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Mechanisms of Glial Swelling by Arachidonic Acid

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Summary

The effect of arachidonic acid (AA, 20:4) was analyzed in vitro by employment of C6 glioma cells and astrocytes from primary culture. The cells were suspended in an incubation chamber under continuous control of pH, pO_2, and temperature. Cell swelling was quantified by flow cytometry. After a control period, the suspension was added with AA at concentrations of 0.01 to 1.0 mM. Administration of AA induced an immediate, dose dependent swelling in C6 glioma cells or astrocytes. AA-concentrations of 0.01 mM led to an increase of the glial cell volume to 103.0 ± 1.0% of control, 0.1 mM to 110.0 ± 1.5%, and 1.0 mM to 118.8 ± 1.5% within 10 min. The swelling response to linoleic acid (18 : 2) was only about half of what was found when AA was administered at a concentration of 0.1 mM, whereas stearic acid (18 : 0) did not induce any cell volume changes. Inhibition of the cyclo- and lipoxygenase pathway by BW 755C did not prevent glial swelling from AA, whereas it was reduced by SOD, or almost completely abolished by the aminosteroid U-74389F, an antagonist of lipid peroxidation. Replacement of Na^+- and Cl^--ions in the suspension medium by choline chloride was also associated with complete abolishment of cell swelling from AA. The results demonstrate an impressive efficacy of arachidonic acid to induce glial swelling which might be attributable to activation of lipid peroxidation by the fatty acid, leading to an increased Na^+-permeability and subsequent influx of water into the cells.

Keywords: Cytotoxic cell swelling; arachidonic acid; C6 glioma cells; astrocytes.

Introduction

Arachidonic acid (AA, 20 : 4) is a major constituent of membrane phospholipids in brain tissue. Normally, the free fatty acid is present only in a small amount, but it accumulates under adverse conditions, such as ischemia or brain injury[1,9]. The release of free fatty acids involves activation of phospholipases and breakdown of membrane phospholipids. AA in particular is considered to mediate pathological processes. The polyunsaturated compound is precursor of prostaglandins, leukotrienes, and oxygen-derived free radicals[11]. In cerebral ischemia concentrations of free AA of up to 0.5 mM/kg were found in brain tissue[9].

Noxious properties of AA have been observed in many investigations. For instance, it was demonstrated that lipid peroxidation or disturbances of respiration in mitochondria are induced by the fatty acid[2,6]. Moreover, AA has a significant role in brain edema. The fatty acid was found to induce swelling of cerebral tissue slices in vitro and to increase permeability of the blood-brain barrier in vivo[3,10]. Since AA may also be involved in cytotoxic brain edema formation, we have currently investigated the glial cell volume response during administration of the fatty acid. Additional experiments were performed to analyze, whether respective effects can be inhibited by blocking of the cyclo- and lipoxygenase pathway, by inhibition of free radical formation or of lipid peroxidation. To assess specificity of AA (20:4) comparative experiments were made using linoleic acid (18 : 2) or stearic acid (18 : 0). The study was carried out in vitro under control of relevant parameters to examine the significance of a single pathophysiological factor in isolation[8]. For that purpose, suspensions of C6 glioma cells or of astrocytes from primary culture were incubated under continuous recording of pH, pO_2, and temperature with assessment of the cell volume by flow cytometry.

Materials and Methods

C6 glioma cells were cultivated as monolayers in Petri dishes using Dulbecco's modified minimal essential medium (DMEM) with addition of 25 mM bicarbonate. The medium was supplemented with 10% fetal calf serum (FCS) and 100 IU/ml penicillin G and 50 µg/ml streptomycin. The cells were grown in a humidified atmosphere of 5% CO_2 and room air at 37°C. Glial cells from

primary culture were prepared from 3-day-old rats according to a modified method of Frangakis and Kimelberg[5]. The culture conditions were identical with those described above. The cells were harvested for the experiment with 0.05% trypsin/0.02% EDTA in phosphate-buffered saline and washed twice thereafter. After resuspension in serum-free medium the glial cells were transferred to a plexiglas incubation chamber which was supplied with electrodes for control of pH, temperature, and pO_2. Details of the method have been published elsewhere[8].

