Analysis of phospholipids associated with pig brain microtubules

ALAN J. HARGREAVES* and W. GRAHAM McLEAN†
Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, U.K.

Microtubules, prepared by one or two cycles of temperature-dependent recyclication in buffer that contains glycerol (Shelanski et al., 1973), are known to be associated with a number of phospholipids (Daleo et al., 1974; Lagnado & Kirazov, 1975; Banks, 1976). Phosphatidylycholine and phosphatidylethanolamine are the major phospholipids associated with microtubules prepared from bovine splenic nerve (Banks, 1976) and rat or chick brain (Daleo et al., 1974; Lagnado & Kirazov, 1975), although all these authors claimed that other phospholipids were present. However, these were not identified nor was their association shown to persist in highly-purified microtubules. The purpose of the present study was to attain a more comprehensive identification of microtubule-associated phospholipids, to quantify their association with highly-purified microtubules and to determine whether specific associations existed between phospholipids and microtubule proteins.

Pig brain microtubules were purified through up to five cycles of temperature-dependent recyclication in the absence of glycerol as described by Larsson et al. (1977). Such extracts, when examined by gel electrophoresis in the presence of 1% sodium dodecyl sulphate, were shown to contain approx. 75% by weight tubulin monomer (mol.wt. 55000) and 25% accessory proteins, which were similar in molecular weight to those described by other workers (Murphy & Borisy, 1975; Cleveland et al., 1977). Phospholipids were extracted from microtubule proteins by homogenization in chloroform/methanol (Folch et al., 1957; Bligh & Dyer, 1959), separated by two-dimensional t.l.c. and identified by their relative mobilities as well as specific spray reagents. Total phospholipid phosphate was assayed by the method of Bartlett (1959).

A reproducible pattern of phospholipids was observed after two-dimensional t.l.c. of chloroform/methanol extracts from microtubules that had been pelleted by centrifugation after one, two and three cycles of purification. The major components were phosphatidylycholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin, along with cholesterol and at least four other unidentified phospholipids. Measurement of total phospholipid phosphate of one chattles of phospholipid phosphate relative to total protein. A

* Present address: Department of Microbiology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.
† Present address: Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Purification and properties of specific cysteine sulphinate decarboxylase (EC 4.1.1.29) from rat brain

PAUL F. URBAN and PAUL REICHERT
Centre de Neurochimie du CNRS, 5 rue Blaise Pascal, 67084 Strasbourg Cedex, France

Taurine (2-aminoethane sulphonic acid) is present in large amounts in vertebrate brain and retina, where it may function as an inhibitory neurotransmitter or, more generally, as a neuromodulator by affecting ionic, especially Ca²⁺, fluxes (for review, see Huxtable & Barbeau, 1976; Barbeau & Huxtable, 1978; Baskin et al., 1981). Its main biosynthetic pathway proceeds through the oxidation of cystine into cysteine sulphinate, which is decarboxylated into hypotaurine and finally oxidized to yield taurine. Purified glutamate decarboxylase (EC 4.1.1.15) may also use cysteine sulphinate as a substrate (Blindermann et al., 1978) and whereas previous attempts to separate glutamate and cysteine sulphinate decarboxylase activities have failed (Urban et al., 1981). We report here the occurrence and purification of a specific cysteine sulphinate decarboxylase in rat brain free of glutamate decarboxylase activity.

The 105000 × g supernatant of a hypo-osmotic homogenate of 50 brains was fractionated on an AH Sepharose 4B column allowing a clear separation of specific cysteine sulphinate decarboxylase and cysteine sulphinate/glutamate decarboxylase. The specific cysteine sulphinate decarboxylase was then purified to homogeneity by hydroxypatite chromatography, preparative isoelectrofocusing with a pH gradient from 3.5 to 10 on an Ultrofoc gel and gel filtration on Ultrogel AcA 34 (Table 1). The yield appears to be rather low, but inactivation during isoelectrofocusing and the unknown specific activity of
the enzyme in the homogenate must be taken into account. On 7.5% polyacrylamide gels at both pH 7.0 and 8.9 in non-denaturing conditions the pure enzyme runs as a single band just behind bovine serum albumin, thus having a molecular weight of 70000 ± 2000. After heating for 1h at 60°C in the presence of sodium dodecyl sulphate and β-mercaptoethanol, the enzyme still runs in the same position relative to albumin on 10% polyacrylamide gels containing detergent by the method of Weber & Osborn (1969). The enzyme is, therefore, monomeric.

