Blunting Effect of Pepsanurin Introduced in the Duodenum on the Atrial Natriuretic Peptide Diuretic Action in Rats (43549)

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Abstract. **Pepsanurin (PU) is a peptide(s) obtained by pepsin hydrolysis of human plasma or its globulin fraction. We have reported that the accelerated renal excretory rate induced by atrial natriuretic peptide (ANP) can be considerably blunted by PU either in the intact rat or in the isolated perfused rat kidney. We explored whether or not PU can be part of a signaling mechanism originated in the digestive tract, involved in the regulation of water and electrolyte homeostasis. PU obtained either from human (0.5 ml) or rat plasma (0.25–0.5 ml) administered into the duodenal lumen of rats, counteracted significantly the diuretic-saluretic action of a 0.5- μg bolus of ANP, reproducing qualitatively the effect of its intraperitoneal administration. Human PU reduced the ANP-stimulated renal excretion by 67–90% for Na ($P < 0.001$) and by 35–54% for water ($P < 0.02$–$P < 0.001$); the inhibition induced by rat PU was 45–96% for Na ($P < 0.05$–$P < 0.01$) and 35–65% for water ($P < 0.05$–$P < 0.01$). Rat PU (0.5 ml) abolished the rise of glomerular filtration rate induced by ANP without affecting fractional Na excretion. All the samples tested decreased K excretion, but in some experiments, the difference did not reach statistical significance. Contrary to the effect of PU, the introduction in the duodenum of (i) isotonic glucose solution, (ii) hydrolysate of bovine serum albumin, or (iii) hydrolysate of casein prepared after the same procedure yielding PU from plasma failed to produce an inhibition of the ANP stimulation of renal excretory rate. In addition, human plasma incubated at 37°C for 24 to 48 hr, prior to pepsin digestion, did not yield PU, which indicates that PU is generated from a substrate sensitive to endogenous enzymes and/or that its stability is vulnerable to endogenous enzymes.**

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We have shown that pepsanurin (PU), a peptide fraction obtained by pepsin hydrolysis of human fresh plasma or its globulin fraction at pH 2.5, counteracts the effect of atrial natriuretic peptide (ANP) on water, Na, and K urinary excretion (1). This blunting effect can be obtained by giving PU either intraperitoneally to anesthetized rats or by adding it to the fluid of a perfused isolated rat kidney (2). Chymotrypsin at pH 7–8 destroys PU activity (3); similar inactivation is obtained by incubation of PU with trypsin (unpublished). These actions provide proof that PU is a peptide. Control peptidic hydrolysates prepared either from human plasma preincubated 48 hr at 37°C (PIHP) or from bovine serum albumin (BSA) or human serum albumin did not inhibit ANP (2). These findings lend support to the existence in fresh blood plasma both of a specific substrate and of an endogenous enzymatic system able to transform and deplete the protein precursor and the released peptide(s) at neutral pH.

PU blocks the rise in glomerular filtration rate (GFR) and reduces the increase in fractional sodium excretion induced by ANP, but it has no effect on basal urinary excretion (2). In addition, PU does not counteract the diuretic action of furosemide, but inhibits the natriuretic effect of amiloride (2). These findings suggest a specific intrarenal mechanism closely related and opposed to ANP glomerular and tubular action (2). Furthermore, previous studies have demonstrated that neither aldosterone (4) nor vasopressin (1) can hinder the diuretic-saluretic action of a bolus of ANP in the

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anesthetized rat, under the experimental condition that allows PU (0.5 ml/100 g body wt) given intraperitoneally to block 80–90% of the typical urinary changes induced by the atrial hormone. To our knowledge, up to now, no other substance possessing such a strong inhibitory effect on the atrial hormone diuretic action has been described. Although PU has been obtained in artificial conditions, we cannot preclude the possibility that it or some related peptide may play a physiologic role.

