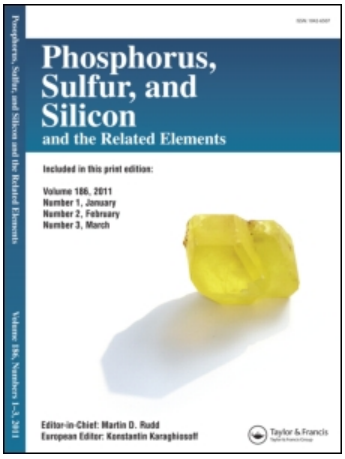

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ISOLATION AND HPLC SEPARATION OF POLYUNSATURATED SPECIES OF RAT BRAIN ACYL-CoA PRODUCED DURING DECAPITATION - ISCHEMIA

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Abstract Acyl-CoA is a crucial metabolic intermediate for the incorporation of fatty acid into membrane phospholipid. The rat brain long chain acyl-CoAs were isolated and quantitated with a procedure involving solid phase extraction with an oligonucleotide purification cartridge, followed by separation and quantitation by HPLC with special emphasis on resolution of the polyunsaturate acyl-CoAs. This procedure is uniquely suited to the rapid analysis of all molecular species of brain acyl-CoA including the polyunsaturate molecular species which are affected by ischemia. A selective 3-4 fold increase in arachidonoyl-CoA was found in rat brain after 3 min of ischemia when compared with microwaved brain. In contrast, the concentration of all other molecular species of acyl-CoA did not change over the time course of the ischemia.

Key Words: Acyl-CoA, Phospholipids, Arachidonic acid, HPLC, Brain ischemia.

INTRODUCTION

Studies on fatty acids released in rat brain during the early phase of decapitation ischemia show a rapid increase in all fatty acids with a selective increase in arachidonate and stearate (1,2). Several reports suggest that arachidonic acid is predominantly liberated from inositol-containing phospholipids by combining action of phospholipase C and diglyceride lipase or from the activity of phosphatidil-choline specific phospholipase and lysophospholipase (1,2,3,4). Changes in the fatty acids levels can affect the long chain acyl-CoAs which are essential metabolic intermediates for the incorporation of fatty acids into lysophospholipids through the action of acyltransferase (5) and for their β-oxidation in mitochondria and peroxisomes (6). Previously we have reported a fast method for quantitation of the long-chain acyl-CoAs from rat brain using isopropanol:acetonitrile extraction followed by purification with an oligonucleotide purification cartridge prior to HPLC separation (7). However, due to difficulties in the HPLC separation and quantification of the polyunsaturated long chain acyl-CoAs, levels of docosahexaenoyl- and arachidonoyl-CoA could not be directly ascertained. We now have a HPLC protocol whereby the acyl-CoAs of these two essential fatty acids can be resolved and quantified. This procedure can be readily applied to the determination of the effect of ischemia on the level of acyl-CoAs. This

is a first step in the analysis of the effect of ischemia on fatty acid flux into brain membrane phospholipids.

MATERIALS AND METHODS

Materials, brain tissue extraction, solid-phase extraction and the HPLC system were as previously described (7). The HPLC separation was performed with a Symmetry C-18, 5 micron column 250 x 4.6 mm; Waters Millipore Corp., (Milford, MA) provided with a stainless steel filter. Chromatography was performed using a combined gradient system including two mobile phases: (A) 75mM potassium dihydrogen phosphate and (B) 100% acetonitrile with a flow rate of 1.0 ml/min. The starting conditions were 56% buffer A and 44% B. B was increased to 49% over 25 min and then increased to 70% during the next 5 min, kept a 70% for 7 min and decreased to 44% over 4 min, and held at 44% for an additional 4 min before returning to the starting conditions.

Animal Preparation: Male Sprague-Dawley laboratory rats (180-210 g) purchased from Charles River Laboratories (Wilmington, MA) were maintained with free access to food and water. Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and killed by focused-beam microwave irradiation (5.5 kW, 3.0 s; Cober Electronics, Stanford, CT). Ischemia was achieved by decapitation of pentobarbital-anesthetized rats. Brains were removed within three minutes. The isolated brains were either frozen in dry ice pellets at three minutes or maintained in a plastic bag at 37°C in a controled-temperature oven for 15 min and then frozen in dry ice.

RESULTS AND DISCUSSION

In this work we report the refinement of previous methodology which allowed us to rapidly analyze the individual molecular species of rat brain acyl-CoA (7). Using a more protracted gradient elution scheme with a suitable system we are able for the

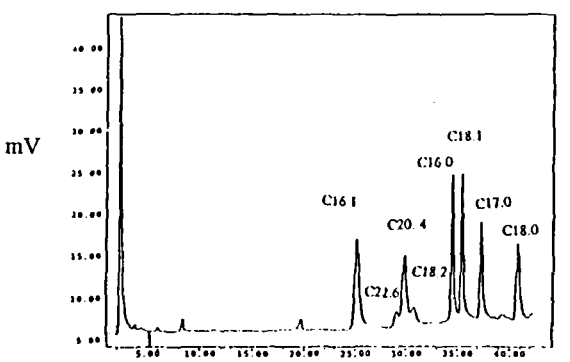

FIGURE 1 HPLC pattern of acyl-CoA standards.

