enzyme (Table 1). This may indicate a changed conformation consequent on the resolution of the flavin-containing peptide(s). Resolution of the latter also explains the failure of component I to catalyse the oxidation of xanthine by NAD$^+$ or by O$_2$ and of NADH by any acceptor (Table 1; cf. Komai et al., 1969; Kanda et al., 1972; Cleere et al., 1974). Component II exhibited little or no activity with xanthine or with NADH as reducing substrate.

The results presented here support the interpretation of Nagler & Vartanyan (1976) that the redox-active groups in these enzymes are deployed in separate globules joined by short peptide loops that may readily be cleaved by proteinases. However, dissociation of the globules after hydrolysis of the loops takes place only under denaturing conditions such as high pH. The pattern of retention and of loss of activity after subtilisin action (Table 1) also provides information on the sites of interaction of oxidizing and reducing substrates with the native enzyme.

Rajagopalan, K. V. & Handler, P. (1964) J. Biol. Chem. 239, 1509–1514

---

The Hydrolysis of Aminoacyl Amides by Guinea-Pig Brain

GERARD O'CUINN

Department of Biochemistry, University College, Galway, Ireland, and Regional Technical College, Galway, Ireland

Many biologically active peptides have now been shown to contain a C-terminal aminoacyl amide. There is also evidence that degradation of such peptides may occur by endopeptidase cleavage and subsequent exopeptidase attack from the N-terminus (Benuck & Marks, 1975). The products of such degradation procedures would include an aminoacyl amide. The present study describes the preliminary investigation of the degradation of aminoacyl amides in guinea-pig brain.

Guinea pigs were killed by cervical dislocation. The brain was removed and homogenized in 10 vol. of 0.3 M sucrose by using six strokes of a Potter–Elvehjem homogenizer. The homogenate was resolved into a soluble and particulate fraction by centrifugation at 30000 g for 20 min. The particulate fraction was washed with 5 vol. of 0.3 M sucrose and finally resuspended in the same volume as the soluble fraction. Various aminoacyl amides were incubated with soluble or particulate fractions for set time intervals and the release of free amino acids was measured by the method of Donlon & Fottrell (1971).

The rates of hydrolysis of various aminoacyl amides by brain fractions are shown in Table 1, which shows that most of the hydrolyase activity against the aminoacyl amides tested resides in the soluble fraction. No hydrolyase activity could be detected against N-terminal tryptophan, methionine or valine in the particulate fraction, yet a large portion of brain hydrolyase activity against N-terminal leucine was present in the particulate fraction. It is not clear whether all aminoacyl amides are hydrolysed by the broad-specificity peptide hydrolases shown to be present in brain (O'Cuinn, 1976). Donlon & Fottrell (1973) have shown that the broad-specificity $\alpha$-peptide hydrolase of guinea-pig intestinal mucosa is capable of hydrolysing N-terminal leucine. It has been
Table 1. Hydrolase activities against aminoacyl amides in soluble and particulate fractions of guinea-pig brain

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Substrate</th>
<th>Amino acid released (μmol/min per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble</td>
<td>L-Leu-NH₂</td>
<td>0.357</td>
</tr>
<tr>
<td>Particulate</td>
<td>L-Leu-NH₂</td>
<td>0.102</td>
</tr>
<tr>
<td>Soluble</td>
<td>L-Phe-NH₃</td>
<td>0.234</td>
</tr>
<tr>
<td>Particulate</td>
<td>L-Phe-NH₃</td>
<td>0.032</td>
</tr>
<tr>
<td>Soluble</td>
<td>L-Trp-NH₂</td>
<td>0.216</td>
</tr>
<tr>
<td>Particulate</td>
<td>L-Trp-NH₂</td>
<td>—</td>
</tr>
<tr>
<td>Soluble</td>
<td>L-Met-NH₂</td>
<td>0.230</td>
</tr>
<tr>
<td>Particulate</td>
<td>L-Met-NH₂</td>
<td>—</td>
</tr>
<tr>
<td>Soluble</td>
<td>L-Val-NH₂</td>
<td>1.516</td>
</tr>
<tr>
<td>Particulate</td>
<td>L-Val-NH₂</td>
<td>—</td>
</tr>
</tbody>
</table>

shown that the broad-specificity peptide hydrolases of intestinal mucosa and of brain in the guinea pig are closely related (O’Cuinn et al., 1975; O’Cuinn, 1976).

Previous work has indicated that the degradation of biologically active peptides takes place in the soluble fraction of brain (Benuck & Marks, 1975; Marks et al., 1973; Griffiths et al., 1975). It is not surprising therefore that the degradative enzymes for the aminoacyl amides should also be present in the cytoplasm.

I thank the Medical Research Council of Ireland for generous financial support.


---

Distribution of the Calcium-Ion-Transport System in Subcellular Fractions from Rabbit Brain

GERALDINE O’DRISCOLL and P. FINBARR DUGGAN

Departments of Biochemistry and Medicine, University College, Cork, Ireland, and Biochemistry Laboratory, St. Finbarr’s Hospital, Cork, Ireland

In the unstimulated nerve cell, the free Ca²⁺ concentration is about 100 nM (Baker, 1977). The surge to a free-Ca²⁺ concentration of 10 μM after excitation affects many reactions within the cell, including neurotransmitter release (Katz & Miledi, 1967). To restore the cell to the resting condition, Ca²⁺ must be either sequestered within the cell or (and) be extruded to the intercellular fluid. An ATP-dependent Ca²⁺ transport system has been identified in the microsomal fraction of brain homogenates (Robinson & Lust, 1968; Ohtsuki, 1969; de Meis et al., 1970).

In a previous report (Duggan & Kelleher, 1975) the activation of Ca²⁺ uptake of rabbit brain microsomal fraction by Na⁺ and K⁺ was described. The stimulation by Na⁺ only became apparent in the presence of high concentrations of phosphate ions. Catecholamines also increased the rate of Ca²⁺ accumulation, and the increase was additive to that due to K⁺ or Na⁺. However, with increasing phosphate concentration the effects of dopamine (3,4-dihydroxyphenethylamine), noradrenaline and 5-hydroxytryptamine