It is well known that peptic hydrolysis of blood plasma yields active peptides, such as angiotensin I, metenkephalin, neurotensin, and bradykinin-like peptides (5–9). In this respect, it is noteworthy to recall that neurotensin (NT)-related peptide(s) was generated by pepsin hydrolysis not only of blood plasma (6) but also rat and chicken gastric tissues (10). In addition, recent observations support the view that NT may play a role in cardiovascular functions and/or body fluid homeostasis (11, 12). However, we have ruled out the possibility that NT can account for the anti-ANP action of PU because it inhibited the effect of ANP on urinary excretion only at doses that lowered blood pressure (13). These considerations led us to explore the possibility that PU, generated within the gastric lumen by the action of pepsin, might participate in the regulation of renal excretory function. Until now little attention has been given to gut peptides as possible mediators of the digestive tract effects on the urinary excretion of water and electrolytes (14). In the present paper, we report the ability of PU introduced in the duodenal cavity to inhibit ANP-stimulated renal excretory rate.

Materials and Methods

ANP (5–28 atriopeptin II, rat form), pepsin (twice crystallized), and casein purified powder from bovine milk were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin was from Calbiochem (San Diego, CA). Tritiated polyethylene glycol was from New England Nuclear Corp. (Boston, MA).

Preparation of PU. As starting material, fresh human and rat blood plasma were used. Human blood from two healthy male donors was obtained in the blood bank. Adults male rats (350 g) anesthetized with pentobarbital (40 mg/ip) served as donors. Blood was drawn from the abdominal aorta and collected in tubes with sodium citrate. Four pools formed with the blood of six rats each, and individual samples drawn from five rats were used. All blood samples were immediately centrifuged, and the plasma was dialyzed overnight (12- to 14-kDa cutoff membrane) against distilled water in a cold chamber (4°C). After acidification with 10 *N* HCl to pH 2.5, pepsin was added to a final concentration of 0.65 mg (2.400 units/ml)/ml of plasma and incubated for 18 hr at 37°C. At the end of this period, the sample was heated in a boiling water bath to inactivate pepsin. The temperature of the sample was kept for 1 min at 80°C and, after cooling, it was centrifuged. The clear supernatant was used, and the pH was adjusted with NaOH immediately before injection. The total protein content (Lowry) was about 58–75 mg/ml and Na concentration was 120–180 μEq/ml after pH adjustments. In this study, excluding one PU sample that was tested at two different doses (0.5 ml and 0.25 ml), all the data presented correspond to the effects of the same dose, the amount of PU obtained from 0.5 ml of rat or human plasma or of the control solutions. The figures expressing the amount of PU given to the rats were adjusted to the volume of plasma from which PU was obtained.

Preparation of Control Hydrolysates. *Preincubated plasma.* In order to produce an endogenous depletion of the presumptive PU substrate, PIHP was prepared as already described (2). One of the fresh human plasma samples was divided into two parts after dialysis: the first half was immediately submitted to the procedure to generate PU, while the second was kept at 37°C for 48 hr after adding 50 units of penicillin and 50 μg of streptomycin (1 ml per 10 ml of the hydrolysate). Bacteriologic tests demonstrated that the antibiotic mixture was effective for preventing microbial growth.

Hydrolyzed bovine serum albumin. BSA (70 mg/ml) dissolved in distilled water was acidified (pH 2.5) by adding 50% HCl solution and was submitted to the digestion procedure with pepsin (0.65 mg/ml) as described to obtain PU.

Hydrolyzed casein. Casein dissolved in water to final concentrations of 70, 140, and 500 mg/ml was dialyzed against distilled water, acidified to pH 2.5, treated with pepsin (0.65 mg/ml), and incubated in a similar way as blood plasma.

Glucose. As a control, 0.5 ml of sterile solution of isotonic glucose (5%) at room temperature was introduced into the duodenum in six rats.

Biologic Assay to Test the Anti-ANP Diuretic-Natriuretic Effect of PU. A previously reported bioassay was adopted with slight modifications (1). Female adult rats (200–220 g) in fasting conditions, and with free access to water, were anesthetized with pentobarbital (40 mg /ip) and heparinized. Five percent isotonic glucose solution (20 μl/min) was infused through a jugular vein; blood pressure was constantly monitored by connecting one femoral artery to a transducer and a Grass polygraph. A bladder cannula allowed the collection of urine and the determination of volume, Na, and K excretions during 10 periods of 20 min each. Identical doses of ANP were given before and after administration of PU. Two intravenous boluses of 0.5 μg of ANP in 100 μl of isotonic glucose were given at the start of the fourth and ninth periods. PU was thawed and its pH was adjusted to 5–5.6 immediately before injections