first time to resolve the individual polyunsaturate molecular species namely

docosahexaenoyl-, arachidonoyl- and linoleoyl-CoA (Fig. 1). A standard mixture of acyl-CoAs was separated by combining a 25 min shallow gradient elution with acetonitrile in 75 mM potassium dihydrogen phosphate followed by a 5 min step gradient. The extraction efficiency using hexadecaenoyl-CoA as internal standard was 100% for arachidonoyl-, linoleoyl-, and docosahexaenoyl-CoAs and 95-100% for palmitoyl-, oleoyl- and 75% for stearoyl- CoAs. In order to increase the accuracy of the quantitation of the late eluting acyl-CoAs, an additional internal standard (heptadecanoyl-CoA), was added. The elution profile of acyl-CoA's isolated from

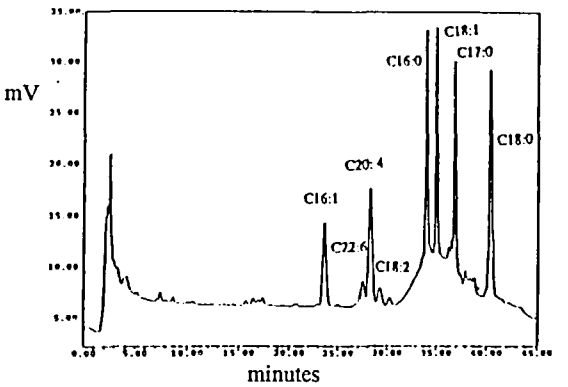

FIGURE 2 HPLC pattern of rat brain spiked with acyl-CoA standards.

microwaved rat brain spiked with the polyunsaturated acyl-CoAs together with the palmitoyl-, oleoyl- and stearoyl-CoAs (Fig 2) was achieved by combining the high performance of the oligonucleotide purification cartridge and optimized interaction of the Symmetry reversed-phase column with the amphipathic acyl-CoAs.

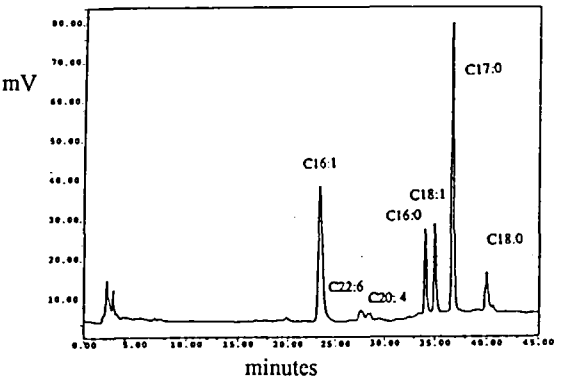

FIGURE 3 HPLC pattern of the microwaved rat brain (control).

In Fig. 3 is shown the pattern of microwaved rat brain spiked with 20 μg of

hexadecanoeoyl-CoA and heptadecanoyl-CoA, as internal standards, extracted and analyzed by HPLC. The docosahexaenoyl-CoA and arachidonoyl-CoA are indicated and linoleoyl-CoA is a smaller unmarked peak eluting at 29.3 min. The values found for the individual molecular species in microwaved brain were; 18:0, 5.1; 18:1, 10.8; 16:0, 8.4; 18:2, 0.9; 20:4, 1.6; 22:6, 1.7 nmole/g cerebral hemisphere giving a total of 28.6 nmole/g. Further, the method allowed us to monitor the selective increase in arachidonoyl-CoA during global ischemia.

Rats were decapitated after pentobarbital anesthesia and the brains excised and frozen in dry ice after 3 min and 15 min at 37°C. The HPLC elution profile of the acyl-CoA molecular species in a single rat brain analyzed after 3 min of decapitation ischemia is shown in Fig 4.

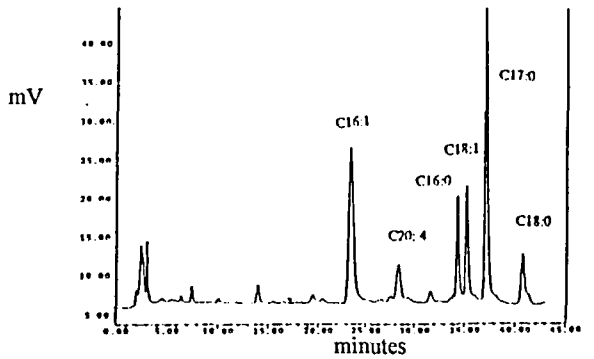

FIGURE 4 HPLC pattern of acyl-CoA obtained from rat brain after ischemia.

It is evident that there is a dramatic increase in the arachidonoyl-CoA with little apparent change in any of the other molecular species. The selective increase in arachidonoyl-CoA is of considerable interest due to the importance of this fatty acid to the stimulus-response coupling in brain and the short life for arachidonate in brain phospholipids. We believe that further measurements of acyl-CoA concentrations will bring in the future a new dimension to the knowledge of cerebral lipid metabolism.

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