Renal Clearance of Pancreatic and Salivary Amylase Relative to Creatinine Clearance in Patients with Renal Disease and Proteinuria


To study the charge-selective properties of the glomerular filter in renal disease, we measured the fractional clearance, relative to creatinine clearance (ECC), of the amylase isoenzymes pancreatic amylase and salivary amylase, which have identical size but different charge. In 63 healthy subjects the mean (and SD) fractional excretion of pancreatic amylase, 4.07% (1.24%), was fourfold that of salivary amylase: 1.02% (0.54%). For 29 patients with renal disease and proteinuria, the mean fractional excretion of pancreatic amylase was significantly lower, 3.31% (1.94%), and that of salivary amylase significantly higher, 2.06% (1.41%), than in controls. In these patients, fractional excretions of both these isoenzymes were negatively correlated with urinary excretion of $\beta_2$-microglobulin and ECC. Evidently, differences in clearances of pancreatic and salivary amylase are a consequence of differences in charge-related glomerular filtration. The relative increase of salivary amylase clearance in patients with renal disease and proteinuria is most probably caused by a loss of the charge-selective properties of the glomerular basement membrane.

Human serum amylase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.1) consists of two major isomers, pancreatic amylase (P-amylase) and salivary amylase (S-amylase), which have identical size (2.9 nm), but different charge, S-amylase (isoelectric point 5.9-6.4) being more anionic than P-amylase (isoelectric point 7.0) (1, 2). The urinary excretion of amylase is governed by glomerular filtration and tubular reabsorption (3, 4). It has gradually become evident that transport of macromolecules through the glomerular filter is determined not only by the size of the molecule, but also to an important degree by its charge (5-7). The negatively charged glomerular basement membrane impairs filtration of anionic proteins such as albumin. Because of the differences in charge of P- and S-amylase, one might expect important differences in renal clearance of these isoenzymes. However, differences in renal processing of amylase isoenzymes have received little attention. Therefore, we measured the renal clearance of amylase isoenzymes in patients with renal disease and proteinuria and compared the results with our values for healthy volunteers. Our results show a preferential increase of clearance of S-amylase over P-amylase in patients with renal disease and proteinuria, pointing to a defect in the charge-selective characteristics of the glomerular basement membrane.

Patients and Methods

Patients. Amylase clearances were measured in 63 healthy controls (group I) and in 29 patients with renal disease and proteinuria (group II).

Group I comprised 34 men and 29 women with a mean (±SD) age of 39 ± 18 years (range 18-85 y). All volunteers had normal renal function (mean serum creatinine 78 ± 10 $\mu$mol/L), and no evidence of any underlying disease.

Group II comprised 19 men and 10 women, with a mean age of 43 ± 16 years (range 17-67 y), a mean serum creatinine of 165 ± 107 $\mu$mol/L, a mean creatinine clearance of 78 ± 42 mL/min, and mean proteinuria of 7.0 ± 3.7 g/24 h (range 0.5-14.5 g/24 h). The underlying renal disease was glomerular in nature in all but one of the patients: membranous glomerulonephritis (n = 11), focal glomerulosclerosis (n = 6), minimal change glomerulonephritis (n = 4), IgA nephropathy (n = 4), mesangiocapillary glomerulonephritis (n = 1), amyloidosis (n = 1), Alport hereditary nephritis (n = 1), and pyelonephritis (n = 1). Informed consent was obtained from all volunteers and patients.

Clearance protocol. Results of pilot experiments showed that both amylase isoenzymes could be accurately measured only if the urinary pH was between 6.8 and 7.2. Therefore the subjects were administered 4 g of sodium bicarbonate orally on the evening before the study and another 2 g in the morning at 2 h before urine collection. All subjects were asked to drink 500 mL of tap water, to promote diuresis. Thereafter a 2-h urine specimen was collected in 10 mL of phosphate buffer (1.0 mol/L, pH 7.0). Blood was sampled in the middle of this 2-h interval.

Determinations. We measured catalytic activity concentrations of total amylase and its isoenzymes, using the "Blue Starch" method (Phadebas; Pharmacia, Upsala, Sweden), at 37°C. To all urine samples we added 1 mg of bovine serum albumin per milliliter. All measurements were done in unfrozen samples within 48 h after collection. We determined P- and S-amylase by the method of O'Donnell et al., using an S-amylase inhibitor (8). Creatinine in serum and urine was determined by the Jaffe technique (9). Proteinuria was measured by the biuret method. In 22 of the patients we concurrently measured the urinary excretion of $\beta_2$-microglobulin by radioimmunoassay ($\beta_2$M-RIA; Pharmacia; upper limit in healthy subjects: 0.15 $\mu$g/min).

Calculations. Clearances were calculated by the usual formula:

$$\text{clearance}_x = \frac{(U_x \cdot V/P_x)}{P_x}$$

where $U_x$ is the concentration of substance $x$ in urine, $V$ is the urine flow rate, and $P_x$ is the concentration of substance $x$ in the plasma.