Table I. Na, K, and H_2O Urinary Excretion Induced by ANP before and after PU or PIHP Administration[a]

Human samples	Route of administration	n	Before PU or PIHP administration: Na (μEq/20 min)	Before: K (μEq/20 min)	Before: H_2O (μl/20 min)	After PU or PIHP administration: Na (μEq/20 min)	After: K (μEq/20 min)	After: H_2O (μl/20 min)
A (PU)	ip	4	31.7 ± 6	9.4 ± 0.9	16 ± 2	10.3 ± 3[b]	6 ± 0.8[c]	8 ± 2[c]
A (PU)	idu	8	23.5 ± 4.7	8.9 ± 0.5	13 ± 1	7.8 ± 2.9[d]	5.3 ± 2[b]	6 ± 1[d]
A (PIHP)	idu	6	33.2 ± 5	14.6 ± 2	16.5 ± 2	54.4 ± 18[e]	15.4 ± 3[e]	25 ± 9[c]
B (PU)	ip	4	48.4 ± 3.2	15.1 ± 2.3	20.6 ± 1.7	12.5 ± 1.9[f]	7.1 ± 0.7[b]	7.6 ± 1.3[f]
B (PU)	idu	6	19.4 ± 2.8	7.9 ± 2.4	16 ± 1.7	1.8 ± 0.49[d]	5.29 ± 1.3[e]	5.6 ± 1.4[f]

[a] Table shows the effects of PU and PIHP prepared from human plasma. The intraperitoneal (ip)- or intraduodenal (idu)-injected amount corresponded to 0.5 ml of two different plasmas (A and B). PIHP was obtained from an aliquot of Plasma A submitted to preincubation. Injections were given 40 min before second ANP intravenous administration.
[b] $P < 0.02$.
[c] $P < 0.05$.
[d] $P < 0.001$.
[e] Not significant.
[f] $P < 0.01$.

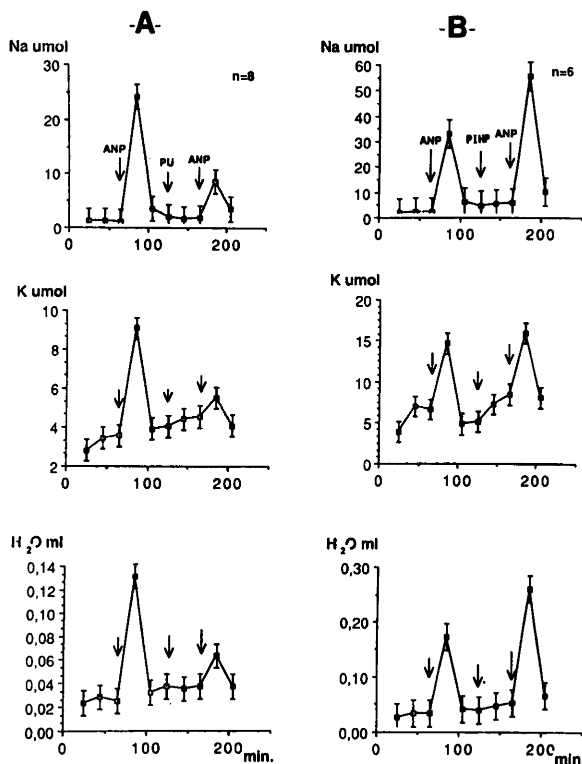

Figure 1. Time course of renal excretion of Na, K, and water in anesthetized rats in response to an intravenous bolus of 0.5 μg of atrial natriuretic peptide before and after treatment with pepsanurin or control preparations (PIHP). (A) PU obtained from 0.5 ml of human plasma was injected into the duodenum of eight rats 40 min before the second ANP injection. (B) PIHP (0.5 ml) obtained from the same plasma as PU, but submitted to an incubation at 37°C for 48 hr before hydrolysis with pepsin, was injected into the duodenum of six rats 40 min before the second injection of ANP. The plots show the means $\pm$ SE of Na, K, and water excreted at each 20-min interval and referred to as 100 g of the rat's body weight.

at the beginning of the seventh period, i.e., 40 min before the second ANP bolus. In control experiments, PU was replaced by the injection of an equal volume of PIHP or BSA or isotonic glucose solution in order to check the role of both hydrolysis-specific effects and mechanical manipulations (distension of the intestinal wall). Their effects were tested in the same bioassay. In all the experiments, each rat served to evaluate the effect of only one sample, either PU or control solutions. Na and K (μEq) and water (μl) excreted in each period were referred to 100 g body wt/20 min. The diuretic-saluretic responses after the first and second ANP injections were submitted to statistical evaluation.

