately 100-fold with the experimental media, and exactly 1 min after, cell volume was measured at the indicated times by electronic sizing using a Coulter Counter (model ZF) associated to a Coulter Channelyzer (model 256). To examine the effect of gramicidin, cell volume was measured at 1 min after the hyposmotic stimulus; 2.5 μM gramicidin or the vehicle (ethanol) was then added to the cell suspension, and the experiment was continued as in controls. Cell volumes were recorded in femtoliters, but results were expressed as relative volume, i.e., the change with time over the initial volume recorded in

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isosmotic medium. In some experiments, results were expressed as percentage of RVD, i.e., the recovery of cell volume between *min 1* (maximal volume) and *min 15*.

The release of osmolytes activated by hyposmolarity was measured in cells in monolayer using two different experimental protocols (29, 32). In the first protocol, the efflux of ^{125}I, [^{3}H]inositol, or [^{3}H]taurine was measured by incubating preloaded astrocytes in isosmotic medium; fractions were then collected every 20–30 s during 2.5 min in isosmotic and hyposmotic media. Measurement of radioactivity released in the medium and that remaining in cells was calculated after the whole incubation period. Efflux rate coefficients for ^{125}I release were calculated as described by Venglarik et al. (32) using the following equation: $r = [\ln(R_1) - \ln(R_2)]/(t_1 - t_2)$, where R_1 and R_2 are percent of counts remaining in cells at *times 1* (t_1) and *2* (t_2), respectively. [^{3}H]inositol and [^{3}H]taurine effluxes were calculated as percent of counts released in every fraction considering the total radioactivity accumulated during the loaded period as 100%. In the second protocol for [^{3}H]taurine or D-[^{3}H]aspartate release, loaded cells were washed with isosmotic medium, and cells were subsequently incubated for 5 min with isosmotic or 50% hyposmotic medium containing the indicated concentration of the tested drugs. Efflux was calculated as percent of tracer released in isosmotic or hyposmotic medium, and results were expressed as net stimulated release, i.e., the difference between these two values.

Cells were preincubated with the fatty acids or drugs at the indicated concentrations for 10 min in isosmotic medium and then during the release period. Control cells were always treated in parallel with the vehicle used to prepare solutions containing the compounds.

The effect of polyunsaturated fatty acids and some arachidonic acid metabolic pathway blockers on cell survival was estimated by measuring the reduction of the tetrazolium derivative 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a colored formazan product (21). Cells grown as described above in 24-well plates with 0.5 ml culture medium were incubated in the presence of MTT (0.06 mg/ml) and the blue formazan product extracted with isopropanol-HCl. The amount of the formazan product formed by cells was estimated spectrophotometrically at 570 nm.

RESULTS

The effect of polyunsaturated (arachidonic, linoleic, and linolenic), monounsaturated (oleic and ricinoleic), and saturated (arachidic, stearic, palmitic) fatty acids was comparatively examined on RVD and on the volume-associated efflux of [^{3}H]taurine, D-[^{3}H]aspartate, [^{3}H]inositol, and ^{125}I.

Regulatory volume decrease. Arachidonic acid and linolenic acid showed a strong, concentration-dependent inhibitory effect on RVD. At concentrations of 2.5–5.0 μM for arachidonic acid (Fig. 1A) and 10 μM for linolenic acid (Fig. 1*B*), RVD was essentially abolished. This effect was not lifted by gramicidin (results not shown). The inhibitory effect of arachidonic acid was essentially the same whether cells were preincubated (10 min) or not with the fatty acid before the hyposmotic stimulus. During cell exposure for 10 min to arachidonic acid and fatty acid removal from the medium, no inhibition of RVD was observed (results not shown). In any condition, the polyunsaturated fatty acids modified cell volume in isosmotic conditions. Linoleic and oleic acids at 5–10 μM caused marked swelling of trypsinized astrocytes, which led to irreversible damage of the cells during the 15 min of the experiment. At concentrations lower than 5 μM, no damage was observed and RVD was unaffected. The saturated fatty acids (palmitic, stearic, arachidic) and ricinoleic acid tested at 10 μM did not affect RVD (results not shown).

