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EFFECT OF ANEMIC HYPOXIA ON FREE FATTY ACID CONTENT IN RAT BRAIN MITOCHONDRIA

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Abstract

The aim of the present investigation was to establish the changes of the level of free fatty acids (FFA) in mitochondria from hypoxic rat brains.
Twenty male Wistar rats at the age of three months were subjected to sodium nitrite-induced anaemic hypoxia. Mitochondrial fraction was isolated and lipids were extracted. The free fatty acid content was measured by gas-liquid chromatography.
In the mitochondria of hypoxic brains, we found 1.6-fold increase of total FFA and increases of the individual FFA. Our results also showed that mitochondria tended to have higher concentrations of long-chain unsaturated fatty acids.

INTRODUCTION

The brain is highly oxidative organ and although it constitutes only 2% of body weight, it accounts for 20% of the total body oxygen consumption. Brain is of special interest for hypoxia studies as it is critically dependent on its oxygen supply. Low oxygen quickly results in ATP depletion and a consequent increase in adenosine. Approximately 50-60% of the brain's energy consumption goes to maintaining ionic gradients [1]. As a result brain suffers energy failure even after a few minutes reduction in oxygen supply.

Hypoxia, as well as ischemia, provokes alterations in the lipid metabolism. Although considerable efforts have been directed at evaluating alterations in hypoxia, lipid metabolism at brain subcellular level has not been fully evaluated. Most of the conducted studies concern the brain as a whole with no attempt of obtaining information on how sensitive to hypoxia brain subcellular structures are. In this connection, the mitochondria are increasingly recognized as a sensitive target for oxidative damage in hypoxia. It is known that mitochondrial oxidative phosphorylation is the primary source of high-energy compounds in the cell [2].

Free fatty acids have numerous well described biological properties some of which are related directly to their effects on mitochondria. Therefore, the studying of hypoxia-induced changes of mitochondrial FFA may be of basic significance for understanding the involved pathomechanism.

MATERIAL AND METHODS

Twenty male Wistar rats at the age of three months, each weighing 190-220 g, were subjected to sodium nitrite-induced anemic hypoxia. Sodium nitrite was administered intravenously at 20 mg/kg body weight (2 ml/kg dosing volume). This model is convenient because no restraint of the animal or special enclosure is required [3]. Hypoxic rats were killed by decapitation.

Mitochondrial fraction was isolated according to the method described by Venkov [4] using two-step sucrose gradient. Lipids were extracted according to the method of

Kates [5] using the following eluates: chloroform: methanol 1:2 (v/v) and chloroform: methanol: water 1:2:0.8 (v/v/v).

The FFA content was determined by gas-liquid chromatography. The fatty acids were converted to fatty acyl methylesters (FAME) by addition of methanol and 25% hydrochloric acid. The FAME were extracted by petroleum ether, then concentrated in a rotary vacuum evaporator and subjected to gas-liquid chromatographic analysis.

A gas chromatograph with flame ionization detector and connected with Trio Vector computing integrator was used. The analysis was performed by injecting 5 µl of the sample into SE-35 column. The temperature was programmed from 85ºC to 205ºC (2.5ºC/min). Nitrogen was used as carrier gas at a flow-rate of 40 ml/min.

The animal experiments were performed in accordance with animal protection guidelines approved by the Ethics Committee for experimental animal use at IEMAM – BAS.

The data were analyzed with Student's t-test.

RESULTS AND DISCUSSION

Mitochondria are intracellular organelles that play an essential role in cellular energy production [6]. In fact, they are the main source of high energy phosphate bond molecules in normal cells. It is known that biochemical functions of mitochondria strongly depend on membrane lipids. The relationship between the membrane lipid environment and its intrinsic enzymes is well documented in mitochondrial membranes [7].

Our data showed that the FFA pool in controls consisted mainly of arachidonic ($C_{20:4}$) and stearic ($C_{18:0}$) acids and they accounted for 32% and for 63% of total FFA, respectively (Fig. 1). We found small amounts of myristic ($C_{14:0}$), myristoleic ($C_{14:1}$), palmitic ($C_{16:0}$) and eicosadienoic ($C_{20:0}$) acids, too. These observations are in accordance to literature data [7]. The presence of arachidonic acid determines the high permeability of mitochondrial membrane which is of great importance for the normal respiratory chain function.

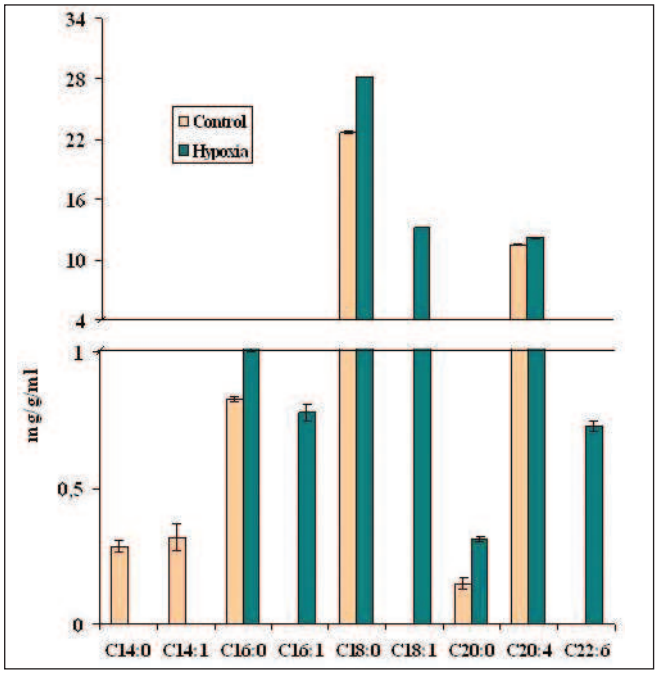

Fig. 1. Changes of the individual FFA in rat brain mitochondria after hypoxia. Values are expressed in mg/g dry lipid residue/ml. p< 0.001