This procedure was slightly modified for intraduodenal injections as follows. In the anesthetized rat, before heparinization, the duodenum was localized by performing a small opening in the middle of the abdominal wall. Forty minutes before the second ANP bolus, the solution containing PU or the control solutions were gently introduced in the intestinal lumen through a fine needle. All solutions were at room temperature at the time of treatment.

Particular attention was given to blood pressure changes. The few experiments in which the second ANP injection lowered blood pressure by 15 mm Hg or more in excess of the decrease observed after the first one were excluded from the analysis.

To throw some light on the mechanism of PU anti-ANP action, measurement of GFR and fractional sodium excretion was undertaken in a group of seven rats as described (2) using tritiated polyethylene glycol as tracer instead of ^{51}Cr-EDTA (15).

The mean and SE of water, Na, and K urinary excretion were calculated for each collecting period. Significant differences were evaluated with Student's paired t test.

Results

Table I shows the inhibitory effect of two batches of human PU (A and B) upon the urinary excretory response to ANP. Intraduodenal administration of PU produced significant inhibition on Na ($P < 0.001$) and

Table II. Na, K, and H_2O Urinary Excretion Induced by ANP before and after Administration of PU[a]

Rat samples	Route of administration	n	Before PU or PIHP administration			After PU or PIHP administration		
			Na (μEq/20 min)	K (μEq/20 min)	H_2O (μl/20 min)	Na (μEq/20 min)	K (μEq/20 min)	H_2O (μl/20 min)
A-PU (pool)	ip	4	16.4 ± 3.5	6.5 ± 0.4	14 ± 2.4	0.66 ± 0.16[b]	2.5 ± 0.3[c]	2.9 ± 0.4[c]
A-PU	idu	7	13 ± 2.55	8.2 ± 1.1	10.6 ± 1.3	2.1 ± 0.8[b]	4.7 ± 1.6[c]	4.6 ± 0.8[b]
B-PU (pool)	ip	6	16 ± 4.9	9.8 ± 1.1	13 ± 2.8	1.55 ± 0.6[c]	3.7 ± 1[b]	3.3 ± 0.6[c]
B-PU	idu	7	32.8 ± 5.3	13.3 ± 2	19 ± 2.6	12.6 ± 3.8[b]	6.8 ± 1.4[b]	9 ± 1.9[b]
C-PU	idu	4	33 ± 0.9	13.5 ± 2.3	18 ± 1.2	17 ± 4.9[c]	10.3 ± 2.1[d]	11 ± 3[c]
D-PU	idu	5	9.5 ± 1.9	9 ± 2.8	9 ± 1.3	0.4 ± 0.1[c]	2.3 ± 0.4[d]	3.5 ± 1[b]
E-PU	idu	6	21 ± 1.2	13.6 ± 2.3	13 ± 0.7	8 ± 1.5[e]	6.9 ± 1.1[b]	6.4 ± 1.6[e]
F-PU (pool)	idu	6	30.3 ± 5.6	11.5 ± 0.9	16.4 ± 2.2	16.7 ± 1.3[b]	9.9 ± 1.7[d]	11 ± 2.3[b]

[a] Table shows the effect of PU prepared from 0.5 ml of rat plasma and injected; A, B, and F were obtained from a pool of blood drawn from three lots of rats; the others were prepared from individual samples.
[b] $P < 0.02$.
[c] $P < 0.05$.
[d] Not significant.
[e] $P < 0.01$.