Osmolyte efflux. The effect of fatty acids on the volume-associated efflux of [^{3}H]taurine, D-[^{3}H]aspartate (used as analogue of glutamate), [^{3}H]inositol, and ^{125}I (used as tracer for Cl^-) was investigated.

Experiments on taurine efflux showed that during the first 5 min of RVD, subsequent to exposure to hyposmotic solutions, astrocytes released 65% of the taurine pool traced by [^{3}H]taurine. A fraction of ~13% is released during the same period in isosmotic medium. Taurine efflux stimulated by hyposmolarity was markedly inhibited by the polyunsaturated fatty acids (linoleic, linolenic, and arachidonic acid) in a concentration-dependent manner. At 10 μM, the decrease in taurine efflux by linoleic, linolenic, and arachidonic acids was 54, 70, and 80%, respectively (Fig. 2). The effect of preincubation time to polyunsaturated fatty acids was exam-

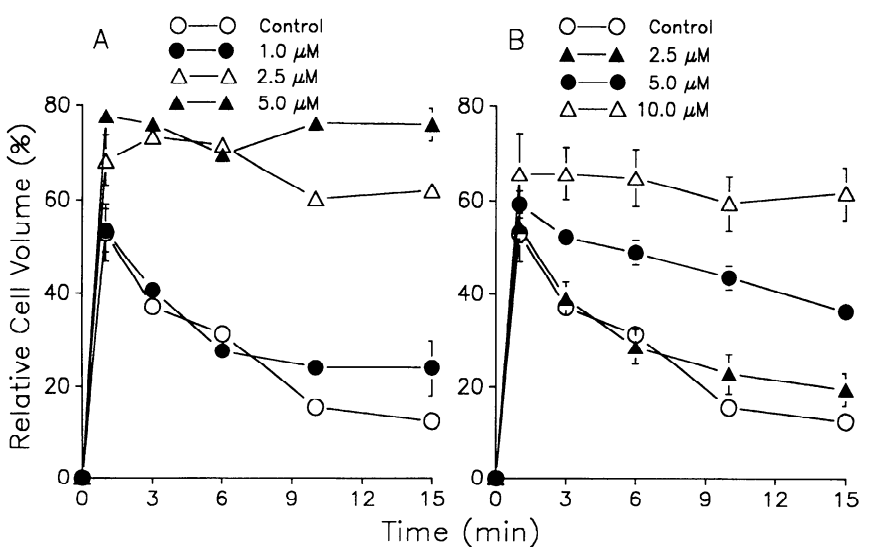

Fig. 1. Effect of arachidonic acid (*A*) and linolenic acid (*B*) on regulatory volume decrease (RVD) in cerebellar astrocytes. Cerebellar cultured astrocytes were detached by treatment with trypsin in a Ca^{2+}-free saline. At zero time, a sample of cell suspension was diluted ~100-fold with isosmotic or 50% hyposmotic media containing arachidonic acid (*A*) or linolenic acid (*B*) at indicated concentrations. Cell volume was measured at indicated times by electronic sizing. Results are expressed as %increase in cell volume relative to isosmotic value. Data are means of 6–9 experiments with SE values shown as vertical bars if they extend beyond the symbols.

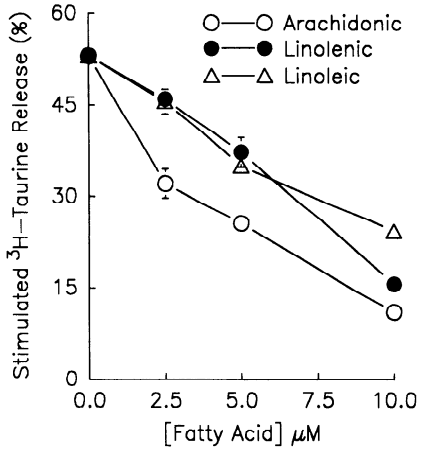

Fig. 2. Effect of fatty acids on [^{3}H]taurine release stimulated by hyposmolarity in cerebellar astrocytes. Preparation of cultures and determination of [^{3}H]taurine release were performed as detailed in MATERIALS AND METHODS. Cells were loaded with [^{3}H]taurine and then preincubated 10 min with 2.5, 5.0, or 10.0 μM arachidonic, linolenic, or linoleic acids. After washing, taurine efflux was measured in a 5-min period in isosmotic or hyposmotic medium. Efflux was calculated as %tracer released in isosmotic or hyposmotic medium, and results were expressed as net stimulated release, i.e., the difference between these 2 values. Data are averages ± SE of 8–10 determinations. At all polyunsaturated fatty acid concentrations, data were significantly different from control by $P < 0.001$ (Student's t-test).

