Exocrine pancreatic enzyme and calcium secretion in health and pancreatitis*

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SUMMARY Calcium, enzyme, and total protein secretion were measured in secretin stimulated pancreatic juice in health, 'early' chronic pancreatitis, and in chronic calcific pancreatitis. Increased concentrations of trypsin, total protein, and calcium, and increased outputs of calcium and protein were shown to be present in the 'early' stages of the disease, indicating that an environment conducive to the formation of protein plugs and possibly later calcification already exists.

The concentration of pancreatic juice enzyme and calcium is increased in established chronic pancreatitis. Supersaturation and precipitation of enzymes in the pancreatic ducts give rise to the protein plugs found in calcific pancreatitis, and are regarded as important in the pathogenesis of the disease. The cause of the raised calcium levels in pancreatic juice in this disease is uncertain.

The purpose of this study was to determine whether the raised calcium and enzyme levels in chronic pancreatitis are found only in the calcific forms of the disease, or whether they may occur in earlier forms of the disease as well. To test this we measured calcium, protein, and trypsin in hormonally stimulated duodenal juice of control subjects, subjects after complete resolution of a documented attack of clinically acute alcohol-induced pancreatitis (post-acute pancreatitis (PAP), and in subjects with alcohol-induced chronic calcific pancreatitis (CCP).

Methods

SUBJECTS

Thirty-two individuals of all races were studied. All gave informed consent for the same investigation. The individuals studied were of three groups: (1) healthy volunteers who acted as controls (12 subjects, six studied with secretin background, and six with a background secretin and cholecystokinin (CCK)); (2) patients at least six weeks after an episode of clinically acute alcohol-induced pancreatitis confirmed by a serum amylase of more than 600 Pimstone units per ml during an attack (11 patients, six with secretin background, and six with a background of secretin and CCK); (3) patients with chronic calcific pancreatitis (CCP) evident radiologically (nine patients, four with secretin background, and six with a background of secretin and CCK).

EXPERIMENTAL DESIGN

After a 10 hour overnight fast a radio-opaque nasogastric tube (Rusch, size 14 or 16) was positioned with its distal tip at the junction of the second and third parts of the duodenum. A second tube was placed in the dependent portion of the stomach. The position of both tubes was frequently checked fluoroscopically throughout the test. Constant siphonage at a pressure of −50 to −80 cm of water and regular manual syringing of the tubes ensured constant patency. After a basal period a constant infusion of secretin (Boots, 1 unit/kg/h) or a combination of secretin (1 unit/kg/h) and CCK (Boots, 1 unit/kg/h) was started, using a constant infusion pump (Braun, Melsungen) and continued for 60 minutes. Duodenal samples were collected under ice at 10 minute intervals, and the volume recorded.

SAMPLE ANALYSIS

Protein concentration was estimated by comparing absorbance at 280 nm with standards of bovine serum albumin. Trypsin was determined spectrophotometrically using benzyl-L-arginine ethyl ester

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as a substrate. Calcium concentration was measured using an atomic absorption spectrophotometer (Varian-Techtron, Melbourne, Australia). Bicarbonate concentrations were determined by adding 1 ml 0.1 M HCl to 0.5 ml of sample, boiling, and backtitrating to pH 7 with 0.2 M NaOH using an automatic titrator (Radiometer, Copenhagen, Denmark). Gastric juice was collected separately and discarded.

**Statistical Analysis**

Statistical analysis was carried out using Student’s t test for unpaired values, and correlation coefficient by the least squares method.

**Results**

**Secretin Stimulated Calcium, Protein, and Trypsin Secretion**

**Calcium secretion**

Calcium concentration and output in secretin-stimulated subjects is shown in Fig. 1. Calcium concentration and output in control subjects was consistently lower than in PAP subjects, both for the mean 60 minute calcium concentration (P<0.01) and peak 10 minute calcium output (P<0.05). There was no significant difference in calcium concentration or output between PAP and CCP subjects.

**Protein secretion**

Protein concentration and output after secretin is depicted in Fig. 2. PAP values were consistently higher than control values for mean 60 minute protein concentration (P<0.05) and 60 minute protein output (P<0.01), while there was no statistical difference between control values and those in CCP subjects.
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Trypsin secretion

Trypsin secretion paralleled that of protein secretion. The mean 60 minute trypsin concentration in controls was 1912 ± 261 (mean ± SEM) BAEE units/ml, and the mean trypsin concentration in PAP subjects was significantly higher, 2913 ± 263 BAEE units/ml, p < 0.05. The trypsin output in controls was 223 ± 59 BAEE units/10 minutes × 10³, and that in PAP subjects 376 ± 87 BAEE units/10 minutes × 10³, which just failed to reach statistical significance.

