Inhibition of Atrial Natriuretic Peptide–Induced Natriuresis by Plasma Hydrolysates Containing Pepsanurin

Mauricio P. Boric, Hector R. Croxatto, Renato Albertini, and Juan S. Roblero

The specificity of antidiuretic actions of pepsanurin, a peptidic fraction obtained by pepsin hydrolysis of plasma, was studied in anesthetized rats and in isolated perfused rat kidneys. Pepsanurin was obtained from fresh dialyzed human plasma digested with pepsin (2,400 units/ml, 18 hours at 37°C, pH 2.5), deproteinized (10 minutes at 80°C), and centrifuged. In the rat, intraperitoneal injections of pepsanurin (0.5 ml/100 g body wt) significantly inhibited the effects of an intravenous bolus of atrial natriuretic peptide (ANP) (0.5 μg) on water, sodium, and potassium excretion without altering systemic blood pressure. In addition, pepsanurin abolished the peak in glomerular filtration rate and reduced the ANP-induced rise in fractional sodium excretion. Pepsanurin also inhibited the natriuretic effects of amiloride (10 μg/100 g body wt i.v.) without changing glomerular filtration rate, but it did not inhibit the potassium-retaining effect of amiloride. In contrast, pepsanurin had no effect on basal urinary excretion, and it did not affect the diuretic response induced by furosemide (doses of 25, 50, or 100 μg i.v.). Control peptidic hydrolysates prepared from human plasma preincubated 48 hours at 37°C (PIPH), bovine albumin (BSAH), or human albumin did not inhibit ANP, amiloride, or furosemide. In perfused kidneys, pepsanurin significantly and reversibly reduced sodium and water excretion. Furthermore, pepsanurin, but not PIPH or BSAH, blocked the natriuretic and diuretic effects of ANP. These results support the existence of a specific plasma substrate able to release a peptide or peptides that counteract distal tubule diuresis and natriuresis by an intrarenal mechanism. (Hypertension 1992;19[suppl II]:II-243–II-250)

Several plasma-borne hormones can be generated by pepsin hydrolysis of plasma substrates. Acidic digestion of plasma or its globulin fraction with pepsin releases a peptidic fraction named pepsanurin because it produced a strong antidiuretic activity when injected intraperitoneally into hyperhydrated rats. More recently, this peptidic fraction was shown to inhibit the natriuretic response to a bolus dose of atrial natriuretic peptide (ANP) in anesthetized rats. In contrast, neither vasopressin nor aldosterone was able to counteract ANP effects in the same experimental model.

The identification of pepsanurin has not been completed, and little is known about the site and mechanisms of its antidiuretic effect. For this reason, we studied the effects of pepsanurin on glomerular filtration rate (GFR) and sodium reabsorption in anesthetized rats, and we assessed the ability of pepsanurin to block diuretic agents other than ANP, such as furosemide and amiloride. Also, to ascertain the specificity of pepsanurin generation, we compared its antidiuretic effects with those of pepsin hydrolysates obtained from either human plasma submitted to prolonged incubation or purified serum albumin. Finally, to test the possibility that pepsanurin actions were intrarenal, we studied its effects on both baseline and ANP-stimulated sodium and water excretion in isolated perfused rat kidneys.

Methods

Materials

Pepsin and ANP (5-28 Atropeptin II, rat form) were obtained from Sigma Chemical Co., St. Louis, Mo., and bovine serum albumin from Calbiochem Corp., San Diego, Calif. Furosemide crystalline powder was graciously provided by Instituto Sanitas, Santiago, Chile; amiloride was generously donated by Laboratorio Andrómaco, Santiago, Chile. Purified human albumin was obtained by standard techniques at the blood bank of the Hospital Clínico, Universidad Católica de Chile. 51Cr-EDTA was purchased...
TABLE 1. Effects of Pepsanurin on Baseline Excretory Parameters