It has been demonstrated that FFA have dual effect on mitochondrial respiration: protonophoric action on the inner mitochondrial membrane (depolarization of the inner membrane due to the uncoupling effect) and inhibitory action on the electron transfer chain [8]. Evidence has been provided that the unsaturated long-chain fatty acids linoleic and arachidonic stimulate reactive oxygen species (ROS) production, whereas saturated fatty acids myristic and palmitic are less active or without effect.

In our experiments we applied a model of sodium nitrite-induced anemic hypoxia. Anemic hypoxia refers to a reduction in hemoglobin's ability to transport oxygen. Sodium nitrite converts hemoglobin to methemoglobin and unlike ferrous form of hemoglobin, methemoglobin does not bind oxygen strongly. Thus the oxygen-carrying capacity of the blood is reduced. It is reported that the oxidation of oxyhemoglobin by nitrite to produce methemoglobin is a complex process that has been characterized by a lag phase followed by an autocatalytic phase [9].

No matter what the cause or type of hypoxia, oxygen limitation is generally considered as an impairment of mitochondrial respiration. Consequences of mitochondrial injury include metabolic failure, oxidative stress, disruption of Ca^{2+} homeostasis, promotion of apoptosis [10]. Total reduction of electron transport chain elements results in the formation of free radicals leading to the initiation of a free radical-mediated peroxidation.

Mitochondrial lipids also are subject to oxidative modifications during hypoxia. Lipid fraction is particularly sensitive to hypoxia, as compared to other macromolecular compounds. The peroxidation of lipids is considered to be a major mechanism of free radicals cell damage. The major phospholipid components of the mitochondrial membrane are rich in unsaturated fatty acids whose double bonds are especially susceptible to oxygen radical attack.

It is known that FFA are the first that undergo changes in different pathological states. Our results showed that free fatty acid changes are well pronounced in the mitochondria of hypoxic brains, too. The concentration of total FFA increased 1.6 times the control value (from 35,666±0,2 to 56.44±0,11 mg/g dry lipid residue/ml, $p<0.001$). Among the individual FFA, palmitic acid increased 1.3-fold (from 0,825±0,01 to 1.038±0,01 mg/g/ml, $p<0.001$), stearic acid – 1.2-fold (from 22,629±0,1 to 28.166±0,04 mg/g/ml, $p<0.001$), eicosadienoic acid – 2.1-fold (from 0,149±0,02 to 0.315±0,01 mg/g/ml, $p<0.001$), arachidonic acid – 1.1-fold (from 11,46±0,08 to 12.202±0,03 mg/g/ml, $p<0.001$). In contrast, myristic and myristoleic acids were not detected after hypoxia (Fig. 1). Probably these short-chain FFA are involved in long-chain FFA synthesis. The increase of free fatty acid pool is a result of their release from membrane phospholipids by the action of phospholipases A_1, A_2, C and D and it is due to the disturbances in the dynamic equilibrium between FFA and the acyl groups of membrane phospholipids. Arachidonic acid is known to inhibit the Na^+, K^+-ATPase activity and thus to alter the energy metabolism. Oxidative metabolism of $C_{20:4}$ is considered to be a major source of ROS in hypoxia, which may generate lipid peroxides and cytotoxic products like 4-hydroxynonenal, acrolein and malondialdehyde [11].

Besides quantitative changes, our data also showed some differences in the composition of FFA pool. The mitochon-

dria of hypoxic brains contained palmitoleic acid ($C_{16:1}$) – 0.775±0,03 mg/g/ml, oleic acid ($C_{18:1}$) – 13.219±0,03 mg/g/ml and docosahexaenoic acid ($C_{22:6}$) – 0.725±0,02 mg/g/ml, which were absent in controls. The presence of docosahexaenoic acid could be explained by the fact that mitochondrial membrane is a basic site for synthesis of $C_{22:6}$, which subsequently is involved in the composition of the membrane phospholipids. The other long-chain FFA are synthesized mainly in the endoplasmaticum reticulum. Alterations in brain docosahexaenoic acid and other polyunsaturated FFA have been associated with changes in physicochemical function (enzyme activity, behaviors, etc.) [12]. The above data indicate a tendency to synthesize long-chain unsaturated fatty acids. The same tendency has been observed in our previous studies in cerebral ischemia [13, 14]. Polyunsaturated FFA have been shown to reverse excitotoxic changes triggered by glutamate[15] and thereby they are considered as neuroprotectors. The general anti-inflammatory effect of docosahexaenoic acid is also well documented [16].

In conclusion, our data provide evidence that anemic hypoxia influences FFA metabolism in rat brain mitochondrial membrane. They also show that brain mitochondria respond to hypoxia by synthesizing a high amount of unsaturated FFA and this is probably involved in the cell survival pathways.

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