ined only for arachidonic acid. The inhibitory action of arachidonic acid was found to be dependent on the exposure time, with maximal inhibition after 5 min of preincubation (Fig. 3). In any case, the polyunsaturated fatty acids affected the efflux of taurine in isosmotic conditions (Table 1). The monounsaturated fatty acids (oleic and ricinoleic acid) and the saturated fatty acids had no effect on taurine efflux either in isosmotic or in hyposmotic conditions (Table 1). The effect of arachidonic acid, the fatty acid with the most potent effect on taurine efflux, was also examined on D-aspartate release associated to cell swelling. A strong inhibition of about 93% was observed at 10 μM arachidonic acid (Fig.4).

Table 1. *Effect of fatty acids on taurine and iodide release in cultured cerebellar astrocytes*

	[^{3}H]taurine Efflux, %		^{125}I Efflux Rate Constant, min^{-1}	
Fatty Acid	Isosmotic	Hyposmotic	Isosmotic	Hyposmotic
None	14.5 ± 0.46	66.9 ± 0.93	0.326 ± 0.019	1.139 ± 0.040
Arachidonic	14.2 ± 0.82	25.4 ± 1.17	0.237 ± 0.018	0.426 ± 0.021
Linoleic	13.8 ± 0.72	33.5 ± 1.49	0.205 ± 0.013	0.462 ± 0.048
Linolenic	15.4 ± 0.55	30.8 ± 1.61	0.327 ± 0.024	0.955 ± 0.082
Oleic	16.3 ± 0.88	60.9 ± 2.56	0.331 ± 0.010	0.995 ± 0.103
Ricinoleic	16.8 ± 1.80	55.3 ± 0.99	0.344 ± 0.071	0.897 ± 0.154
Stearic	16.8 ± 0.95	62.5 ± 1.73		
Palmitic	16.2 ± 1.58	65.8 ± 1.91	0.360 ± 0.067	1.214 ± 0.167
Arachidic	14.2 ± 0.82	52.4 ± 1.17	0.316 ± 0.028	0.831 ± 0.062

Values are averages ± SE of 6–10 different experiments. Preparation of cultures and determination of [^{3}H]taurine release and ^{125}I were performed as detailed in MATERIALS AND METHODS. Cells were loaded with ^{125}I or [^{3}H]taurine and preincubated 10 min with fatty acids (10 μM). Cells were washed and release was measured. For ^{125}I, cells were exposed to isosmotic and then to 50% hyposmotic medium, and 5 fractions of each medium were collected at 20-s intervals; results are expressed as the efflux rate constant. For [^{3}H]taurine, a 5-min period in isosmotic or hyposmotic medium was collected; results are expressed as %tracer released.

The effect of arachidonic acid was also examined on [^{3}H]inositol efflux stimulated by hyposmolarity. The release of inositol from astrocytes in response to swelling was markedly lower than that of D-aspartate or taurine. For this reason, inositol efflux was followed by collecting medium samples every 30 s. The amount of [^{3}H]inositol released during 2 min of exposure to hyposmotic medium corresponded to ~12% of the total accumulated during loading. Arachidonic acid at 10–20 μM reduced this efflux only by ~30%, showing a notably less potent effect than in the case of amino acids. As taurine (and D-aspartate) efflux was measured using a different experimental procedure, with the labeled amino acid released in the medium collected only at the end of the incubation period (see MATERIALS AND METHODS), to