<table>
<thead>
<tr>
<th>Collection period</th>
<th>Volume (μl/min)</th>
<th>Sodium (μeq/min)</th>
<th>Potassium (μeq/min)</th>
<th>Glomerular filtration rate (ml/min)</th>
<th>Fractional sodium excretion (%)</th>
<th>Mean arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>1.69±0.45</td>
<td>0.104±0.079</td>
<td>0.313±0.079</td>
<td>0.802±0.122</td>
<td>0.098±0.068</td>
<td>114±5.2</td>
</tr>
<tr>
<td>20 min</td>
<td>1.55±0.27</td>
<td>0.092±0.056</td>
<td>0.286±0.054</td>
<td>0.658±0.181</td>
<td>0.152±0.106</td>
<td>107±5.0</td>
</tr>
<tr>
<td>40 min</td>
<td>1.46±0.21</td>
<td>0.073±0.044</td>
<td>0.257±0.047</td>
<td>0.692±0.123</td>
<td>0.090±0.056</td>
<td>108±2.8</td>
</tr>
<tr>
<td>60 min</td>
<td>1.72±0.30</td>
<td>0.092±0.061</td>
<td>0.233±0.046</td>
<td>0.677±0.060</td>
<td>0.099±0.067</td>
<td>111±3.0</td>
</tr>
<tr>
<td>80 min</td>
<td>1.68±0.25</td>
<td>0.092±0.056</td>
<td>0.218±0.044</td>
<td>0.708±0.074</td>
<td>0.107±0.073</td>
<td>107±3.8</td>
</tr>
</tbody>
</table>

*Values expressed per 100 g body weight.
†Basal values taken 20 minutes before intraperitoneal injection of pepsanurin.

from Comisión Chilena de Energía Nuclear, Santiago, Chile. All other reagents were of analytical grade from E. Merck, Darmstadt, FRG.

Animals. Male and female adult Sprague-Dawley rats were bred and kept at the animal facilities of the Faculty of Biological Sciences, Universidad Católica de Chile.

Generation of Pepsanurin

Fresh human plasma, obtained from normal volunteers less than 3 hours before, was dialyzed overnight against distilled water (4°C, 12-14 kDa cutoff membrane). The plasma was then acidified to pH 2.5 with HCl and digested with pepsin (2,400 units/ml) for 18 hours at 37°C. The hydrolysis was stopped by a 10-minute incubation in a boiling bath and was centrifuged (10,000 rpm, 10 minutes). This step also served to deproteinize the solution and to inactivate the pepsin. The clear supernatant containing 50-65 mg/ml total protein was aliquoted and stored at -20°C (crude pepsanurin). Throughout this procedure, bacterial growth was prevented by addition of an antibiotic mixture (50 units penicillin plus 50 μg streptomycin per 100 ml solution).

The specificity of pepsanurin generation was studied using human plasma preincubated before pepsin digestion to allow for endogenous depletion of the presumptive pepsanurin substrate. In these experiments, the fresh plasma samples were dialyzed and divided into two parts: the first half was immediately submitted to the procedure to generate pepsanurin, and the second was kept at 37°C for 48 hours under sterile conditions. The preincubated plasma was then acidified, digested, and centrifuged as described and

TABLE 2. Effects of Pepsanurin and Control Hydrolysates on Atrial Natriuretic Peptide

<table>
<thead>
<tr>
<th>Collection period</th>
<th>Volume (μl/min)</th>
<th>Sodium (μeq/min)</th>
<th>Potassium (μeq/min)</th>
<th>Arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsanurin (n=14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.14±0.07</td>
<td>0.013±0.014</td>
<td>0.262±0.026</td>
<td>105±3.1</td>
</tr>
<tr>
<td>1st ANP</td>
<td>10.39±1.14</td>
<td>2.025±0.210</td>
<td>0.836±0.059</td>
<td>99±2.8</td>
</tr>
<tr>
<td>Pepsanurin</td>
<td>1.24±0.14</td>
<td>0.024±0.007</td>
<td>0.222±0.040</td>
<td>109±2.0</td>
</tr>
<tr>
<td>2nd ANP</td>
<td>5.43±1.03§</td>
<td>0.671±0.177§</td>
<td>0.549±0.078*§</td>
<td>105±2.2</td>
</tr>
<tr>
<td>PIPH (n=13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.14±0.07</td>
<td>0.024±0.007</td>
<td>0.253±0.032</td>
<td>111±3.7</td>
</tr>
<tr>
<td>1st ANP</td>
<td>10.47±1.08</td>
<td>1.949±0.283</td>
<td>0.840±0.071</td>
<td>104±2.6</td>
</tr>
<tr>
<td>PIPH</td>
<td>1.53±0.20</td>
<td>0.061±0.019</td>
<td>0.265±0.042</td>
<td>111±3.9</td>
</tr>
<tr>
<td>2nd ANP</td>
<td>11.87±1.53§</td>
<td>2.338±0.330§</td>
<td>1.203±0.091§</td>
<td>106±3.8</td>
</tr>
<tr>
<td>BSAH (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.93±0.12</td>
<td>0.018±0.006</td>
<td>0.157±0.026</td>
<td>131±2.9</td>
</tr>
<tr>
<td>1st ANP</td>
<td>7.71±1.73</td>
<td>1.149±0.298</td>
<td>0.539±0.125</td>
<td>128±3.8</td>
</tr>
<tr>
<td>BSAH</td>
<td>1.32±0.18</td>
<td>0.082±0.051</td>
<td>0.174±0.043</td>
<td>127±3.7</td>
</tr>
<tr>
<td>2nd ANP</td>
<td>9.21±1.30§</td>
<td>1.202±0.203§</td>
<td>0.824±0.124§</td>
<td>124±3.7</td>
</tr>
</tbody>
</table>

