likely that kynurenine is indeed synthesized from tryptophan within the brain. The assay method described should be useful both in the study of tryptophan and kynurenine metabolism in vivo, and in the assay in vitro of tryptophan pyrrolase and indolamine 2,3-dioxygenase.

We thank Dr. G. Slavin (Department of Histopathology, Northwick Park Hospital) who arranged the supply of autopsy material and Dr. P. Davies (Brain Metabolism Unit, Edinburgh) and Dr. D. M. Bowen (Institute of Neurology, London) for the material from therapeutic neurosurgery.

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Rat Brain Acetoacetyl-Coenzyme A Deacylase

TARUN B. PATEL and JOHN B. CLARK

Department of Biochemistry, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, U.K.

The formation of acetoacetate directly from acetoacetyl-CoA was first demonstrated by Stern & Miller (1959) in liver homogenates. Williamson et al. (1968) also demonstrated the presence of acetoacetyl-CoA deacylase in rat liver homogenates and reported that the major portion of the activity was cytosolic. However, Burch & Wertheim (1973) have shown that rat liver acetoacetyl-CoA deacylase is distributed in both the mitochondrial and cytosolic compartments, the mitochondrial activity forming 60% of the total rat liver deacylase activity. The existence of a mitochondrial acetoacetyl-CoA deacylase in human adipose tissue has been reported, and the possible importance of acetoacetate in fatty acid synthesis in this tissue discussed (Rous, 1976). The present communication presents data on the subcellular distribution of rat brain acetoacetyl-CoA deacylase and its possible role in the brain cytosolic biosynthetic processes.

Rats used were of the Wistar strain. The brains of these animals were removed after decapitation; only the forebrain without the olfactory bulbs was used.

Acetoacetyl-CoA deacylase was assayed by the method of Williamson et al. (1968). The rate of disappearance of acetoacetyl-CoA in the presence of iodoacetamide was monitored at 303 nm, with 373 nm as the reference wavelength on an Aminco–Chance dual-wavelength spectrophotometer in a dual-wavelength mode. Citrate synthase (EC 4.1.3.7) was assayed by the method of Coore et al. (1971). Lactate dehydrogenase was measured by the method of Clark & Nicklas (1970). The microsomal marker NADPH-cytochrome c reductase (rotenone-insensitive) (EC 1.6.99.2) was assayed by the method of Duncan & Mackler (1966).

Table 1 shows the specific activities, relative specific activities and percentage activities of all enzymes assayed in the various fractions. The total recovery of all enzyme activities was in excess of 90%, except for lactate dehydrogenase (total recovery 86.1%).

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Table 1. Subcellular distribution of acetoacetyl-CoA deacylase

The fractions were obtained as follows. Nuclear pellet from centrifugation at 2000 g for 3 min; crude mitochondrial pellet from centrifugation at 15000 g for 10 min; microsomal pellet from centrifugation at 99000 g for 60 min; cytosolic supernatant from 99000 g centrifugation for 60 min. The enzymes in various fractions were assayed as described above. Specific activities are expressed as mean±s.d. for three determinations. Relative specific activity is the ratio of the mean specific activity of a fraction to the mean specific activity of the homogenate. Units of specific activity are nmol/min per mg of protein.

<table>
<thead>
<tr>
<th></th>
<th>Homogenate</th>
<th>Nuclear</th>
<th>Crude mitochondrial</th>
<th>Microsomal</th>
<th>Cytosolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetoacetyl-CoA deacylase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of total homogenate activity</td>
<td>100</td>
<td>30.1</td>
<td>62.1</td>
<td>1.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Specific activity</td>
<td>5.3±0.3</td>
<td>4.2±0.2</td>
<td>9.8±0.4</td>
<td>1.5±0.1</td>
<td>0.7±0.02</td>
</tr>
<tr>
<td>Relative specific activity</td>
<td>1</td>
<td>0.8</td>
<td>1.84</td>
<td>0.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of total homogenate activity</td>
<td>100</td>
<td>30.1</td>
<td>63.2</td>
<td>3.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Specific activity</td>
<td>145±4</td>
<td>106±5</td>
<td>275±16</td>
<td>70±8</td>
<td>7.3±0.8</td>
</tr>
<tr>
<td>Relative specific activity</td>
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<td>0.73</td>
<td>1.9</td>
<td>0.48</td>
<td>0.05</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of total homogenate activity</td>
<td>100</td>
<td>22.5</td>
<td>22.6</td>
<td>3.8</td>
<td>37.2</td>
</tr>
<tr>
<td>Specific activity</td>
<td>348±3</td>
<td>189±7</td>
<td>217±4</td>
<td>213±11</td>
<td>702±20</td>
</tr>
<tr>
<td>Relative specific activity</td>
<td>1</td>
<td>0.54</td>
<td>0.62</td>
<td>0.6</td>
<td>2.02</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of total homogenate activity</td>
<td>100</td>
<td>33.7</td>
<td>39.8</td>
<td>22.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Specific activity</td>
<td>3.6±0.2</td>
<td>2.8±0.2</td>
<td>4.5±0.1</td>
<td>13.3±1.0</td>
<td>0.6±0.03</td>
</tr>
<tr>
<td>Relative specific activity</td>
<td>1</td>
<td>0.8</td>
<td>1.2</td>
<td>3.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The subcellular distribution of acetoacetyl-CoA deacylase mimics the distribution of citrate synthase almost exactly when percentages of total homogenate activity and relative-specific-activity values are compared. Preliminary work on further fractionation of the crude mitochondrial fraction into synaptosomes, free mitochondria and myelin has further emphasized the similarity of distribution between acetoacetyl-CoA deacylase and citrate synthase. Brain acetoacetyl-CoA deacylase is therefore almost entirely mitochondrial, with a specific activity of 38.5±1.3 (3)nmol/min per mg of mitochondrial protein, and a total activity of 1.1±0.03 μmol/min per g wet wt. of brain.

