Journal of Neuroscience Research 24:247–250 (1989)

Brain Mitochondrial Swelling Induced by Arachidonic Acid and Other Long Chain Free Fatty Acids

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Polyunsaturated fatty acids (PUFAs), arachidonic acid in particular, are well known, potent inducers of edema in the brain, while monounsaturated and saturated long chain fatty acids do not possess this quality. This investigation has compared the ability of some free fatty acids (FFAs), known to be released during cerebral ischemia, to induce brain mitochondrial swelling in vitro. The PUFAs tested, especially arachidonic acid (20:4), were more potent in causing swelling than saturated or monounsaturated ones, as measured by the decrease in light absorbance of the mitochondrial suspension. This finding is in line with the unique potency of 20:4 to induce brain edema. Incubation of brain mitochondria with 20:4 for 20 min caused a dose-dependent swelling. ATP-$MgCl_2$ both prevented and reversed this swelling, while binding of the 20:4 by the addition of bovine serum albumin could only prevent but not reverse the swelling. The contraction of the swollen mitochondria appeared to be mediated by a mechanism dependent upon high-energy phosphates, potentiated by $MgCl_2$.

The concentration of 20:4 required to induce swelling was about 20 times higher than the concentration required to induce inhibition of mitochondrial respiratory function (L Hillered and P H Chan: J Neurosci Res 19:94–100, 1988a). Morover, reversal of the swelling occurred without recovery of respiratory function. These results suggest that swelling is a phenomenon of minor importance as an indicator of brain mitochondrial dysfunction, at least when induced by 20:4 in vitro.

Key words: mitochondria, edema, recovery

INTRODUCTION

It is well documented that polyunsaturated fatty acids (PUFAs), particularly arachidonic acid (20:4), are potent inducers of brain edema when incubated with cortical slices (Chan and Fishman, 1978) and when injected into the brain (Chan et al., 1983; Black and Hoff, 1985). Docosahexaenoic acid (22:6) is less effective in causing edema than 20:4, while monounsaturated (e.g., oleic acid, 18:1) and saturated (e.g., palmitic acid, 16:0) fatty acids do not have this ability. Although free fatty acids (FFAs), especially the medium and long chain saturated ones, are well known to affect adversely several mitochondrial functions in general (reviewed by Wojtzak, 1976), little information exists about the pathophysiological effects of long chain PUFAs at the cellular/subcellular level in the brain. We have reported that 20:4, in concentrations relevant to ischemic conditions in vivo, markedly inhibited respiratory activities of isolated brain mitochondria (Hillered and Chan, 1988a). More recently, we found that such mitochondrial dysfunction was partly involved in the mitochondrial response to cerebral ischemia (Hillered and Chan, 1988b). Kuwashima et al., (1976) reported that PUFAs were more potent than monounsaturated and unsaturated FFAs in inhibiting brain mitochondrial respiration and ATP production in vitro. We now extend previous work by comparing the capacity of the long chain FFAs palmitic (16:0), stearic (18:1), 20:4, and docosahexaenoic (22:6) acids to induce brain mitochondrial swelling and by studying the reversibility of such swelling.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200–300 g) with unlimited access to rat pellets and tap water were used. The following substances were obtained from Sigma (St. Louis, MO): adenosine, adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), adenosine

Received December 27, 1988; revised April 17, 1989; accepted April 18, 1989.

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5′-triphosphate (ATP), arachidonic acid (20:4), bovine serum albumin (BSA; fatty acid free), docosahexaenoic acid (22:6), ethyleneglycol-bis-(β-amino-ethylether)N, N′-tetraacetic acid (EGTA), oleic acid (18:1), and palmitic acid (16:0). Ficoll 400 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Other chemicals were commercial products of the highest available purity.