The specific cysteine sulphinate decarboxylase has a rather broad pH optimum around 7.0 and its \( K_m \) for cysteine sulphinate is 37 \( \mu \)M at the last step of the purification, which is a much lower value than that reported for the non-specific enzyme with a \( K_m \) of 3.8 \( \mu \)M (5.4 mM for cysteine sulphinate in the case of purified glutamate decarboxylase). The high affinity of the specific decarboxylase also reduces the importance of cysteine sulphinate transamination as a regulator of taurine biosynthesis, since both the transaminases L-cysteine sulphinate synthetase and L-aspartate:2-oxoglutarate aminotransferase (EC 2.6.1) and L-aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.1) have \( K_m \) values for cysteine sulphinate in the 5–10 \( \mu \)M range (Recasens & Mandel, 1980). A \( K_m \) for pyridoxal phosphate cannot be determined because the enzyme remains saturated with cofactor even after gel-filtration columns with pyridoxal phosphate-free elution buffer. The enzymic activity assay in the absence of pyridoxal phosphate or increasing concentrations of co-factor from 100 \( \mu \)M to 0.2 mM yields the same activity, but pre-incubation of the enzyme for 15 min with 10 \( \mu \)M DL-penicillamine, a known pyridoxal phosphate complexor, reduces its activity to half the maximum value (Guion-Rain et al., 1975). A similar situation has been reported for the rat liver enzyme, and in both brain (Rassin & Sturman, 1975) and liver, cysteine sulphinate decarboxylase (Hope, 1955) activity disappears during vitamin B-6 deficiency.

We report here a method for the purification of specific cysteine sulphinate decarboxylase and their homologues from rat brain. As the assay of cysteine sulphinate decarboxylase activity based on the measurement of \(^{14} \text{CO}_2\) will also simultaneously measure glutamate decarboxylase activity in dissected brain regions, this method is no longer valid. A separation of both enzymes before microassays of the enzymic activities in tissue fragments being too laborious, the need for a radioimmunoassay and the immunohistochemical localization of specific cysteine sulphinate decarboxylase in different brain regions as an index for taurine-synthesizing neurons is evident.

### Table 1. Purification of specific cysteine sulphinate decarboxylase from rat brain

<table>
<thead>
<tr>
<th>Step</th>
<th>Total activity (nmol/h)</th>
<th>Protein (mg)</th>
<th>Specific activity (nmol/h per mg)</th>
<th>Yield (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo-osmotic homogenate from 50 brains</td>
<td>560</td>
<td>8000</td>
<td>70</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>105000 g supernatant</td>
<td>320</td>
<td>1600</td>
<td>200</td>
<td>57</td>
<td>3</td>
</tr>
<tr>
<td>AH Sepharose 4B</td>
<td>3</td>
<td>130</td>
<td>230</td>
<td>5.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Hydroyapatite</td>
<td>3</td>
<td>40</td>
<td>750</td>
<td>5.4</td>
<td>10.7</td>
</tr>
<tr>
<td>Isolelectrofocusing (pH 3.5–10)</td>
<td>0.6</td>
<td>3.6</td>
<td>1660</td>
<td>1.1</td>
<td>23</td>
</tr>
<tr>
<td>Ulrogl A C A 34 (filtration gel)</td>
<td>0.4</td>
<td>0.4</td>
<td>100000</td>
<td>0.7</td>
<td>140</td>
</tr>
</tbody>
</table>

---

**Study in vitro** of the effects of α-aminoadipate and its homologues on the electroretinogram of the isolated frog retina

P. F. URBAN, M. FRAULI, P. MANDEL and G. VINCENTON

Centre de Neurochimie de CNRS, 5 rue Blaise Pascal, 67084 Strasbourg Cedex, France

α-Aminoacidopate, a dicarboxylic amino acid, and its homologues, aspartate, glutamate and α-aminopimelate exhibit well characterized neuroexcitatory effects on the neurons of cat spinal cord. Certain dicarboxylic amino acids (like α-amino-adipate) have been shown to be selectively glutotoxic, whereas other homologues (aspartate, glutamate) exhibit neurotoxic properties (Olney et al., 1980). Studies in vitro on the cytotoxic effect of α-aminoacidopate have been reported recently (Szmajer et al., 1981; N. Bonaventure, G. Roussel & N. WioIand, unpublished work). Our aim was to investigate the effects of α-aminoacidopate on the waves of the electroretinogram in vitro, to establish the dose–response relationships, the concentration range of the eventual recovery of the different waves and finally to compare these data with the effects induced in the presence of L-glutamate and DL-α-aminopimelate.

This study has been performed on retinas from dark-adapted (4h) frogs (Rana esculenta). The isolated retina mounted in a perfusion chamber is superfused with normal frog Ringer solution and control values of the electroretinogram recorded. The test amino acid is added at different concentrations to the medium and the changes of the waves observed. After stable amplitudes of the waves were obtained the amino acid containing medium was replaced by the normal frog Ringer and the kinetics of the recovery of the waves was recorded. The intensity–voltage curves for the a and b waves were established before and after drug administration. The concentration range for each amino acid investigated starts from 10 mM to the lowest concentration that permits detection of measurable effects on the electroretinogram. The most important effects have been recorded for all three amino acids on the b wave and the dose–effect relationships are shown in Table 1. For all amino acids we observed inhibition of the b wave. The most potent amino acid is L-glutamate and the least potent, DL-α-aminopimelate. L-α-Aminoacidopate has a very similar efficiency to the DL-racemate. The threshold values for L-glutamate, DL- or