Clearance of creatinine was used as marker of glomerular filtration rate. Fractional excretions of amylase and its isoenzymes were calculated as clearance of amylase divided by creatinine clearance, and expressed as percentages.

Statistical analysis. We used the Wilcoxon test for unpaired observations. Correlation was calculated according to Spearman. A P-value of <0.05 was considered significant. Unless otherwise mentioned, all values are given as means ± SD. In cases of nonparametric distribution, median values are also given.

Results

Table 1 gives values for total amylase and its isoenzymes in serum and for fractional excretions. Two of the healthy volunteers showed no S-amylase in serum, and in a further 11 no S-amylase was found in urine. Therefore, fractional
excretions of S-amylase could only be determined in 50 of the 63 normal controls. By contrast, urinary S-amylase could not be demonstrated in only one of the 29 patients. When we compared the 50 individuals with detectable urinary S-amylase and the 11 individuals with undetectable urinary S-amylase we found a significant difference in serum isoenzyme pattern. The percentage of P-amylase in serum was 47.0 ± 15.8% in the former and 62.8 ± 12.5% in the latter group (P < 0.01), whereas results for total amylase in serum were not significantly different (187 ± 60 and 206 ± 54 U/L, respectively).

It is evident from Table 1 that, in the patients with renal disease, the fractional excretion of P-amylase was significantly less than in controls, whereas the fractional excretion of S-amylase was significantly greater. As a result the ratio of fractional excretion of P-amylase to fractional excretion of S-amylase (P/S ratio) was significantly lower, with a median value of 1.60 (range 0.66–6.71), as compared with 3.53 (range 1.79–49.5) for the normal controls (P < 0.001). In the patients, fractional excretion of amylase and amylase isoenzymes correlated significantly with proteinuria: FEamy vs proteinuria: r = 0.49; P < 0.02, FEPamy vs proteinuria: r = 0.53; P < 0.01, FESamy vs proteinuria: r = 0.54; P < 0.01.

Fractional excretion of total amylase correlated significantly with creatinine clearance (Figure 1).

The fractional excretion of amylase isoenzymes similarly correlated with creatinine clearance (FEamy vs ECC: r = −0.48; P < 0.01; FEpamy vs ECC: r = −0.67; P < 0.001). In the 22 patients in whom we measured the urinary excretion of β2-microglobulin (Uβ2M), we found a significant correlation of this rate with creatinine clearance (r = −0.69; P < 0.001). In these patients the fractional excretion of total amylase and amylase isoenzymes also correlated significantly with urinary β2M excretion (FEamy vs Uβ2M: r = 0.60; P < 0.01, FEPamy vs Uβ2M: r = 0.59; P < 0.01, FESamy vs Uβ2M: r = 0.67, P < 0.001). The patients could be divided according to β2M microglobulin excretion rate (Table 2). In nine patients β2M excretion was normal or only slightly increased (<1.5 μg/min; mean 0.36 ± 0.13 μg/min). In the others, β2M excretion clearly exceeded 1.5 μg/min (mean 22.5 ± 19.6 μg/min). On comparing these groups of patients we observed a significantly lower creatinine clearance accompanied by significantly increased fractional excretions of total amylase, P-amylase, and S-amylase in the group of patients with the high urinary β2M excretion. The P/S ratio was not different, however. When we categorized patients according to their original renal disease, the P/S ratio was lowest in patients with minimal-lesions glomerulonephritis (P/S ratio = 1.37 ± 0.26; n = 4), as compared with 2.00 ± 0.44, 2.25 ± 0.91, and 2.67 ± 0.43 in patients with membranous glomerulonephritis (n = 11), focal glomerulosclerosis (n = 6), and proliferative glomerulonephritis (n = 5), respectively.

Discussion

For the normal volunteers the fractional clearance of total amylase averaged 2.4%, a value similar to those reported in the literature, which range from 1.24 to 3.1% (1, 10–16). Fractional excretion of P-amylase was 4.07%, a value higher than those reported in the literature, which range from 1.75 to 3.5% (11, 12, 14, 17–19). The fractional excretion of P-amylase exceeded that of S-amylase by three- to fourfold. This preferential loss of P-amylase in control subjects has already been reported: ratios of the fractional excretion of P-amylase and fractional excretion of S-amylase reportedly range from 1.6 to 6 (11, 12, 14, 16–21). The differences in the results of these studies can partly be explained by methodological differences, several different techniques being used to determine amylase isoenzymes, e.g., cellulose acetate electrophoresis (12), diethylaminoethyl ion-exchange chromatography (14, 21), polyacrylamide gel electrophoresis (16), and thin-layer isoelectric focussing (17, 18). However, O’Donnell and coworkers (11, 19) found values of 2.64% and 1.64% for the fractional excretion of P- and S-

Table 1. Results of Amylase Measurements in Control Subjects and Patients with Renal Disease

