A Fragment of Human Kininogen Containing Bradykinin Blunts the Diuretic Effect of Atrial Natriuretic Peptide (43999)

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Abstract. **A synthetic 15 amioacids kinin, named PU-15, is able to block the diuretic natriuretic action of Atrial Natriuretic Peptide (ANP). The structure of PU-15 is Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ser-Ser-Arg-Iso, having the aminoacid sequence of a fragment of human kininogens. The increase in the urinary excretion of sodium, potassium, and water, elicited by a bolus of 0.5 μg of ANP in anesthetized rats, is blocked by PU-15 (100–150 ng) given either intravenously 3 min before ANP injection, or injected intraperitoneally or in the duodenal lumen, 40 min before ANP. This ANP blockade, which mimics the action of pepsanurin, is only obtained with doses of PU-15 in a narrow range around 100 picomol/rat, and do not modify blood pressure. Larger doses, 2- to 8-fold the effective dose, either do not change the response to ANP or raise the excretion of sodium and water. The administration of HOE-140, a bradykinin B2 receptor blocker, prior to PU-15, completely abolishes the anti-ANP action of PU-15. These findings lend support to the proposal that kinins released from the intestinal tract during prandial period can modulate renal excretory function.** [P.S.E.B.M. 1996, Vol 212]

The name pepsanurin (PU) was coined to designate a semipurified peptide fraction originating from the incubation of blood plasma globulins with pepsin, whose main effect is to inhibit urinary excretion in hyperhydrated rats (1, 2). Besides PU, two other fractions were described: one with vasopressor and another with oxytocic action, later identified with angiotensin I (3) and met-lys bradykinin (4) respectively. PU did not show antidiuretic action in normally hydrated rats (2). However, the intraperitoneal (ip) administration of PU, generated from 0.5–1.0 ml of human plasma, to anesthetized rats caused a 70%–90% inhibition of the sodium, potassium, and water excretion induced by an ANP bolus (0.5 μg), without altering the blood pressure (5–7). Moreover, PU counteracted the natriuretic effect of amiloride, but it did not affect the diuretic response to furosemide (6). PU also inhibited ANP when injected into the duodenal lumen (id) (7). In hydrolysates of fresh human plasma incubated with pepsin, a rather high accumulation of kinin-like material was found in a radioimmunoassay against bradykinin (BK) (unpublished results). Neither kinin-like material, or anti-ANP activity was found when, prior to pepsin addition, the plasma was submitted to a prolonged incubation at 37°C (6). This finding led us to explore the effect of kinins released from plasma by pepsin hydrolysis. Habermann, isolated two main peptides from purified ox plasma kininogens under pepsin hydrolysis, one of 14 and another of 16 aminoacids (8). Both contained the full sequence of met-lys-BK, and their oxytocic activity was 100-fold less than that of BK (8), but their action on diuresis was not tested. Recently, we demonstrated that one of these peptides (of 16 aminoacids) inhibits ANP, given either iv or id (9). Moreover, it

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Table I. Effects of iv Administration of PU-15 or vehicle on ANP Response and MAP

	First bolus ANP before PU-15				Second bolus ANP after PU-15				*n*
	Na (μEq)	K (μEq)	Volume (μl)	MAP (mm Hg)	NA (μEq)	K (μEq)	Volume (μl)	MAP (mm Hg)	
PU-15									
100 ng	15.3 ± 2.6	6.9 ± 0.7	128 ± 19	98 ± 4	11.9 ± 2.9	8.2 ± 1.2	125 ± 25	101 ± 7	8
150 ng	19.0 ± 1.9	7.3 ± 0.5	154 ± 17	112 ± 5	4.4 ± 1.1[a]	3.8 ± 0.3[a]	57 ± 8[a]	117 ± 4	8
300 ng	8.5 ± 2.4	4.9 ± 0.6	84 ± 12	111 ± 7	7.4 ± 2.6	5.4 ± 0.9	86 ± 21	117 ± 8	6
500 ng	10.5 ± 2.2	5.1 ± 1.0	97 ± 12	114 ± 5	15.8 ± 2.7	11.0 ± 1.5[b]	156 ± 23[c]	117 ± 4	6
1000 ng	10.8 ± 1.5	8.4 ± 0.9	110 ± 11	96 ± 3	20.2 ± 3.4[c]	12.3 ± 1.3[c]	171 ± 14[b]	96 ± 5	10
Vehicle (isotonic glucose solution)									
50 μl	23.4 ± 5.9	7.9 ± 2.3	170 ± 37	102 ± 3	31.1 ± 4.7	10.6 ± 1.3	242 ± 24	102 ± 2	5