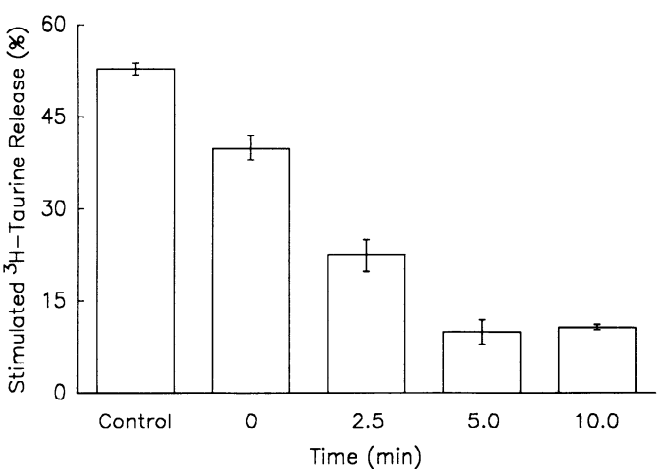

Fig. 3. Effect of preincubation time on inhibitory action of arachidonic acid on release of [^{3}H]taurine stimulated by hyposmolarity in cerebellar astrocytes. Cells were preincubated during indicated times, and [^{3}H]taurine release was measured as detailed in Fig. 2 and MATERIALS AND METHODS. Bars represent net stimulated release. Results are averages ± SE of 6 determinations.

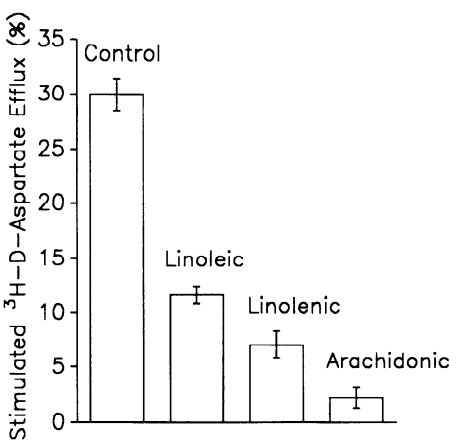

Fig. 4. Effect of fatty acids on D-[^{3}H]aspartate release stimulated by hyposmolarity in cerebellar astrocytes. Determination of D-[^{3}H]aspartate release was performed as detailed in MATERIALS AND METHODS and Fig. 2. Cells were preincubated with 10 μM arachidonic, linoleic, or linolenic acid for 10 min and then D-[^{3}H]aspartate release induced by a 50% hyposmotic medium containing the corresponding drugs was measured. Bars represent net stimulated release. Results are expressed as detailed in Fig. 2 and are averages ± SE of 6 determinations.

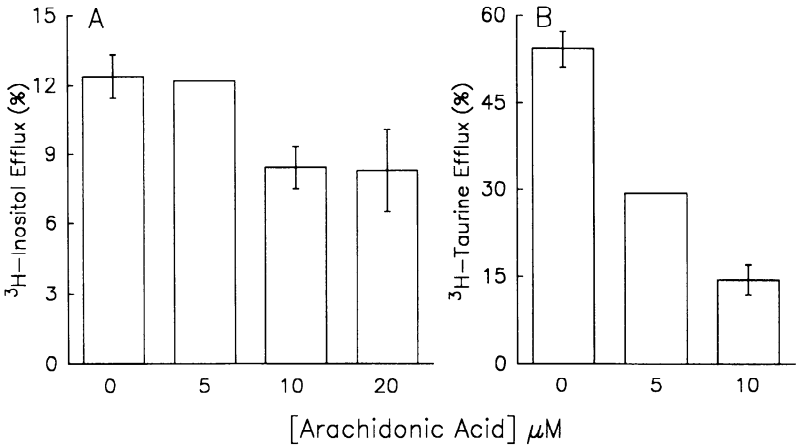

Fig. 5. Effect of arachidonic acid on [^{3}H]inositol release stimulated by hyposmolarity in cerebellar astrocytes. Determination of [^{3}H]inositol (*A*) and [^{3}H]taurine release (*B*) was performed as detailed in MATERIALS AND METHODS. Cells were preincubated 10 min with indicated concentrations of arachidonic acid. Preloaded cells were washed with isosmotic medium and then fractions were collected every 30 s during 5 min in isosmotic and hyposmotic media containing arachidonic acid. Efflux was calculated as percent of counts released in every fraction, considering total radioactivity accumulated during the loading period as 100%. Results represent percent of tracer released in 4 largest fractions. Bars are averages ± SE of 6–8 experiments.

accurately compare the effect of arachidonic acid on inositol and taurine, experiments in parallel were carried out with [^{3}H]taurine and [^{3}H]inositol using the same experimental procedure. Results in Fig. 5 show that inositol efflux is clearly less sensitive to arachidonic acid than taurine efflux.