Results are pooled from five pairs of samples of pepsanurin and hydrolysate of preincubated human plasma (PIPH). Plasma units were divided in halves and digested by pepsin, either before (pepsanurin) or after (PIPH) prolonged incubation at 37°C. Each sample was tested in two to three rats. A pepsin hydrolysate of bovine serum albumin (BSAH) was also tested in eight rats. ANP, atrial natriuretic peptide.

*Values expressed per 100 g body weight.
†Values from periods immediately before and after the ANP boluses (0.5 μg). Periods denoted PU, PIPH, and BSAH started 20 minutes after intraperitoneal injections of the respective hydrolysates.
‡p<0.05 vs. first ANP injection, paired t test.
§p<0.001 when comparing all four periods by analysis of variance.
ANP Blockade by Pepsanurin

Figure 1. Time course of changes in renal excretory parameters elicited by two intravenous boluses of atrial natriuretic factor (ANP) (0.5 μg) given at indicated times. The intraperitoneal injection of pepsanurin (PU) (0.5 ml/100 g body wt) 40 minutes before the second ANP bolus produced a significant reduction in the sodium (UNa), potassium (UK), and water (UV) excretory peaks. PU also prevented the ANP-induced peak in glomerular filtration rate (GFR) and reduced the increase in fractional sodium excretion (FENa, broken line, top panel). Mean arterial pressure (MAP) was reduced after the first ANP bolus and remained stable thereafter. *p<0.01 vs. first response, paired t test.

is referred to as PIPH. Two additional control preparations were used to test the specificity of pepsanurin effects. These consisted of a solution of 6.5% bovine serum albumin treated as described for the generation of pepsanurin, referred to as BSAH, and a similar hydrolysate prepared from a sample of human albumin, referred to as HSAH.

Experimental Protocols

Anesthetized rats. We used the bioassay described by De Bold et al5 with slight modifications.34 Fasted female rats (250-300 g) were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and heparinized. Polyethylene catheters were placed in the trachea, left jugular and femoral veins, and right carotid and femoral arteries. A continuous infusion of 5% dextrose was started (20 μl/min, jugular), and the urinary bladder was cannulated with a Silastic catheter via the urethra. After a 20–30-minute equilibration period, urine was collected during 10 periods of 20 minutes each. Mean arterial pressure (carotid) was monitored constantly.

Inhibition of ANP effect was assessed by comparing the responses to identical doses of the hormone given before and after administration of pepsanurin. Each rat served as its own control. Two intravenous boluses of ANP (0.5 μg in 50 μl isotonic dextrose, femoral) were given at the start of the fourth and ninth periods. Pepsanurin was thawed, neutralized with NaOH to pH 6.5–7.5, and injected (0.5 ml/100 g body wt) in the peritoneal cavity at the beginning of the seventh period, that is, 40 minutes before the second ANP bolus. In the control experiments, pepsanurin was replaced by an equal volume of neutralized PIPH or BSAH.

Inhibition of furosemide effect was studied in a similar protocol, but doses of 25, 50, or 100 μg furosemide were given in periods 4 and 9 instead of ANP. HSAH was used as the peptidic control in this series of experiments.

Amiloride was given as a single intravenous injection (10 μg/100 g body wt), because this diuretic agent produced a delayed and prolonged response. In parallel experiments, pepsanurin, PIPH, or BSAH (0.5 ml/100 g i.p.) were given 40 minutes after or 40 minutes before amiloride.

An additional experimental series was performed to test the effects of pepsanurin on basal urinary function. In this case, the rats received only one
intraperitoneal injection of pepsanurin at the begin-
ning of period 4.