Brain mitochondria were isolated by a modification of the method of Clark and Nicklas (1970) as described elsewhere (Hillered and Ernster, 1983). In summary, the tissue was disintegrated manually and enzymatically in a mannitol-sucrose-EGTA medium containing BSA (2.5 mg/ml). The crude mitochondrial pellet was purified using a Ficoll density gradient. This method consistently yields mitochondrial preparations with respiratory control ratios between 7 and 10. The maximal respiratory rates and the purity (0.17–0.23 nmol cytochrome a + a_3 per mg of mitochondrial protein) of the preparations are similar to those reported in other studies using the same technique (Clark and Nicklas, 1970; Rehncrona et al., 1979; Hillered and Ernster, 1983; Hillered et al., 1984). Mitochondrial swelling was measured as the decrease in optical density of the mitochondrial suspension at 520 nm at room temperature. This is a widely used method for measuring the change in volume of suspended mitochondria (Koch, 1961; Lehninger, 1962), and such measurements correlate well with ultrastructural changes (Stoner and Sirak, 1969). Brain mitochondria (0.9 mg of protein; 0.3 mg/ml were suspended in 3.2 ml of a reaction medium consisting of 150 mM KCl and 10 mM phosphate buffer (pH 7.4). The change in absorbance was recorded continuously during 20 min in the presence of 20:4 (0–600 nmol/mg protein; 0–169 μM) using a Diode Array Spectrophotometer (Hewlett-Packard 8451A). Prevention and reversal of the swelling effect was studied by the addition of BSA (2 mg/ml) or ATP (5 mM)-$MgCl_2$ (3 mM; final concentrations) before or during the experiment. In a separate series of experiments the swelling-inducing capacity of palmitic (16:0), oleic (18:1), arachidonic (20:4), and docosahexaenoic (22.6) acid (600 nmol/mg of protein; 169 μM, respectively) was compared. The FFAs used were dissolved in a small volume (0.3 ml) of ethanol and diluted in a large volume (11–28 ml) of the reaction medium immediately before the experiment was started. The initial rate of swelling was the change in absorbance during the first 2 min and the total swelling during 20 min following the addition of the fatty acid to the reaction medium.

One-way analysis of variance and multiple range analysis for the means using 95% confidence intervals (Statgraphics, STSC Inc., Rockville, MD) was employed for the comparison of multiple means in Table I.

TABLE I. Comparison Between Various Long Chain Free Fatty Acids in Their Ability To Induce Brain Mitochondrial Swelling*

Free fatty acid	Initial rate (abs × 100)	Total swelling (abs × 100)
Control	1.5 (0.3)	7.5 (0.6)
Palmitic (16:0)	4.8 (1.2)[a]	19.8 (1.4)[a]
Oleic (18:1)	6.8 (1.3)[a]	21.8 (1.9)[a]
Arachidonic (20:4)	14.8 (3.6)[a,b,c,e]	26.7 (3.2)[a,b,c]
Docosahexaenoic (22:6)	7.8 (2.4)[a,b,d]	25.9 (1.8)[a,b,c]

*Initial rate of swelling was the decrease in absorbance (520 nm) during the first 2 min and the total swelling that occurred during 20 min of incubation with each FFA at 600 nmol/mg protein (169 μM). Values were the means of five preparations and SD. Statistically significant difference from [a]control, [b]16:0, [c]18:1, [d]20:4, and [e]22:6.

Differences with a P value < 0.05 were considered statistically significant.

RESULTS

Table I shows the relative capacity of the long chain saturated (16:0), monounsaturated (18:1), and polyunsaturated (20:4, 22:6) fatty acids (600 nmol/mg protein; 169 μM, respectively) to induce brain mitochondrial swelling. The initial rate of swelling (first 2 min) was almost twice as high with 20:4 as with the other FFAs. The total swelling during 20 min was more pronounced (19–35%) with the PUFAs as compared with 16:0 and 18:1.

Incubation of brain mitochondria with 20:4 (150-600 nmol/mg protein, 42–169 μM) resulted in a dose-dependent swelling effect probably reflecting a passive influx of K^+ and Cl^- owing to a FFA-induced increase in membrane permeability (Lehninger, 1962; Brierley, 1976). The time course of this effect is illustrated in Figure 1, summarizing the data from several preparations. Figure 2 shows examples of recordings demonstrating the reversibility of the 20:4-induced swelling using BSA and ATP-$MgCl_2$. The spontaneous swelling of untreated mitochondria was partly prevented and partly reversed by ATP-$MgCl_2$, but was not affected by BSA. When BSA or ATP-$MgCl_2$ was present during the incubation, 20:4 did not cause swelling. The observation that 2 mg/ml of BSA prevented swelling even in the presence of the highest concentration of 20:4 (abs × 100 during 20 min being 7.7, which is within the control range) indicates that the amount of BSA used was sufficient to bind the added FFAs. Addition of ATP-$MgCl_2$ 10 min following the addition of 20:4 reversed the swelling. At the higher concentration of 20:4 the swelling was reversed beyond the control level. ATP alone was less effective in reversing the swelling than ATP-$MgCl_2$, while $MgCl_2$ alone had no effect. ADP-$MgCl_2$ was almost as effective as ATP-$MgCl_2$, while AMP-$MgCl_2$ only partly reversed