Note. Sodium, potassium, and volume excreted in the urine during the 20-min period following the administration of the first and second bolus of ANP (0.5 μg). Three minutes before the second bolus. PU-15 was given iv. Five different doses of PU-15 were tested in separated groups of rats. In addition, the administration of vehicle was assayed in a control group. Values of MAP in the periods after ANP injections are also shown, in the respective columns. Values represents means ± SEM/100 g body wt.
[a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$ versus first ANP.

was found that BK itself injected iv in a 100- to 150-ng range, 3 min prior to ANP, reproduces the anti-ANP effect (10). Considering that in most of our studies, human plasma was employed as starting material, we decided to explore the effect of a synthetic kinin of 15 aminoacids named PU-15 (MW:1 763.06), whose sequence corresponds to a human kininogen fragment (either from high or low molecular weight) starting with met-lys-BK, analogous to the peptides obtained from the ox kininogen sequence. We compared the ability of PU-15 to counteract ANP effects when administered by three different routes on anesthetized rats: iv, ip, and id. In addition, we assessed whether the anti-ANP effects of PU-15 are mediated by BK B2 receptors by using the specific BK antagonist HOE-140 (11).

Materials and Methods

ANP (ANF 5-28, atriopeptin II, rat form), was purchased from Sigma Chemical Co. (St. Louis, MO). HOE-140 was a gift of Hoescht, Frankfurt an Main, Germany. PU-15 was synthesized by BIOS CHILE, Ingienería Genética (Santiago, Chile).

Biologic Assay to Test the Anti-ANP Effect of PU-15. A bioassay was employed as previously reported (5–7). In brief, fasted female Sprague-Dawley rats (200–220 g) were anesthetized with sodium pentobarbital (40 mg/kg ip) and heparinized. For intestinal injections, small incisions on the skin and on the white line of the abdominal wall were performed, before heparin administration (7). Polyethylene cannulas were introduced in the right femoral artery, left femoral vein, left jugular vein, and trachea. Urine was collected during 10 periods of 20 min each, by means of a Silastic catheter introduced in the bladder. Arterial pressure was continually recorded on a polygraph, and a constant infusion of 0.6 ml/hr isotonic glucose solution was given through the jugular vein. Two intravenous boluses of 0.5 μg ANP in 50 μl isotonic glucose solution were administered at the start of the fourth and ninth periods. PU-15, alone or associated with HOE-140, was administered through different routes prior to the second ANP bolus as described below. For a quantitative expression of the anti-ANP effects of PU-15, the urinary excretion of sodium, potassium, and volume brought about by the second bolus of ANP were compared with the respective values following the first bolus, considered the control.

Doses and Administration of PU-15. PU-15 was dissolved in sterile, isotonic glucose solution, aliquoted, immediately frozen, and kept at −60°C until performing the experiments. For the intravenous injections, PU-15 was given in a volume of 50 μl, 3 min prior to the second ANP bolus (10). For the duodenal or the peritoneal injections, a volume of 0.25 or 0.5 ml was given, 40 min before ANP. Duodenal injections were performed at a distance of 5–15 cm from the pylorus, using a 30-gauge needle (7). In each animal only a single dose of PU-15 was tested. To assess the effect of BK receptor blockade, 2.5 μg of HOE-140 was injected iv 45 min prior to the administration of the second bolus of ANP (10).

Statistical Analysis of Data. All values are given as mean ± SEM. Excretion of sodium, potassium, and water is expressed in μEq (Na and K) or μl (water) per 100 g body wt, mean arterial pressure (MAP) is expressed in mm Hg. A paired *t* test was performed to determine the statistical significance of the differences between the first and second ANP response for each parameter. In addition, the percentage change is given in the text, to emphasize the extent of inhibition.