The volume of the glial cells was determined by flow cytometry using an advanced coulter system with hydrodynamic focusing[7]. The experiments with administration of AA were performed after a 45 minute control period used for measurements of normal cell volume and medium osmolality. Subsequently, the suspension was added with AA (20 : 4) in a dose range of 0.01 mM to 1.0 mM (final concentration). Cell volume and viability were measured for 90 min during incubation with AA. Comparative studies using linoleic- (18 : 2) or stearic acid (18 : 0) were made at concentrations of 0.1 mM. Further experiments with AA at this concentration were conducted with inhibition of the cyclo- and lipoxygenase pathway by BW755C (0.2 mM), with scavenging of superoxide radicals by superoxide dismutase (SOD, 300 U/ml), or during blocking of lipid peroxidation by the aminosteroid U-74389F (0.1 mM). Inhibitors were administered to the suspension 15 min prior to addition of AA. In further experiments cell swelling by 0.1 mM AA was studied in Na^+-free medium, where Na^+-ions were replaced for that purpose by choline, and bicarbonate by 10 mM HEPES.

Results and Discussion

Administration of AA to the suspension caused an immediate dose-dependent swelling of C6 glioma cells. AA-concentrations as low as 0.01 mM led to an increase of the cell volume to 103.0 ± 1.0% (mean ± SEM) of control within 10 min ($p < 0.01$). A volume increase to 110.0 ± 1.5% (Table 1) or 118.8 ± 1.5% of control was obtained, when the cells were administered with AA at 0.1 or 1.0 mM, respectively ($p < 0.001$). After initial and rapid swelling the increased cell volume was largely constant for the remaining observation period (Table 1). Swelling of C6 glioma cells by AA was confirmed in experiments using astrocytes from primary culture. Addition of AA at a dose level of 0.05 or 0.1 mM led to swelling of astrocytes ($p < 0.01$), which was comparable to that of C6 glioma cells. In order to assess specificity of the AA-induced glial swelling, cell swelling inducing properties of 0.1 mM linoleic (18 : 2) or of 0.1 mM stearic acid (18 : 0) were tested (Table 1). While stearic acid was not found to

Table 1. *Volume Response of C6 Glioma Cells to Administration of AA (0.1 mM) to the Suspension Medium.* Cell swelling was significant ($p < 0.01$) during the entire observation period as compared to a control group without addition of AA (not shown). The cell volume response to AA is further shown during inhibition of the cyclo- and lipoxygenase pathway by BW755C (0.2 mM), in the presence of SOD (300 U/ml), U-74389F (0.1 mM), or in Na^+-free incubation medium. Cell volume is given in percent of the cell size obtained during the last 15 min of the control period. Mean ± SEM of 4–7 experiments per group are shown

	Control			Incubation with free fatty acids					
Time	−15	−10	−5	1	5	10	30	60	90
AA 0.1 mM	100.40	99.93	99.67	104.45	110.04	112.17	111.00	109.58	106.85
	0.26	0.17	0.25	1.16	1.49	1.90	2.23	2.20	1.72
LA 0.1 mM	100.36	99.63	100.01	103.05	106.53	105.88 *	105.66	108.22	105.53
	0.61	0.35	0.75	0.71	0.82	0.76	0.82	1.38	1.41
SA 0.1 mM	100.63	99.07	100.30	100.07 **	100.45 **	99.42 **	99.36 **	99.46 **	100.25 **
	0.37	0.64	0.28	0.54	0.96	0.26	1.44	2.13	2.23
AA 0.1 mM	99.88	99.90	100.23	103.84	109.60	111.18	112.36	107.57	106.91
+ BW 755 C	0.29	0.20	0.33	1.77	1.58	2.04	2.32	2.58	3.86
AA 0.1 mM	99.84	100.25	99.91	104.59	108.28	109.42	106.79	103.19 **	103.05
+ SOD	0.09	0.44	0.43	2.03	1.98	1.17	0.82	1.03	0.80
AA 0.1 mM	100.27	99.40	100.33	99.41 **	100.18 **	98.93 **	99.47 **	100.97 **	101.39 **
+ U-74389F	0.33	0.49	0.35	0.70	1.29	1.35	1.37	1.11	1.53
AA 0.1 mM	98.63	101.25	100.12	100.87 **	101.65 **	103.77 **	100.01 **	102.00 **	103.46
Na^+-free M.	0.75	0.40	0.44	0.46	0.41	0.86	1.82	1.99	2.51