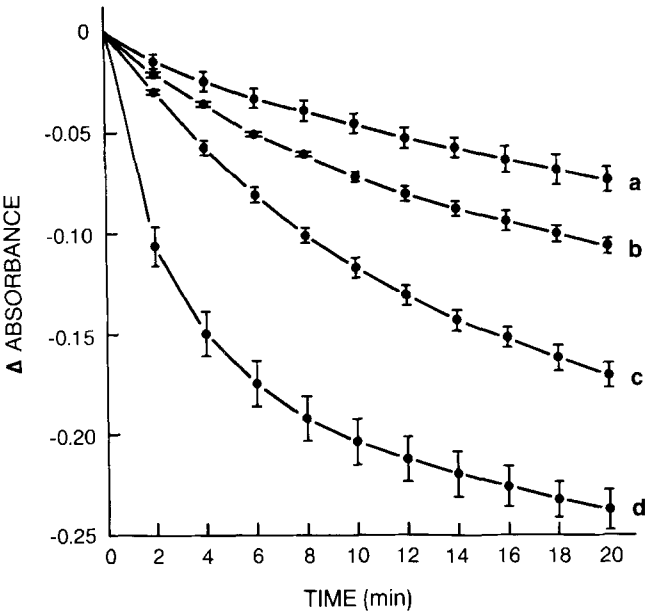

Fig. 1. Swelling of isolated brain mitochondria incubated with arachidonic acid measured as the decrease in optical density (520 nm) of the mitochondrial suspension. Values were the means (and SD) from five preparations showing the time course of the swelling effect. Mitochondria (0.3 mg/ml) were suspended in 3.2 ml of a medium consisting of 150 mM KCl and 10 mM phosphate buffer, pH 7.4, and incubated **a:** without, **b:** with 150, **c:** 300, and **d:** 600 nmol/mg protein of arachidonic acid.

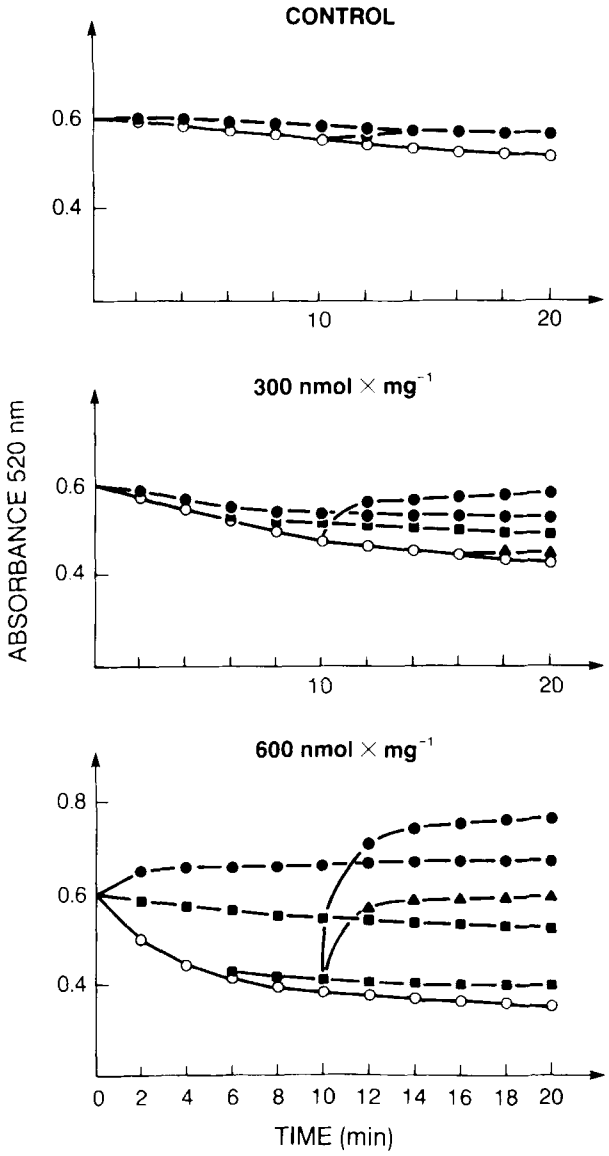

Fig. 2. Swelling of isolated brain mitochondria incubated with arachidonic acid measured as the decrease in optical density of the mitochondrial suspension. Examples of recordings illustrating the reversibility of the swelling effect. When indicated, 5 mM ATP and 3 mM $MgCl_2$ (closed circles), 5 mM ATP (closed triangles), or 2 mg/ml bovine serum albumin (BSA, closed squares) was added to the medium containing mitochondria and arachidonic acid (open circles).

the swelling. Adenosine-$MgCl_2$ had no effect (data not shown). BSA added 2 min after the addition of 20:4 ameliorated the swelling slightly but had no effect when added at 10 min (Fig. 2). The swelling effect of 20:4 was similar in the presence of respiratory substrates such as malate and glutamate (4.5 mM, respectively; data not shown).

