Peptidase content of the bile canalicular membrane

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The results of a number of independent studies suggest that circulating regulatory peptides may be inactivated in the liver. Circulating levels of [Leu]- and [Met]-enkephalin increase in patients with cholestatic liver disease [1] and in rats following bile duct ligation [2]. Furthermore, cholecystokinin octapeptide (CCK-8) and tetrapeptide [3] and [Leu]-enkephalin [4] radio-labelled with [3H]-Bolton and Hunter reagent are efficiently taken up by the isolated, perfused rat liver and then rapidly excreted in bile from where they can be recovered in a largely metabolised form. Short-chain gastrins and somatostatin also appear to be subject to hepatic inactivation.

Nothing is known of the enzymes responsible for hepatic peptide metabolism but the inactivation of regulatory peptides in the CNS has been extensively studied (see e.g. [5]). For example, enkephalin inactivation in brain is mediated by the combined actions of aminopeptidase N (AP-N) and endopeptidase-24.11 (E-24.11; neprilysin) [6] and these same enzymes are also involved in the metabolism of CCK-8 in brain [7]. Although E-24.11 has a broad role in the inactivation of regulatory peptides the enzyme is present at extremely low levels in liver [8]. In contrast, AP-N has been located on the bile canalicular membrane [9].

Proposing that some circulating peptides may be inactivated by hydrolysis at the bile canalicular membrane following internalisation via a transport protein in the sinusoidal membrane, we have prepared from rat liver a plasma membrane preparation approximately forty times enriched in the bile canalicular membrane [10] and examined this preparation for the presence of a number of membrane peptidases.

E-24.11 and angiotensin converting enzyme were assayed by using h.p.l.c. methods with [DAla2, Leu3]-enkephalin and Hip-His-Leu as substrate respectively; enzyme specificities were controlled by inhibition with phosphoramidon (1 uM) and enalaprilat (10 uM). Membrane dipeptidase (MDP) was assayed by using Gly-D-Phe as substrate and specificity checked by inhibition by cilastatin (0.1 mM). Aminopeptidase P (AP-P) was assayed with Gly-Pro-Hypro (1 mM) as substrate. AP-N and dipeptidyl-peptidase IV (DPP-IV) were assayed by fluorimetric methods. Phosphoramidon-sensitive endothelin converting enzyme (ECE) activity was measured by a radioimmunoassay procedure in which the endothelin-l produced from big endothelin-l (1 uM) was quantified. ECE activity was distinguished from E-24.11 by its insensitivity to inhibition by 10uM thiorphan [11].

Table 1. Comparison of Peptidase Activity in the Bile Canalicular Membrane and the Kidney Brush Border

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bile Canalicular Membrane</th>
<th>Kidney Brush Border*</th>
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<tbody>
<tr>
<td>AP-N</td>
<td>303 ± 50</td>
<td>524</td>
</tr>
<tr>
<td>DPP-IV</td>
<td>290 ± 36</td>
<td>606</td>
</tr>
<tr>
<td>MDP</td>
<td>3.3 ± 0.3</td>
<td>121</td>
</tr>
<tr>
<td>ECE</td>
<td>0.073 ± 0.011</td>
<td>–</td>
</tr>
<tr>
<td>E-24.11</td>
<td>nd</td>
<td>283</td>
</tr>
<tr>
<td>ACE</td>
<td>nd</td>
<td>12</td>
</tr>
<tr>
<td>AP-P</td>
<td>nd</td>
<td>68</td>
</tr>
</tbody>
</table>

All enzyme activities in nmol/min/mg of membrane protein except for endothelin converting enzyme activity in pmol/min/mg.

Activities in the bile canalicular membrane are the means of 3 or 4 observations ± SEM.

*nd : not detected

*taken from reference 11

In conclusion, the canalicular plasma membrane is enriched in a number of membrane peptidases that are known to play a role in regulatory peptide metabolism. The spectrum of peptidases differs considerably, however, from those present in the renal brush border membrane. In addition, two novel peptidases have been identified in this membrane fraction: an enkephalin hydrolysing activity distinct from E-24.11 and ECE, a zinc metallopeptidase which shows considerable homology with E-24.11. Whether any of these peptidases plays any direct role in the uptake and metabolism of circulating peptides by the liver remains to be established.