The efflux of ^{125}I was inhibited by arachidonic acid in a concentration-dependent manner with a decrease of ~70% at 10 μM. The effect of arachidonic acid required that the cells be preincubated with the fatty acid (result not shown). Linolenic and linoleic acids were both less effective inhibitors than arachidonic acid, the less potent being linolenic acid (Fig. 6).

Effect of blockers of arachidonic acid metabolism on RVD and taurine and I efflux. The requirement for a preincubation time of ~5 min to exhibit maximal effects of arachidonic acid on osmolyte efflux raises the possibility that accumulation of metabolite products rather than the fatty acid itself might be responsible for the observed effects. To investigate this possibility, the metabolic pathways of arachidonic acid were inhibited using *1*) indomethacin to block the cyclooxygenase pathway *2*) nordihydroguaiaretic acid (NDGA) and esculetin to block the lipoxygenase pathway and *3*) naphthoflavone and metyrapone to inhibit the *P*-450 pathway. The effect of these inhibitors on RVD and on the volume-sensitive efflux of taurine and I was examined, first, in the absence of arachidonic acid to exclude any effect of the inhibitors per se and, second, together with arachidonic acid to examine if the inhibitory effect of the fatty acid was prevented by blocking its metabolism.

Indomethacin did not affect RVD or taurine efflux and was also unable to modify the inhibitory action of arachidonic acid (Table 2). The inhibitor of the lipoxygenase pathways, NDGA, showed, per se, a marked, concentration-dependent inhibitory effect on RVD (Fig. 7) and on taurine and I efflux (Table 2). According to some effects of NDGA described in other preparations (12, 14), NDGA inhibitory action on RVD could be because of *1*) decreasing calcium influx, *2*) increasing sodium accumulation, thus resulting in cell swelling, and *3*) blockade

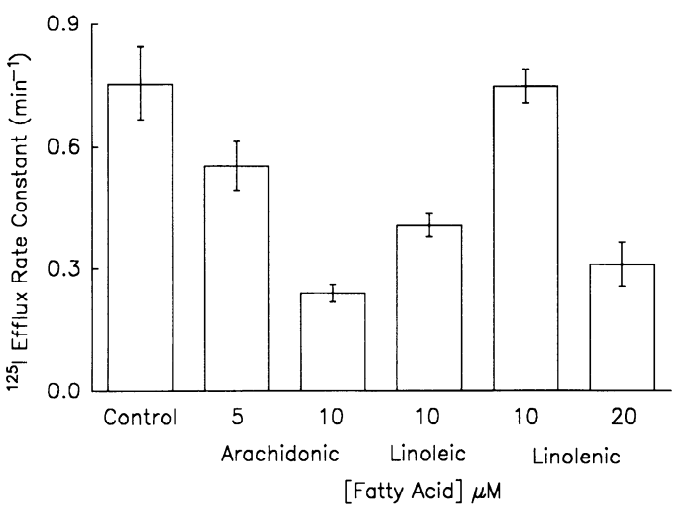

Fig. 6. Effect of fatty acids on release of ^{125}I stimulated by hyposmolarity in cerebellar astrocytes. Preparation of cultures and determination of ^{125}I release were performed as detailed in MATERIALS AND METHODS. Cells were loaded with ^{125}I for 15 min and preincubated 10 min with arachidonic, linolenic, or linoleic acids at indicated concentrations. After washing, cells were exposed to 50% hyposmotic medium, and 5 fractions of medium were collected at 20-s intervals. Results are expressed as difference between efflux rate constant in isosmotic medium and maximal value in hyposmotic medium. Bars are averages ± SE of 8 different experiments.