DISCUSSION

The aim of this study was to compare the individual abilities of saturated (e.g. 16:0), monounsaturated (e.g. 18:1), and polyunsaturated (e.g. 20:4 and 22:6) FFAs to induce swelling of isolated brain mitochondria and to study some features of mitochondrial swelling induced by 20:4. The finding that the PUFAs, especially 20:4, were clearly more potent than 16:0 or 18:1 in causing mitochondrial swelling correlates with the unique ability of these FFAs to induce brain edema (for references, see Introduction) and suggests that they may affect mitochondrial and other membranes by a common mechanism.

The data presented in Figure 2 demonstrate that 20:4-induced mitochondrial swelling is reversible upon the addition of ATP-$MgCl_2$. The ability of ATP-$MgCl_2$ to induce contraction of isolated mitochondria following FFA-induced swelling in general is well known (for references, see Lehninger, 1962). To our knowledge this is the first report of 20:4-induced brain mitochondrial swelling and extends previous observations by indicat-

ing that the contraction was mediated by a mechanism dependent upon high-energy phosphates and potentiated by magnesium ions. Our results are compatible with the contractile mechanism as an explanation for the energy-dependent contraction of the swollen mitochondria. According to this hypothesis a conformational change occurs in the membrane upon energization, leading to the extrusion of cations, anions, and water as the membrane contracts (Lehninger, 1962; Brierley, 1976). This type of contraction is not affected by respiratory inhibitors or by uncouplers of oxidative phosphorylation (Lehninger, 1962). This fits well with our observation that the contraction occurs in spite of an inhibition of respiration and an uncoupling caused by 20:4 (Hillered and Chan, 1988a). The mechanism of the contraction may be the incorporation of FFAs into membrane phospholipids upon the addition of ATP-$MgCl_2$ (Wojtzak and Lehninger, 1961). This is in agreement with our finding that binding of the 20:4 by the addition of BSA could prevent but not reverse the swelling (Fig. 2), suggesting that the FFAs were incorporated into the membrane or accumulated intramitochondrially. Since the transport system for FFAs across the mitochondrial membrane (carnitine, acetylcarnitine, and carnitine acetyltransferase) is poorly developed in brain mitochondria (Marquis and Fritz, 1965), an intramitochondrial effect is less likely.

The present results raise the following question: Does mitochondrial swelling, a well-known histopathological feature of ischemic cell damage (Trump et al., 1976) underlie cellular edema induced by 20:4 in the brain, or, in other words, does mitochondrial swelling correlate with an inhibition of mitochondrial respiratory capacity? To address this question it seems relevant to compare the present results with our previous study on the effect of 20:4 on brain mitochondrial respiratory activities (Hillered and Chan, 1988a). In that study we found that incubation of mitochondria with 20:4, 7–53 nmol/mg protein (i.e., the estimated concentration range to which the mitochondria are exposed in vivo during ischemia) markedly inhibited their respiratory capacity. Since mitochondrial swelling required much higher concentrations of 20:4, 150–600 nmol/mg protein (Fig. 1), and since the respiratory inhibition was not reversed by ATP-$MgCl_2$ (not shown), it seems clear that respiratory inhibition is a much more sensitive indicator of 20:4-induced mitochondrial dysfunction than mitochondrial swelling and that severe inhibition of respiratory capacity may be present in mitochondria with normal volume. In conclusion, mitochondrial swelling may be a phenomenon of minor importance as an indicator of brain mitochondrial dysfunction, at least when induced by 20:4 in vitro.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Robert A. Fishman for constructive criticism and continuous support. This project was supported by N.I.H. grants NS-14543 and NS-25372, the Swedish Medical Research Council (Project No. 7888), the Swedish Society of Medicine, the Trygg-Hansa Foundation, Pharmacia AB, and Volvo of America Corporation.

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