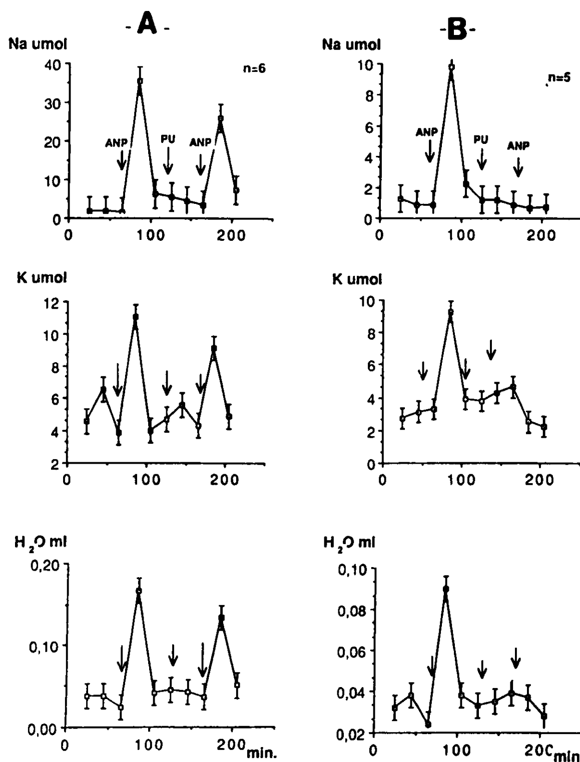

Figure 2. For details, see legend to Figure 1. (A) Effect of 0.25 ml of rat PU and (B) effect of 0.5 ml of the same rat PU injected into the duodenum in eight and six rats, respectively.

water ($P < 0.01$–$P < 0.001$) excretion, comparable to that produced by intraperitoneal administration. The effect of intraduodenal PU injection on K was less constant and never as prominent as the fall in Na excretion. The decrease in K excretion produced by intraduodenal administration of the B sample was not significant. Intraduodenal administration of PIHP failed to hinder ANP effect but it increased water excretion, a feature already observed after intraperitoneal injection of PIHP (2) and also observed in the present work when watery samples such as isotonic glucose solution were introduced in the duodenum cavity (Table III). Figure 1, A and B, illustrates the time course of the excretory rate in rats injected with PU ($n = 8$) or with PIHP ($n = 6$) obtained from the same human plasma.

Table II shows the effects of PU prepared from six different batches of rat plasma (A through F) given by intraduodenal injection and two batches (A and B) also given intraperitoneally. Intraduodenal and intraperitoneal administration of 0.5 ml of rat PU inhibited Na ($P < 0.05$–$P < 0.01$) and water ($P < 0.05$–$P < 0.01$) excretion. Significant inhibitory effect was also obtained with PU prepared from a pool of rat plasma (data not tabulated in Table II, but illustrated in Fig. 2, A and B). Figure 2 shows the difference of the inhibitory response induced by 0.5 ml and 0.25 ml of rat PU given by intraduodenal injection.

The typical increase in GFR rate induced by ANP was significantly inhibited by rat PU given in the duodenal cavity of a group of seven rats. The first ANP injection increased GFR from 0.57 ± 0.11 to 0.96 ± 0.09 ml/min/100 g body wt ($P < 0.001$), but the second ANP injection, after 0.5-ml intraduodenal rat PU injection, did not change this parameter (from 0.46 ± 0.03 to 0.35 ± 0.07 ml/min/100 g body wt, NS). In contrast, this dose of rat PU did not modify sodium reabsorption. Fractional sodium excretion was increased by the first ANP bolus (from 0.018 ± 0.003 to 1.215 ± 0.414%, $P < 0.001$), and increased to a similar extent by the second ANP bolus (from 0.072 ± 0.020 to 1.101 ± 0.247%, $P < 0.001$). The corresponding urinary sodium excretion values were increased from 0.32 ± 0.08 to 23.57 ± 5.85 μEq/20 min/100 g by the first ANP injection and from 0.72 ± 0.20 to 8.09 ± 1.21 μEq/20 min/100 g by the second ANP injection

Table III. Na, K, and H_2O Urinary Excretion Induced by ANP before and after Administration of Control Solutions[a]