Table 2. *Effect of blockers of metabolic pathways of arachidonic acid on hyposmolarity-induced release of osmolytes and RVD in cultured cerebellar astrocytes*

Condition	RVD, %	[^{3}H]taurine Efflux, %	^{125}I Efflux Rate Constant, min^{-1}
None	72.2 ± 1.55	55.8 ± 1.16	0.812 ± 0.039
Indomethacin (50 μM)	62.3 ± 1.46	45.0 ± 0.94	
Esculetin (100 μM)	84.7 ± 1.36	52.8 ± 0.05	
NDGA (10 μM)	0.0	33.6 ± 1.74	0.444 ± 0.037
Naphtoflavone (25 μM)	0.0	30.0 ± 1.46	0.390 ± 0.049
Metyrapone (100 μM)	75.7 ± 2.36	59.1 ± 2.80	
Arachidonic acid (5 μM)	3.9 ± 1.31	14.0 ± 2.42	
+Esculetin	3.6 ± 1.18		
+Metyrapone	0.0		
+Indomethacin	0.0		

Values are averages ± SE of 4 different experiments. Preparation of cultures and determination of [^{3}H]taurine release, ^{125}I, and regulatory volume decrease (RVD) were performed as detailed in Fig. 1 and Table 1. For release experiments, cells were preincubated 10 min with drugs at indicated concentrations. Release was calculated as the efflux rate constant for ^{125}I and %[^{3}H]taurine released. Results are expressed as the delta between isosmotic and hyposmotic release values. RVD results are expressed as %RVD, i.e., %recovery of cell volume between *min 1* (maximal volume) and *min 15*.

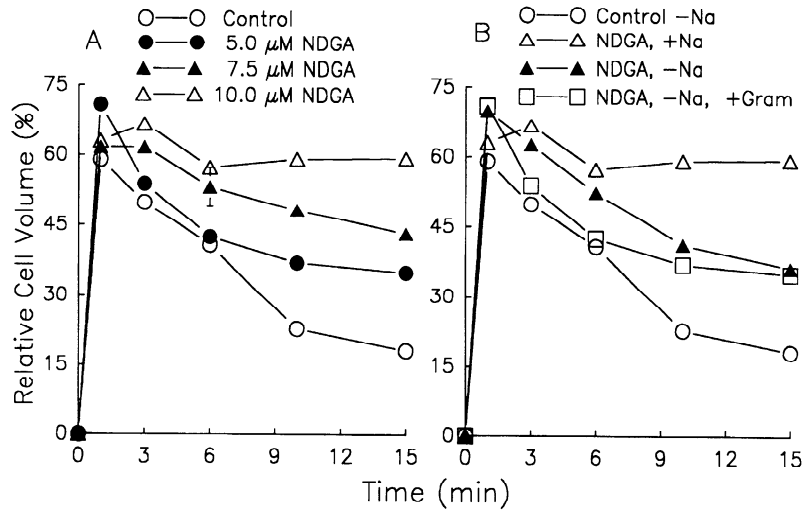

Fig. 7. Effect of nordihydroguaiaretic acid (NDGA) on RVD in cerebellar astrocytes. Cell volume was measured in cultured cells as described in Fig. 1. Cells were exposed to 50% hyposmotic medium with or without 5–10 μM NDGA (*A*), and cell volume was measured. *B*: effect of gramicidin (2.5 μM) on inhibitory action of 10 μM NDGA on RVD. Gramicidin was added to cell suspension after 1 min of exposure to hyposmotic medium, and cell volume measurement proceeded as in control. NaCl was replaced by choline chloride. Results are expressed as %increase in cell volume relative to isosmotic value. Data are means of 6–9 experiments with SE values shown as vertical bars if they extend beyond the symbols.

of K^+ channels. To test these different possibilities, the effect of NDGA on RVD was examined in calcium-free or sodium-free media and in the presence of gramicidin. The inhibitory effect of NDGA persisted in the absence of extracellular calcium (results not shown) but was decreased when sodium in the medium was replaced by choline chloride (Fig. 7). The inhibition by NDGA on RVD was not lifted by gramicidin (Fig. 7).

Esculetin, another inhibitor of lipoxygenase pathways, did not affect RVD or taurine efflux and failed to reverse the inhibitory effect of arachidonic acid (Table 2). Naphthoflavone, a blocker of the *P*-450 pathway, exhibited a potent inhibitory effect on RVD and on the volume-associated efflux of taurine and I (Table 2). In contrast, metyrapone, another blocker of this same pathway, had no effects per se and did not reverse the effect of arachidonic acid on [^{3}H]taurine or I efflux (Table 2).

