Glutaminase activity in rat skeletal muscle mitochondria[1]

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Summary. Isolated rat skeletal muscle mitochondria took up about 40-ng-atoms O per min per mg protein, with glutamine as the only respiratory substrate. The mitochondria incubated in the presence of glutamine and KCN formed both ammonia and glutamate in equivalent amounts. The experiments reported here provide suggestive evidence that rat skeletal muscle mitochondria contain glutaminase (L-glutamine amidohydrolase EC 3.5.1.2.) activity.

It has been known for a very long time that muscular work is accompanied by ammonia production[2]. Experimental evidence was presented indicating that purine nucleotide cycle is the main process leading to the release of ammonia[3]. Much less is known about other enzymes such as glutamate dehydrogenase and glutaminase, which could provide ammonia from amino acids. The glutamate dehydrogenase activity in skeletal muscle is very low, and it is generally assumed that this enzyme does not play any role in the release of ammonia in skeletal muscle[3]. Skeletal muscle contains active glutamine synthethase[4] and is known to release considerable amounts of glutamine into the circulation[5]. It has also been shown that acute exercise lowered the levels of glutamine in plasma and in some tissues as liver, kidney and gastrocnemius muscle[6]. Glutaminase activity has been demonstrated in many rat tissues[7,8]; however, to our knowledge, the activity of this enzyme in skeletal muscle has not yet been described. Therefore the purpose of this study was to determine the activity of glutaminase in skeletal muscle. Taking into consideration that in many tissues phosphate-dependent glutaminase iso-enzyme has mainly mitochondrial localization[9–11], we looked for the activity of this enzyme in mitochondria.

Materials and methods. L-glutamine, L-glutamate, L-malate, fumarate, malonate, amino-oxyacetate, cytochrome c and glutamate dehydrogenase were from Sigma Chemical Co., ADP was from Lódz, Poland. All other chemicals were of the purest grade available and made up in twice quartz-distilled and deionized water.

Mitochondria were prepared as described previously[12]. Mitochondrial protein concentration was determined by ultraviolet absorption as described previously[13]. Respiration was measured with a Clark oxygen electrode at 25 °C in 2.5 ml medium containing: 50 mM potassium phosphate buffer, pH 8.0, 1 mM ADP, 1 mM $MgSO_4$, 10 μM cytochrome c and 2 mg of mitochondrial protein.

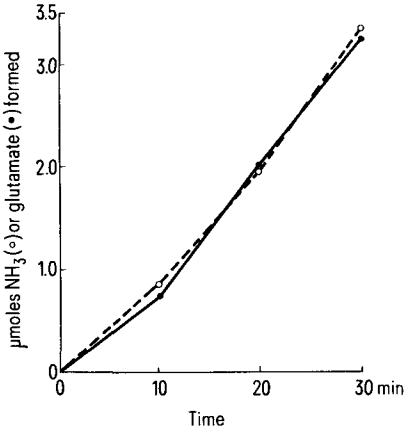
Glutaminase activity was assayed by measuring the rate of ammonia and glutamate formation at 30 °C. The incubation mixture contained: 50 mM potassium phosphate buffer, pH 8.2, 1 mM KCN, 20 mM L-glutamine and 3.2 mg of mitochondrial protein. Final volume was 1 ml. The reaction was started by the addition of mitochondria and stopped by the addition of 0.25 ml 10% $HClO_4$. The samples were centrifuged, the supernatant fluid neutralized with KOH and analyzed for glutamate and ammonia. The enzymic determination of glutamate was done as described by Bernt and Bergmeyer[14]. Ammonia was determined as described by Chaney and Marbach[15].

Results and discussion. It may be seen from the table that rat skeletal muscle mitochondria are able to oxidize L-glutamine. With L-glutamate as substrate for respiration, the mitochondria took up oxygen 3–4 times faster than

Oxidation of glutamine and glutamate by rat skeletal muscle mitochondria

Substrate	Respiration rate (ng-atoms of O/min per mg of protein)
20 mM L-glutamine	42.5
20 mM L-glutamate	150.0

Oxygen uptake was measured in the conditions outlined in 'materials and methods'.



The formation of ammonia (○) and glutamate (●) from glutamine by rat skeletal muscle mitochondria. Experimental conditions as outlined in 'materials and methods'.