The major circulating ketone body in suckling rats is β-hydroxybutyrate (Hawkins et al., 1971). The direct formation of acetoacetyl-CoA from circulating acetoacetate by the cytosolic acetoacetyl-CoA synthetase (Buckley & Williamson, 1973) will be minimal in terms of contribution of carbon for lipids and acetylcholine synthesis. Furthermore Patel & Owen (1977) have shown that carbon from ketone bodies is incorporated more efficiently into lipids than is that from glucose in developing rats; also D'Adamo & D'Adamo (1968) have demonstrated that rat brain citrate lyase activity is low. The presence of acetoacetyl-CoA deacylase in mitochondria allows a controlled availability of acetoacetate from β-hydroxybutyrate for export to the cytosol for incorporation into lipids and acetylcholine, the acetoacetate being converted
initially into acetoacetyl-CoA by the very active mitochondrial 3-oxo acid-CoA transferase and, depending on the acetoacetyl-CoA concentration, may then be converted back into acetoacetate.


A Method for the Rapid Separation of Cytosolic and Particulate Components of Rat Brain Synaptosomes

ROBERT F. G. BOOTH and JOHN B. CLARK

Department of Biochemistry, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, U.K.

Metabolic studies on intact synaptosomes have been limited by difficulties in measuring metabolite concentrations in each of the cytosolic and mitochondrial compartments. For a complete understanding of factors influencing metabolic fluxes, such as the redox and phosphorylation state, the metabolite-indicator method has been developed (Holzer et al., 1956). However, the application of this method requires a knowledge of cytosolic and mitochondrial metabolite concentrations.

A method has been developed by Zuurendonk & Tager (1974) for the rapid separation of mitochondria and cytoplasm in isolated liver cells by selective destruction of cell membranes with digitonin. It has been found that by modifications to this procedure and also that of Siess & Wieland (1975), a rapid separation of mitochondria and cytosol can be obtained with synaptosomes. In the present paper we report preliminary observations on the experimental procedure so far developed. To conduct such experiments, modifications of the techniques currently in use for the isolation of synaptosomes were necessary to decrease the contamination of synaptosomes by free mitochondria to minimal values (less than 3% on a protein basis), without impairing the metabolic competence of the synaptosome preparation. This preparation has been successfully developed and characterized (R. F. G. Booth & J. B. Clark, unpublished work).

Separation of soluble and mitochondrial components was achieved as follows. Synaptosomes (20 mg/ml) were incubated in Krebs-Henseleit medium at 25°C. A portion of this mixture was injected into 1 ml of an ice-cold solution containing 0.25 M sucrose, 20 mM-Mops* buffer, pH 7.2, 3 mM-EDTA and digitonin at a given concentration. This mixture was vortex-mixed for 5 s and then a sample was carefully transferred to an Eppendorf cup containing 0.1 ml of 12% (w/v) HClO₄ below 0.5 ml of the silicone oil Versilube F.50, which had been cooled to 0°C. The cup was then transferred to an Eppendorf centrifuge and was centrifuged for 1 min at a given time after mixing with digitonin (20–40 s). Enzyme activities were subsequently measured in the top layer and compared with the total activity in synaptosomes. The mean results of two experiments are shown in Table 1.

The results indicate a substantial release of the cytosolic marker enzyme lactate dehydrogenase at digitonin concentrations of 0.5 mg/ml, indicating a substantial lysis of the synaptosomal membrane. Furthermore, at the digitonin concentrations used the

* Abbreviation: Mops, 4-morpholinepropanesulphonic acid.