The main metabolic product of arachidonic acid in astrocytes, 12-hydroxyeicosatetraenoic acid (12-HETE), is formed via the lipoxygenases pathway. This compound (1 μM) did not modify RVD or taurine or I efflux (results not shown).

The effect of arachidonic acid (2.5–10 μM), naphthoflavone (25 μM), NDGA (up to 20 μM), metyrapone (100 μM), and esculetin (100 μM) on cell viability and on the oxidative metabolism of astrocytes was assessed by the conversion of MTT to formazan, a reaction occurring only in viable cells with an intact mitochondrial function (21). There was no difference between control and experimental conditions in this reaction (results not shown).

DISCUSSION

Results of the present work show that polyunsaturated fatty acids, particularly arachidonic acid, are among the most potent inhibitors of RVD in primary cultures of astrocytes. A similar marked inhibitory effect of polyunsaturated fatty acids on RVD has been previously observed in Ehrlich ascites tumor cells (15). The presence of double bonds in the fatty acid chain appears to be a requirement for their inhibitory action, as shown by the relative potency of effects of polyunsaturated, monounsaturated, and saturated fatty acids. Similar structure requirements have been reported for the effects of fatty acids on a number of transport processes in astrocytes (2, 33, 34).

The effect of polyunsaturated fatty acids on RVD is the consequence of inhibition of the corrective fluxes of the various osmolytes, ions as well as organic molecules, activated upon swelling in astrocytes. The volume-sensitive ionic fluxes in astrocytes occur through separate but interdependent K^+ and Cl^- channels (24) so that blockade of one channel results in inhibition of efflux of both osmolytes. A blockade by fatty acids restricted to K^+ channels without affecting Cl^- efflux seems unlikely, since RVD inhibition could not be reversed by gramicidin, a ionophore that opens an alternate pathway for K^+. Therefore, Cl^- and organic osmolyte pathways are more likely candidates for fatty acid inhibition. That this is the case was demonstrated in the present work by the potent inhibitory effects exhibited by fatty acids on Cl^-, taurine, and inositol release activated by hyposmolarity in primary astrocytes and previously in ascitic cells (15) and in C6 glioma cells (10, 31), although in these later preparations, higher concentrations of polyunsaturated fatty acids were used. The effluxes of Cl^- and taurine were similarly and markedly sensitive to fatty acids, whereas inositol appeared less affected. This is somewhat surprising in view of recent evidence suggesting that taurine, inositol, and Cl^- fluxes activated by hyposmolarity occur through the same pathway (10, 30).

The common osmolyte pathway activated by swelling in astrocytes is presumably an anionic channel (10, 29, 30, 31), and this channel could then be the target of fatty acids. A large-conductance anion channel activated by hyposmolarity has recently been described by Jalonen (11) in rat cortical astrocytes in culture. This channel might correspond to the volume-activated pathway suggested for Cl^- and organic osmolytes (10, 30). Fatty acids are known to affect the function of ion channels, activating cationic channels and blocking anionic channels, including those activated by hyposmolarity. The

effect of fatty acids seems to be exerted on the channel itself rather than on intracellular messenger systems modulating the channel activity, since fatty acid effects persist in experiments performed on patches of membranes excised from the cell and consequently devoid of soluble intracellular modulators (22).

The present results in astrocytes, although showing clear inhibitory effects of fatty acids on RVD and osmolyte fluxes, were not conclusive about the mechanisms of this action. A direct effect of fatty acids on the volume-activated pathway itself is suggested by the rapid and fully reversible actions of fatty acids on trypsinized astrocytes. However, it cannot be excluded that transduction signals for activation of osmolyte fluxes may also be affected by fatty acids, particularly by arachidonic acid, which is known to modulate a number of second messenger systems (7, 16, 19, 27). Elucidating this point must, however, await further knowledge about the involvement of intracellular signals for RVD in astrocytes, which has not been extensively examined so far (3, 4), or, alternately, must await results from studies on the effects of fatty acids on isolated membrane patches.