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 63)</th>
<th>Patients (n = 29)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total amylase, U/L</td>
<td>201 ± 49</td>
<td>238 ± 93</td>
<td>NS</td>
</tr>
<tr>
<td>P-amylase, U/L</td>
<td>98 ± 34</td>
<td>126 ± 63</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>S-amylase, U/L</td>
<td>105 ± 48</td>
<td>112 ± 77</td>
<td>NS</td>
</tr>
<tr>
<td>FEamy%</td>
<td>2.43 ± 0.74</td>
<td>2.71 ± 1.63</td>
<td>NS</td>
</tr>
<tr>
<td>FEPamy%</td>
<td>4.07 ± 1.24</td>
<td>3.35 ± 1.94</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>FESamy%</td>
<td>1.02 ± 0.54b</td>
<td>2.06 ± 1.41c</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>6.58 ± 8.02b</td>
<td>2.09 ± 1.25c</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviations: P = pancreatic; S = salivary; FEamy = fractional excretion of total amylase; FEpamy = fractional excretion of pancreatic amylase; FESamy = fractional excretion of salivary amylase; P/S ratio = FEamy/FESamy. NS, not significant. *n = 61; †n = 50; ‡n = 28.

Fractional excretion of total amylase correlated significantly with creatinine clearance (Figure 1).

Table 2. Results of Amylase Measurements in Patients with Renal Disease in Relation to β2-Microglobulin (Uβ2M) Excretion Rate

<table>
<thead>
<tr>
<th></th>
<th>Uβ2M &lt; 1.5</th>
<th>Uβ2M &gt; 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg/min</td>
<td>(n = 9)</td>
<td>(n = 13)</td>
</tr>
<tr>
<td>ECC, mL/min</td>
<td>114 ± 29</td>
<td>59 ± 33</td>
</tr>
<tr>
<td>FEamy%</td>
<td>1.55 ± 0.63</td>
<td>3.44 ± 1.78</td>
</tr>
<tr>
<td>FEPamy%</td>
<td>1.96 ± 0.74</td>
<td>4.27 ± 2.09</td>
</tr>
<tr>
<td>FESamy%</td>
<td>1.18 ± 0.67</td>
<td>2.62 ± 1.60</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>2.30 ± 1.84</td>
<td>2.06 ± 1.37</td>
</tr>
</tbody>
</table>

Abbreviations: ECC, endogenous creatinine clearance. For other abbreviations, see Table 1.
amylose, respectively, and they used an inhibitor technique similar to ours. We cannot easily explain the differences between results of their and our study, but two points need to be considered. First, they determined amylose in serum and urine samples that had been stored at −20 °C for five days. Second, for their control subjects they found values for P-amylose in serum (averaging 65% of total amylose) that clearly exceeded values reported by others, which range from 40 to 50% (10, 12, 14, 16, 17, 21–24).

Some of these authors also used an inhibitor technique (23, 24). It can be expected that in the subjects of O'Donnell et al. the proportion of P-amylose in urine as compared to total amylose would easily exceed 90%. At this high percentage of urinary P-amylose the inhibitor method is insensitive (25) and measurements will give falsely high values for S-amylose (8). In this respect, it is important to note that, in our study, fractional excretion of both amylose isoenzymes could be determined in only 50 of the 63 volunteers (79%), and in 11 subjects we could detect no S-amylose in urine. In these subjects, serum P-amylose (expressed as percentage of total amylose) was significantly higher. In view of the higher clearance of P-amylose it can be calculated that the percentage of P-amylose relative to total amylose in the urine will exceed 90% in these subjects.

In the patients with renal disease and proteinuria, fractional excretion of P-amylose was decreased and that of S-amylose was increased as compared with control values. In the patients, the fractional excretions of amylose and amylose isoenzymes were negatively correlated with ECC, in agreement with others (10, 12, 13, 15, 19). This finding can be explained by the decrease of tubular reabsorptive capacity as renal insufficiency progresses. Comparing patients with normal and abnormal tubular function, we observed no differences in the P/S ratio. This indicates that both isoenzymes are processed by the glomerular tubules in the same way. Therefore, the current opinion of most authors that the differences in urinary excretion of P-amylose and S-amylose are a consequence of difference in tubular reabsorption is not supported by our results.

The restricted transport of S-amylose must be attributed to its negative charge, which is consistent with recent observations that the net electric charge on a molecule is an important determinant of its fractional clearance (6, 7). The decreased fractional excretion of P-amylose in patients with proteinuria fits well with recent findings that the transglomerular transport of neutral dextrans of 2.0–4.6 nm size is restricted in patients with nephrotic syndrome (26). The relative increase of fractional excretion of S-amylose points to a defect in the charge selectivity of the glomerular capillary wall. Such a defect has been demonstrated in several experimental models of glomerulonephritis and in humans with diabetes mellitus, congenital nephrotic syndrome, and minimal lesions glomerulonephritis (6). In agreement with the latter observations, the P/S ratio was lowest in the patients with minimal-lesions glomerulonephritis. It seems worthwhile to study further the possible usefulness of fractional excretions of P- and S-amylose as markers of glomerular basement membrane charge.

J. F. M. W. is supported by a grant from the Netherlands Foundation for Medical Research (Medigon). Pharmacia—The Netherlands supplied part of the kits for determination of amylose. We thank Ms. Edena for secretarial assistance.

References