Control solutions		*n*	Before control solution			After control solution		
			Na (μEq/20 min)	K (μEq/20 min)	H_2O (μl/20 min)	Na (μEq/20 min)	K (μEq/20 min)	H_2O (μl/20 min)
Hydrolyzed BSA	idu	6	20.3 ± 2.7	11.5 ± 0.9	12.6 ± 0.02	25.6 ± 4.4	13.8 ± 2.1	15.9 ± 1.2
Hydrolyzed casein								
70 mg/ml	idu	4	42 ± 8	12.4 ± 1.1	22 ± 4	67 ± 13	19 ± 1.2	37.6 ± 8.6
140 mg/ml	idu	6	36.5 ± 7	10.2 ± 1.3	20.7 ± 3	36 ± 6	17 ± 2.7	21.8 ± 3.7
500 mg/ml	idu	3	39.8 ± 12	11.3 ± 2.4	18 ± 3.5	41 ± 17	15.4 ± 0.9	23.5 ± 4.5
Glucose 5%	idu	6	27.3 ± 2.8	11.8 ± 0.7	18 ± 1.3	35.8 ± 4.2	12.6 ± 0.7	27.8 ± 4.1

[a] Table shows the effects of control solutions (0.5 ml) introduced into the duodenal lumen 40 min before the second 0.5-μg ANP bolus.

(60.0 ± 8.2% of inhibition, $P < 0.05$, between both ANP responses).

Table III shows the effects of intraduodenal injections of control solutions: hydrolyzed BSA, hydrolyzed casein (three different concentrations), and sterile 5% isotonic glucose solution. All of them, injected at the dose of 0.5 ml in the duodenal lumen, were not only unable to reproduce the blunting effect of PU, but enhanced the natriuretic and diuretic action of ANP.

Discussion

A major finding in this study was that PU administered directly into the digestive tract produced a blunting effect on ANP diuretic-saluretic action that qualitatively did not differ from the one observed after intraperitoneal administration. The experiment, designed to determine changes in GFR and tubular sodium reabsorption, showed that the antidiuretic effects of rat PU given intraduodenally were associated with abolishment of the GFR peak, as seen in rats receiving human PU intraperitoneally (2), but the tubular effects of PU were not observed in this case. This lack of inhibitory effect on tubular Na reabsorption could be accounted for by the rather low capacity to counteract ANP action present in the PU sample used in this group of rats, as compared with other batches. This would imply that PU inhibitory action is more readily observed upon the glomerular than the tubular effects of ANP. It is also interesting that the time elapsed between PU injection and the inhibition of the response to the second ANP bolus was the same (40 min) in both series of experiments, a fact that supports the assumption that a similar mechanism counteracting ANP diuretic-saluretic properties could be involved. However, experiments were not yet performed leading to a precise determination of optimal timing between administration of PU (intraduodenal and intraperitoneal) and the ANP injections to obtain maximal inhibitory effects. In general, the percentage of the decrease in natriuresis did not show significant differences on the anti-ANP effect between the same dose of PU given in the digestive tract and given intraperitoneally. In this context, this result is in keeping with the relevant features of intestinal peptides, which show high luminal concentration and high stability and absorbability through the mucosa, despite the occurrence of proteolytic enzyme activities in the lumen (14). In the rat, NT located in the gut is released into the blood and into the lumen of the bowel (11). We ruled out NT as the agent responsible for anti-ANP effects observed with our PU preparations. Although we cannot exclude that they could have some traces of NT, previous experiments showed us that its antidiuretic effect could only be observed at doses that induced marked hypotensive action (13). Furthermore, the present data allowed us to preclude an indirect effect derived from changes in the systemic blood pressure, because whichever the route of injection, no significant systemic hemodynamic changes were observed. In addition, the inhibition cannot be accounted for by a nonspecific effect of the bulk of peptides other than PU, as demonstrated by experiments with PIHP, hydrolyzed BSA, and hydrolyzed casein. Although the chemical identification of PU is still underway, it is interesting to consider the demonstration that some, but not all, peptides instilled on the duodenal mucosa can exert a clear-cut inhibition of ANP action on its renal excretory role, a fact that highlights the digestive tract mucosa modifying kidney excretory functions. The blunting effect of PU described here can be mediated by the nervous system and/or by the release of some additional humoral factor(s) elaborated by the endocrine cells of the digestive tract.

It is tempting to speculate that a peptide similar to PU can be set free by enzymes in the digestive tract (an appropriate candidate could be pepsin of gastric juice) acting upon some globulin precursor secreted by the mucosa. The amount of the inhibitory peptide could be regulated according to the water content and osmolality of the ingested food and the need to produce the digestive juices in order to satisfy both the digestive process and the homeostatic requirements.