Table 1 Mean bicarbonate concentration and peak bicarbonate output in controls, postacute pancreatitis, and in chronic calcific pancreatitis.

<table>
<thead>
<tr>
<th></th>
<th>Controls n=12</th>
<th>Post-acute pancreatitis n=11</th>
<th>Chronic calcific pancreatitis n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate (mmol/l)</td>
<td>110 ± 7</td>
<td>89 ± 1</td>
<td>69 ± 8</td>
</tr>
<tr>
<td>Bicarbonate (mmol/10 min)</td>
<td>±3.48 ± 7.1*</td>
<td>±3.48 ± 5.6†</td>
<td>±3.48 ± 2.3†</td>
</tr>
</tbody>
</table>

Data are mean ± SEM from secretin and combined secretin and CCK stimulated subjects. *p < 0.05, †p < 0.01 compared with control values.

In CCP subjects, trypsin concentration and output was 1505 ± 352 BAEE units/ml, and 155 ± 65 BAEE units/10 minutes respectively, which was not significantly different from control values.

Effect of combined secretin and CCK on protein and calcium secretion

Calcium secretion

Combined infusion of secretin and CCK increased both calcium concentration and output in control subjects (p < 0.05, p < 0.01) and PAP subjects (p < 0.02, p < 0.01) (Fig. 3). In contrast, calcium secretion in CCP subjects, which was high in response to secretin, showed no increase in either concentration or output after CCK.

Protein secretion

The 60 minute protein output after secretin and CCK in control subjects was 4198 ± 852 mg (mean ± SEM). In PAP subjects the mean protein output was comparable with 4442 ± 818 mg, while in CCP subjects protein output was only 1985 ± 620 mg.

Table 2 Calcium concentrations and outputs after secretin, and secretin and cholecystokinin in controls, post-acute pancreatitis, and in chronic calcific pancreatitis.

<table>
<thead>
<tr>
<th></th>
<th>Controls (mmol/l)</th>
<th>Post-acute pancreatitis (mmol/l)</th>
<th>Chronic calcific pancreatitis (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretin</td>
<td>0.26 ± 0.06</td>
<td>0.62 ± 0.06</td>
<td>1.38 ± 0.65</td>
</tr>
<tr>
<td>Secretin and CCK</td>
<td>0.93 ± 0.26</td>
<td>205 ± 44</td>
<td>142 ± 0.34</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data represented are the mean ± SEM. The addition of CCK produces a significant rise in both concentration and output of calcium in controls and post-acute pancreatitis, but not in chronic calcific pancreatitis.
CORRELATION BETWEEN CALCIUM AND PROTEIN SECRETION

There was no correlation in secretin and CCK stimulated calcium and protein secretory values in control subjects or in subjects with pancreatic disease (data not shown).

Bicarbonate secretion

Peak bicarbonate secretory values are shown in Table 1, and data have been pooled for secretin stimulated, and secretin and CCK stimulated subjects. In PAP subjects there was significantly decreased bicarbonate concentration but not output, while in CCP there was reduced bicarbonate concentration and output in relation to control values.

Discussion

The vast majority of patients with clinically acute alcohol-induced pancreatitis have established pathological and functional changes at the time of the first attack, and were considered to be a suitable group of patients for studying the changes in the 'early' form of the chronic disease. Bicarbonate values in Table 1 suggest that function in these subjects is impaired, while protein secretion after secretin and CCK (Table 2) indicate that such impairment is minimal.

The results of the study indicate that secretin stimulated enzyme and calcium secretion is increased in 'early' disease. Both concentration and output of calcium and protein are increased, showing that this is not simply a decrease in volume flow, but an absolute increase in production. This suggests that an environment conducive to the formation of protein plugs, and possibly later calcification, already exists in an early stage of pancreatitis.

Various factors may be involved in the raised calcium and protein levels in pancreatitis. Damage to the pancreatic duct system in pancreatitis may allow increased 'leak' of calcium into pancreatic juice, and, as calcium in serum is higher than that normally present in pancreatic juice, this could account for the rise in juice calcium. However, individual 10 minute calcium outputs, particularly in calcific pancreatitis, occasionally greatly exceeded serum values, and this cannot, therefore, be the complete explanation. Secondly, it is interesting to speculate whether the high protein and calcium levels in pancreatitis may not perhaps be related to the allegedly high levels of CCK in patients with chronic pancreatitis.

The present study confirms the observations of previous workers regarding the increased pancreatic juice calcium levels after CCK stimulation, but has failed to show the positive correlation between calcium and enzyme secretion reported by Goebell and his co-workers in the experimental animal. Biliary contamination of pancreatic juice may have obscured possible correlation, but, alternatively, our results could indicate that calcium enters the pancreatic juice by more than one route.

References