For the determinations of GFR, a bolus dose of
30,000 cpm $^{51}$Cr-EDTA was given intravenously, fol-
lowed by a constant infusion of 1,000 cpm/min in the
5% dextrose solution. Thirty minutes was allowed for
tracer equilibration before the first collection period
was started. Arterial blood samples (0.6 ml) were
taken at the end of the second, sixth, and 10th
periods. After each sample, an equal volume of blood
from a donor rat was given to keep volemia and
arterial pressure constant. $^{51}$Cr-EDTA and sodium
were determined in plasma and urine samples to
calculate GFR and fractional sodium excretion.

Isolated kidneys. Male rats (250–280 g) were used.
After the rat was anesthetized with pentobarbital,
the right kidney was isolated and perfused at a fixed
flow (35–40 ml/min) in a 50-ml closed circuit as
previously described.\textsuperscript{6,7} Urine was collected every 15
minutes, and perfusion pressure was monitored. The
first experimental protocol was designed to test the
effect of pepsanurin on baseline renal function. After
two basal periods, 1 ml pepsanurin was added to the
perfusing fluid, and urine was collected during three
successive periods. Then, pepsanurin was washed out
of the perfusion system by replacing the medium, and
urine was collected again for two periods. The second
protocol tested the inhibition of ANP action by
pepsanurin. After two baseline collection periods, 0.5
fig ANP was added to the perfusing medium. Two
collection periods later, 1 ml pepsanurin was added,
still in the presence of ANP, and urine was collected
for two more periods. In separate experiments,
BSAH or PIPH was used instead of pepsanurin.

Analysis. Urinary volume was determined gravimet-
ically. Urinary sodium and potassium levels were
measured by flame spectrophotometry (Eppendorff,
FRG). $^{51}$Cr-EDTA was measured in a gamma counter
(Pharmacia LKB Biotechnology, Uppsala, Sweden).
Statistical significance of differences was estimated by
Table 3: Effects of Pepsanurin and Pepsin Hydrolysate of Human Albumin on Furosemide

<table>
<thead>
<tr>
<th>Collection period†</th>
<th>Volume (µl/min)</th>
<th>Sodium (µeq/min)</th>
<th>Potassium (µeq/min)</th>
<th>Arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 µg FUR (n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.41±0.26</td>
<td>0.133±0.008</td>
<td>0.140±0.031</td>
<td>138±7.3</td>
</tr>
<tr>
<td>1st FUR</td>
<td>7.63±2.99</td>
<td>0.388±0.158</td>
<td>0.389±0.059</td>
<td>137±7.4</td>
</tr>
<tr>
<td>Pepsanurin</td>
<td>2.12±0.30</td>
<td>0.127±0.034</td>
<td>0.332±0.077</td>
<td>129±8.3</td>
</tr>
<tr>
<td>2nd FUR</td>
<td>4.58±1.29</td>
<td>0.430±0.140</td>
<td>0.532±0.094$§</td>
<td>130±8.4</td>
</tr>
<tr>
<td>25 µg FUR (n=11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.51±0.27</td>
<td>0.083±0.026</td>
<td>0.243±0.059</td>
<td>118±7.6</td>
</tr>
<tr>
<td>1st FUR</td>
<td>3.96±0.80</td>
<td>0.334±0.091</td>
<td>0.453±0.129</td>
<td>118±8.6</td>
</tr>
<tr>
<td>HSAH</td>
<td>2.62±0.59</td>
<td>0.248±0.136</td>
<td>0.441±0.107</td>
<td>122±6.3</td>
</tr>
<tr>
<td>2nd FUR</td>
<td>6.98±1.89</td>
<td>0.550±0.247</td>
<td>0.729±0.167</td>
<td>121±8.4</td>
</tr>
<tr>
<td>100 µg FUR (n=17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.035±0.010</td>
<td>0.94±0.08</td>
<td>0.150±0.029</td>
<td>115±5.2</td>
</tr>
<tr>
<td>1st FUR</td>
<td>1.29±0.194</td>
<td>11.52±1.51</td>
<td>0.694±0.099</td>
<td>114±4.6</td>
</tr>
<tr>
<td>Pepsanurin</td>
<td>0.186±0.078</td>
<td>2.01±0.43</td>
<td>0.335±0.051</td>
<td>115±4.3</td>
</tr>
<tr>
<td>2nd FUR</td>
<td>1.15±0.298</td>
<td>16.11±2.23$¶</td>
<td>0.815±0.090$¶</td>
<td>115±3.9</td>
</tr>
<tr>
<td>100 µg FUR (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.43±0.24</td>
<td>0.10±0.06</td>
<td>0.083±0.019</td>
<td>117±6.7</td>
</tr>
<tr>
<td>1st FUR</td>
<td>14.64±5.14</td>
<td>1.36±0.82</td>
<td>0.347±0.050</td>
<td>119±6.0</td>
</tr>
<tr>
<td>HSAH</td>
<td>2.18±0.38</td>
<td>0.22±0.18</td>
<td>0.263±0.099</td>
<td>115±6.2</td>
</tr>
<tr>
<td>2nd FUR</td>
<td>14.58±2.53$§</td>
<td>1.22±0.31</td>
<td>0.307±0.072</td>
<td>114±6.6</td>
</tr>
</tbody>
</table>