There is much to be learned concerning PU as a possible physiologic humoral factor having a regulatory role. In this regard, it is worthwhile to mention the

significant increase in the yield of PU of plasma of patients with advanced congestive heart failure and notorious insufficiency in water and electrolytes excretion which may underlie the decreased sensitivity to ANP (16). It is feasible to visualize that the disclosure of the overall process involved in the PU action can contribute to a better understanding of the interplay between the digestive tract and kidney functions, and of the decreased sensitivity of the kidney to ANP action observed in experimental and pathologic conditions.

In addition, it is pertinent to mention that in anesthetized rats, PU significantly inhibits urinary water and sodium excretion induced by a 10% volume expansion with 7% BSA intravenously, as compared with control rats that receive hydrolyzed BSA instead of PU (unpublished results). This finding supports the hypothesis that PU is able to inhibit endogenous ANP.

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1. Croxatto HR, Rosas R. Atrial natriuretic factors (ANF) and antidiuretic agents. Acta Physiol Pharmacol Latinoam **38**:1–9, 1988.
2. Borić MP, Croxatto HR, Roblero JS, Albertini RB. Inhibition of ANP induced natriuresis by plasma hydrolisates containing pepsanurin. Hypertension [Suppl] **II**:II-243–II-250, 1992.
3. Croxatto HR. Polypeptides with posterior-pituitary-like activities. Proceedings of the eighth Symposium of the Colston Research Society. London: Butterworths Scientific Publications, pp51–63, 1956.
4. Croxatto HR, Rosas R, Gengler J. Potentiating effect of aldosterone on the diuretic action of atrial extract. Experientia **43**:604–606, 1987.
5. Croxatto HR. How many peptidic hormones can derive from blood plasma proteins? News Physiol Sci **5**:201–204, 1990.
6. Carraway RE, Mitra SP, Ferris CF. Pepsin treatment of mammalian plasma generates immunoreactive and biologically active neurotensin-related peptides in micromolar concentrations. Endocrinology **119**:1519–1526, 1986.
7. Singer EA, Mitra SP, Carraway RE. Plasma protein(s) yields met-enkephalin-related peptides in near-micromolar concentrations when treated with pepsin. Endocrinology **119**:1527–1533, 1986.
8. Herrmann C, Cuber JC, Abello J, Dakka T, Bernard C, Chayvialle JA. Release of ileal neurotensin in the rat by neurotransmitters and neuropeptides. Regul Pept **32**:181–192, 1991.
9. Giraud AS, Parker L, Reichman C, Familari M, Smith AI, Funder J. Generation of met-enkephalin Arg6Phe7 immunoreactivity by proteolytic cleavage of mammalian plasma precursors by pepsin. Endocrinology **124**:1711–1716, 1989.
10. Mashford ML, Nilsson G, Rokaeus A, Rosell S. Release of neurotensin-like immunoreactivity (NTLI) from the gut in anaesthetized dogs. Acta Physiol Scand **104**:375–376, 1978.
11. Unwing RJ, Ganz MD, Sterzel RB. Editorial review. Brain-gut peptides, renal function and cell growth. Kidney Int **37**:1031–1047, 1990.
12. Kivlighn SD, Jandhyala BS. Antidiuretic effects of neurotensin in chloralose anaesthetized dogs. Clin Exp Pharmacol Physiol **17**:401–412, 1990.
13. Croxatto HR. Blood plasma proteins as substrates for the formation of peptidic hormones. In: Chiou SH, Wang KT, Wu SH, Eds. International Symposium on Biologically Active Proteins and Peptides. Republic China, Taipei: Institute of Biological Chemistry, Academia Sinica, Taipei, TAIWAN, R.O.C. 1988.
14. Rao RK. Biologically active peptides in the gastrointestinal lumen. Life Sci **48**:1685–1704, 1991.
15. Nakane H, Nakane Y, Reach G, Corvol P, Menard J. Aldosterone metabolism in isolated perfused kidney. Am J Physiol **234**:E472–E479, 1978.
16. Guarda ES, Corbalan RH, Croxatto HR, Silva RN, Vergara TL, Valenzuela CP, Albertini RB. Pepsanurina en la insuficiencia cardiáca congestiva. Rev Med Chile **119**:137–141, 1991.