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with L-glutamine as substrate assayed under the same conditions. However, the apparent K_m for glutamine as measured by the respiration rate was several times lower than for glutamate (1.4 mM and 16.6 mM for glutamine and glutamate respectively). To investigate whether glutamine oxidation is taking place through glutaminase pathway (L-glutamine amidohydrolase EC 3.5.1.2.) but not through transamination with keto acids catalyzed by glutamine transaminase (L-glutamine: 2 oxoacid aminotransferase, EC 2.6.1.15), the formation of the reaction products was determined. The figure shows the dependence on time of ammonia and glutamate formed in the presence of an inhibitor of respiratory chain. It appears that the first step of glutamine oxidation by rat skeletal muscle mitochondria is the hydrolysis of glutamine by glutaminase (L-glutamine amidohydrolase EC 3.5.1.2). Ammonia production from glutamate was very low under these conditions: at the end of the experimental period, less than 0.2 μmoles from glutamate was formed. Inhibition of glutamine and glutamate oxidation by malonate and amino-oxyacetate (not shown) suggests that these amino acids are oxidized mainly through the transamination pathway similarly as in mitochondria from other tissues[16–19]. Glutamate added or formed by hydrolysis of glutamine would undergo transamination with oxaloacetate, to form aspartate and 2-oxoglutarate. The latter would be oxidized through the Krebs cycle supplying further oxaloacetate for transamination. Inhibition by malonate of glutamine and glutamate oxidation could have been completely reversed by a low concentration of either L-malate or fumarate (not shown). These results also indicate that these amino acids are oxidized mainly through the transamination pathway. In this case oxaloacetate may be formed only from the malate or fumarate added, as malonate prevents the conversion of 2-oxoglutarate to oxaloacetate by inhibiting succinate dehydrogenase.

The results obtained in this study indicate that isolated rat skeletal muscle mitochondria are able to oxidize glutamine and that the first step of this process is deamination of glutamine via the pathway catalysed by glutaminase. It seem reasonable to postulate that in the muscle of the exercising rat, glutamine may be oxidized as one of the energy sources. The observation that in the exercising rat a significant decrease in plasma and tissues glutamine concentration occurs[6], may be pertinent in this respect.

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Search for sex-dependent and gestation-induced changes in choline and ethanolamine phosphorylating activities

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Summary. Choline kinase and ethanolamine kinase of liver, brain and kidney had nearly the same activity in 4-month-old male and virgin female mice. Ethanolamine kinase activity was almost doubled in the liver and brain of mice in advanced pregnancy compared with the virgin, while choline kinase activity was unaltered.

Sex-linked differences in enzyme activities and sex-specific enzyme activities and pregnancy-induced changes in enzyme activities have been documented[2]. Gulonolactonase in adult rat kidney, but not liver, exhibits sex dependence in activity and is controlled by androgens. The renal level of the enzyme in the male was 10–15-fold that in the female[2]. The activities of enzymes involved in the metabolism of steroid hormones in rat liver exhibit distinct sexual differences[3–5]. There is a striking sex-difference in the activity of liver histidine ammonialyase which was ascribed to induction of the enzyme forming system by oestrogen during pubertal development[6].

Several of the enzymes showing sex-dependent changes in activity are membrane-associated. Phospholipids are known to influence the activity of a number of membrane-bound enzymes, and in many of these cases the requirement is for a specific phospholipid. It was, therefore, of interest to examine whether sex-dependent change existed in choline- and ethanolamine kinase activity in liver, brain and kidney tissues, which are the active tissues involved in the phospholipid synthesis. During the gestation period there is additional requirement for phospholipids, and it is possible that there are differences in the requirement for choline phospholipids and ethanolamine phospholipids. The following study is a search for a possible sex-dependence in the phosphorylative activity towards choline and ethanolamine, and for any changes in the activity in the pregnant animal. A difference in the ratio of ethanolamine kinase to choline kinase between the male and the female adult, and between the virgin and gravid female, would be indicative of separate proteins catalyzing the phosphorylation of choline and ethanolamine.

Materials and methods. The mice used were 4-month-old. The 17–20 days pregnant animals used in the study had conceived for the 1st time. The animals were housed under standard conditions and fed the standard colony diet. The animals were sacrificed by cervical dislocation and decapitation. The liver, brain and the pair of kidneys were excised rapidly and kept chilled. The gall bladder from the liver was cut and discarded. After chilling and

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