FUR, furosemide; HSAH, pepsin hydrolysate of human albumin.

†Values correspond to periods immediately before and after intravenous FUR injections. Periods marked pepsanurin and HSAH started 20 minutes after intraperitoneal injections of the respective hydrolysate.

$P<0.05 vs. first FUR injection, paired t test.

§/?<0.01, /?/0.001, comparing all four periods by analysis of variance.

a one-way analysis of variance followed by Newman-Keuls test when comparing several groups or time course of responses. In addition, the Student’s paired t test was used to compare a priori specified responses in the same animal or preparation.

Results

Effects of Pepsanurin on Baseline Excretion

Unstimulated urinary excretion was very stable during the course of the experiment. In addition, the intraperitoneal injection of pepsanurin did not modify any of the urinary or vascular parameters under study (Table 1).

Effects of Pepsanurin on Atrial Natriuretic Peptide

Pepsanurin significantly blunted the diuretic, natriuretic, and kaliuretic effects of the second dose of ANP, whereas injections of PIPH prepared from the same plasma samples or of BSAH had no demonstrable effect (Table 2). The antidiuretic effects of pepsanurin were associated with abolishment of the GFR peak normally observed after ANP administration but also with a significant reduction in the fractional sodium excretion peak induced by ANP (Figure 1). These pepsanurin-induced changes cannot be attributed to variations in pressure, because mean arterial pressure did not change significantly during these collection periods (Table 2, Figure 1).

Effects of Pepsanurin on Furosemide

In contrast to the effect on ANP, pepsanurin was unable to inhibit the diuretic response to different doses of furosemide (Table 3). In fact, the response to the second furosemide bolus tended to be higher than the first, regardless of whether the rats had received pepsanurin or HSAH (Table 3). Clear-cut results were obtained in a series in which the same pepsanurin sample, known to inhibit 0.5 µg ANP, was unable to counteract an equipotent dose of 50 µg furosemide (Figure 2).

Effects of Pepsanurin on Amiloride

Injection of amiloride (10 µg/100 g) produced a delayed and prolonged rise in urinary sodium and fractional sodium excretion, accompanied by a rather moderate increase in diuresis, a drastic but transient reduction in urinary potassium, and no changes in GFR (Figure 3). The injection of pepsanurin at the time of peak response, 40 minutes after amiloride, produced a mild reduction in mean arterial pressure and a significant reduction in sodium excretion toward baseline (Figure 3). In contrast, PIPH-treated rats responded similarly to control, even when they had the lowest mean arterial pressure level (Figure 3). Pepsanurin counteracted the natriuretic effect but did not antagonize the potassium retention effect of...
Effects of Pepsanurin on Isolated Kidneys

Addition of pepsanurin (1 ml/50 ml) to the perfusion medium produced a significant reduction in urinary volume and urinary potassium and sodium levels, without concomitant changes in perfusion pressure (Table 4). Furthermore, the effects on urinary sodium and urinary volume were reversed after pepsanurin was washed out with fresh medium (Table 4). However, the pepsanurin inhibitory effect was more marked on the natriuresis and diuresis elicited by ANP (Table 4). These effects were observed in the absence of a significant fall in perfusion pressure. In comparison, the addition of unspecific peptidic material to the perfusing fluid had no effect, except for a significant increase in perfusion pressure observed after PIPH (Table 4).

Discussion

The present results confirm the striking inhibitory action of pepsanurin on renal responses to ANP. Furthermore, for the first time, this effect was seen both in intact rats and in isolated perfused kidneys. This inhibition cannot be accounted for by the unspecific effect of the bulk of peptides, other than pepsanurin, present in the peritoneal cavity, because neither outdated or preincubated plasma nor bovine or human serum albumin hydrolysates similarly treated with pepsin were able to release this putative antinatriuretic factor or factors.