AA arachidonic acid, *LA* linoleic acid, *SA* stearic acid.
* $p < 0.05$ vs. AA 0.1 mM.
** $p < 0.01$ vs. AA 0.1 mM.

induce volume changes of the glioma cells, cell volume was significantly increased by linoleic acid ($p < 0.01$). Nevertheless, the volume increase to linoleic acid was only about 50% of what was found when AA was administered at the same concentration ($p < 0.05$; Table 1). Additional experiments were performed to analyze mechanisms of the AA-induced glial swelling. Inhibition of the metabolization of AA by cyclo- and lipoxygenase using the dual pathway inhibitor BW755C did not affect glial swelling from AA (Table 1). Administration of SOD to scavenge superoxide radicals did not influence the initial volume response but reduced glial swelling by AA significantly at 60 min after addition of the fatty acid ($p < 0.01$). Preincubation with the aminosteroid U-74389F, however, prevented cell swelling from AA practically completely ($p < 0.01$). Similar results were obtained when the experiments were conducted with choline for replacement of Na^+-ions in the suspension medium and HEPES as buffer compound instead of bicarbonate (Table 1).

The present results demonstrate a powerful potential of AA to induce glial swelling. Exposure of glial cells to the fatty acid led to a dose-dependent cell volume increase at concentrations, which have been observed in brain tissue in vivo under pathophysiological conditions, such as focal injury or ischemia[1,9].

Not only AA but also its metabolites, such as prostaglandins or leukotrienes, can be considered to have mediator functions in the brain under respective circumstances[11]. The present results on inhibition of the cyclo- and lipoxygenase pathway by BW755C suggest, however, that AA itself was the swelling inducing agent but not these metabolites. On the other hand, since administration of the aminosteroid U-74389F was almost completely inhibiting cell swelling from AA, lipid peroxidation must be taken into consideration as major factor of AA-induced cell swelling. Lipid peroxidation commences, if oxygen-derived free radicals accumulate in the presence of free fatty acids[2,4]. It is conceivable that superoxide radicals generated by the conversion of AA were interacting with AA as substrate, thereby initiating the formation of lipid- and lipidperoxide radicals. The marginal success of SOD to reduce glial swelling from AA suggests that superoxide radicals formed in the intracellular compartment were not available for the enzyme, since SOD as a large, hydrophilic molecule is unlikely to penetrate the plasma membrane. Accumulation of lipid- and lipidperoxide radicals, however, may result in a chain reaction, mainly acting on fatty acids of cell membranes, consequently leading to damage of the double-lipid layer[2]. The results indicate further that the swelling-inducing properties of free fatty acids are related with their number of non-saturated chemical bonds. Accordingly, the production of superoxide- and lipid radicals from free fatty acids by glial cells appears to be directly correlated with the number of non-saturated bonds. This is supported albeit indirectly by findings that formation of reactive radical species is minimal from saturated fatty acids as precursors[4]. Finally, glial swelling from AA was more or less completely prevented, if the experiments were conducted in a Na^+-free suspension medium making obvious the significance of the cellular uptake of Na^+, and thereby water as ultimate mechanism of the AA-induced cell volume increase.

Taken together, AA causes swelling of C6 glioma cells and of astrocytes from primary culture, probably by activation of lipid peroxidation among others. The lipid peroxides by damaging lipid bilayers of cell membranes are likely to increase Na^+-permeability and, thus influx of water into the cells. The resulting cellular accumulation of Na^+ together with Cl^- and water must be viewed as the final step of glial cell swelling.

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