The effects of fatty acids, particularly arachidonic acid, could be exerted by intermediates of the arachidonic acid metabolic cascade. The enzyme systems for the three main arachidonic acid metabolic pathways are present in astrocyte primary cultures, but arachidonic acid metabolism in this preparation seems to proceed mainly via the lipoxygenase pathway, originating 12-HETE as the main metabolite (1, 8, 9). Interestingly, in C6 glioma cells, the cyclooxygenase pathway appears to be predominant (8). The present results, showing that blockade of each of the arachidonic acid metabolic avenues with indomethacin (cyclooxygenase), esculetin (lipoxygenase), or metyrapone (*P*-450) failed to reverse the arachidonic acid inhibitory effect, suggest that further metabolism of arachidonic acid is not required for the inhibition of RVD and associated osmolyte fluxes in astrocytes. A consistent lack of effect of indomethacin on either RVD or osmolyte fluxes has been reported in other preparations (6, 12, 15, 31), excluding a role for prostaglandins, prostacyclins, and thromboxanes on RVD. Intermediates of the lipoxygenase pathway seem not to be involved in RVD in astrocytes, since *1*) in cultured primary astrocytes, 12-HETE, the main metabolite of the lipoxygenase pathway, did not modify RVD or osmolyte efflux (present results); *2*) in C6 glioma cells, the intermediates of the lipoxygenase pathway are present in small amounts (18); *3*) their concentration is not affected during swelling or RVD (18); and *4*) inhibitors of the lipoxygenase pathway either have effects per se or did not affect RVD or osmolyte fluxes (Ref. 18 and present results). The most noticeable example is ketoconazole, which rapidly and reversibly blocks volume-sensitive anion channels in glioma cells (18), suggesting a direct effect of this compound on the anion conductance, not secondary to arachidonic acid metabolic conversion. Altogether, these results suggest that arachidonic acid effects are not mediated by metabolic products and support the suggestion of a direct effect of arachidonic acid on the main osmolyte permeability pathway activated during RVD.

A potent inhibitory effect of some blockers of the lipoxygenase pathway, such as NDGA, ketoconazole, 5,8,11,14-eicostetraynoic acid, and gossypol, has been observed in a number of preparations (6, 12, 15, 17) including C6 glioma cells (8, 31), but the mechanisms subserving these actions are unclear at present. An effect of NDGA as blocker of calcium channels has been described (14), but this could not explain its effects on RVD and osmolyte fluxes, which in our preparation are independent of extracellular calcium. Besides, the effects of NDGA persist in the absence of external calcium. NDGA may also reduce RVD by increasing sodium accumulation (12), a likely possibility because NDGA has a less pronounced inhibitory effect on RVD in sodium-free medium. However, since even in these conditions a substantial portion of the inhibition persists, other mechanisms must be involved as well. All these observations, although suggestive of a role for components of the arachidonic acid cascade as modulatory signals on RVD, may not be related to the effect of arachidonic acid. Moreover, the fact that arachidonic acid metabolites seem not to affect RVD or osmolyte fluxes (except LTD_4 in Ehrlich ascites cells) (15) supports the interpretation of direct effects of arachidonic acid on RVD and related osmolyte fluxes.

The physiological meaning of the inhibitory effects of arachidonic acid and other polyunsaturated fatty acids on RVD in astrocytes is unclear at present. It is not known whether swelling results in arachidonic acid mobilization from membranes and whether this intracellularly released arachidonic acid has access to the osmolyte pathways as does the externally applied arachidonic acid. The consequences of swelling on the intracellular levels of arachidonic acid metabolites are also unknown in astrocytes. Clearly, more work is required to investigate whether arachidonic acid or some of its metabolites are effectively functioning as intracellular signals for RVD in this preparation. The inhibitory effect of arachidonic acid on RVD in astrocytes has, in contrast, clear implications in damage to nervous tissue cells observed in situations of hyperexcitability, such as ischemia, epilepsies, and excitotoxicity, where arachidonic acid is indeed released from membrane phospholipids (5, 20, 26). As cell swelling occurs under all these pathological conditions, increased arachidonic acid, by preventing the volume-corrective fluxes of osmolytes, contributes to the injury cascade triggered by hyperexcitability.

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