Pepsanurin inhibited the glomerular and tubular actions of ANP,8-9 abolishing the ANP-induced rise in GFR and decreasing ANP-induced elevation in fractional sodium excretion. These results suggest that pepsanurin could act as an anti-ANP agent. Until now, no specific anti-ANP substances have
been reported.\textsuperscript{10,11} Enalapril can inhibit ANP in normal human subjects,\textsuperscript{12} but this effect was apparently mediated by the hypotensive effect of this substance. In contrast, we found that the inhibitory action of pepsanurin on ANP occurred while blood pressure remained constant. In the isolated kidney, inhibition of ANP by pepsanurin was also observed in the absence of major hemodynamic changes. As described,\textsuperscript{13} ANP increased perfusion pressure in vasodilated preparations, but the subsequent inhibition of ANP by pepsanurin was not associated with a decrease in this parameter. We cannot preclude, however, that the inhibition of ANP by pepsanurin could be mediated by subtle changes in intrarenal hemodynamics and peritubular forces, which have been recognized as important determinants for the action of this hormone.\textsuperscript{10,11}

In this regard, the different results obtained with pepsanurin on the diuretic actions of furosemide and amiloride suggest that pepsanurin could act on sodium-transporting mechanisms at the distal tubule.\textsuperscript{14,15} Moreover, the finding that pepsanurin did not inhibit the potassium retention effect of amiloride would support the concept of an enhanced neutral sodium reabsorption, perhaps in association with chloride or in exchange by hydrogen ions.\textsuperscript{14,16} On the other hand, there is a possibility that the blunted response to amiloride could have been mediated, at least partially, by the decrease in mean arterial pressure observed after the injection of that particular pepsanurin sample. This contention is not supported, however, by the finding that there were no differences in blood pressure between rats treated with pepsanurin and control hydrolysates during the period of response to amiloride.

A better characterization of the mechanisms of the pepsanurin inhibitory action on ANP and amiloride requires further purification of pepsanurin, which is still underway. One of the problems in pepsanurin purification is the rather burdensome bioassay needed to test its inhibitory effect on renal function. Nevertheless, at the present stage, we know that pepsanurin can be partially purified by ultrafiltration and ion-exchange chromatography. Also, pepsanurin inhibitory action on ANP and amiloride suggest that pepsanurin could act on sodium-transporting mechanisms at the distal tubule.\textsuperscript{14,15} Moreover, the finding that pepsanurin did not inhibit the potassium retention effect of amiloride would support the concept of an enhanced neutral sodium reabsorption, perhaps in association with chloride or in exchange by hydrogen ions.\textsuperscript{14,16} On the other hand, there is a possibility that the blunted response to amiloride could have been mediated, at least partially, by the decrease in mean arterial pressure observed after the injection of that particular pepsanurin sample. This contention is not supported, however, by the finding that there were no differences in blood pressure between rats treated with pepsanurin and control hydrolysates during the period of response to amiloride.

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Although pepsanurin has been obtained in artificial conditions, we cannot preclude the possibility that it may play a physiological role. We demonstrated that pepsanurin activity is eliminated by plasma incubation at 37°C before pepsin hydrolysis. We extended this observation by showing that pepsanurin activity is progressively reduced with increasing preincubation times between 2 and 48 hours,\textsuperscript{17} a finding consistent with the idea that an endogenous plasma substrate is being depleted. Furthermore, plasma obtained from patients with severe, decompensated congestive cardiac failure yields higher pepsanurin activity than plasma obtained from patients with mild cardiac failure or normal volunteers.\textsuperscript{18} This observation is interesting, because congestive cardiac failure has been related to volume expansion, sodium retention, and refractoriness to ANP.\textsuperscript{19} Moreover, it is well known that under peptic hydrolysis from plasma precursors, active hemodynamic factors such as angiotensin I, bradykinin, bradykinin-like substances, metenkephalin-related peptides, and neurotensin-like peptides have been identified.\textsuperscript{1} Particularly relevant is the case of neurotensin, which has antidiuretic properties,\textsuperscript{20} although it apparently does not exert its action directly on the kidney.\textsuperscript{21} In this context, it has been proposed that one or a combination of neurointestinal peptides may be part of a signaling mechanism influencing renal excretory rate,\textsuperscript{21,22} and it seems pertinent to explore to what extent pepsanurin and its plasma precursor could be involved in this process.

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References


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