Results

Effect of PU-15 Given iv. Table I shows sodium, potassium, and volume excretion and MAP in the 20-min period following iv injections of ANP given before

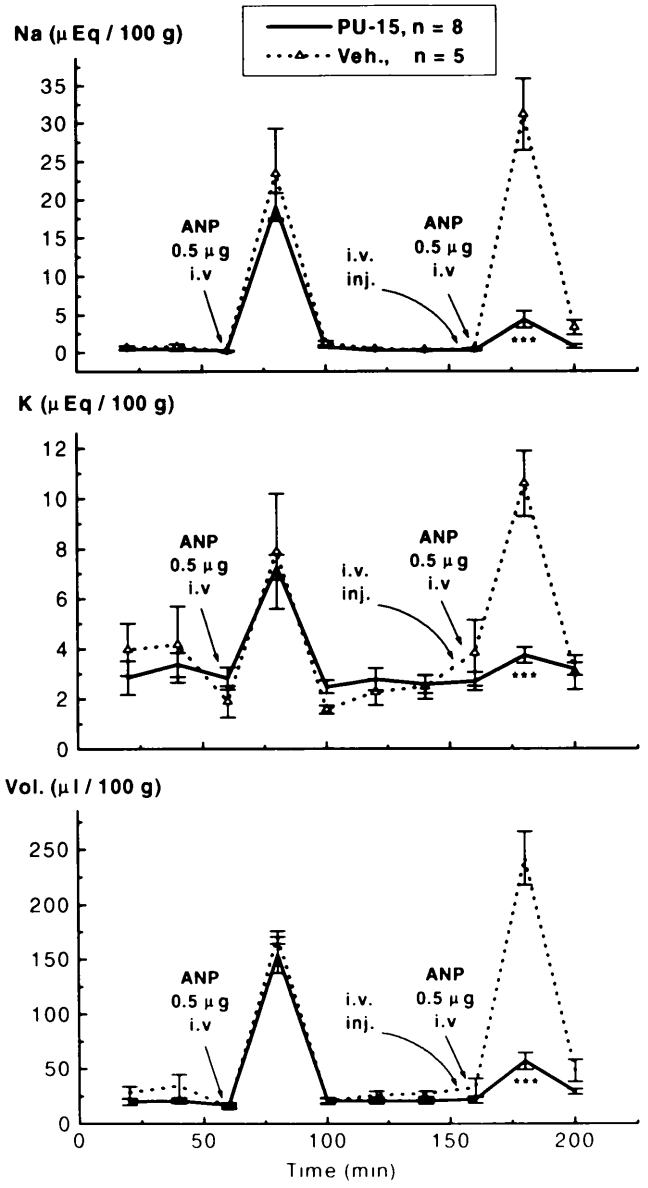

Figure 1. Effect of PU-15 given intravenously. Time course of urinary excretion of sodium, potassium, and volume in two groups of rats injected twice with ANP (0.5 μg, iv). One group received 150 ng in 50 μl of isotonic glucose solution iv 3 min before second ANP bolus. Control group was given with only the vehicle (Veh.), 50 μl glucose solution. ***$P < 0.001$ compared with the first ANP response.

and after the iv administration of different PU-15 doses. None of the assayed doses of PU-15 modified the blood pressure. Of all the doses tested, only 150 ng of PU-15 blunted the effect of ANP on sodium, potassium, and volume excretion ($P < 0.001$). The three parameters decreased by 79% ± 5%, 48% ± 3% and 63% ± 4%, respectively. No significant changes in the urinary excretion where observed when 100 ng PU-15 was given. A larger dose, such as 300 ng, was also ineffective, whereas 500 ng of PU-15 augmented significantly potassium and volume excretion induced by ANP. The dose of 1 μg of PU-15 induced an even greater increment of sodium (+108% ± 47%; $P < 0.025$), potassium (+55% ± 19%; $P < 0.05$) and volume (+66% ± 19%; $P < 0.005$) (Table I). In control rats injected with vehicle, the second response to ANP was similar to the first one (Fig. 1 and Table I).

The complete time course of the experiment with the dose of 150 ng of PU-15 is shown in Figure 1, which demonstrates the stability of urinary excretion during the periods without ANP injections, and the striking reduction in the magnitude of the second response to ANP provoked by PU-15 on all three parameters.

Effect of PU-15 Given in the Duodenal Lumen. The effects of PU-15 or vehicle introduced in the duodenum are summarized in Table II. The results resemble those seen after iv administration, and provide evidence that PU-15 injected in the intestine is able to keep its attribute to counteract the diuretic-natriuretic action of ANP, similar to that described for kinins of 16 and 18 aa from ox kininogen (9). Fifty nanograms of PU-15 was below the threshold, but 100 ng of PU-15 was effective in inhibiting sodium and volume excretion. The amount of 150 ng almost reproduced the blockade intensity observed with the same dose given intravenously, on sodium (−73% ± 5%; $P < 001$) and volume (−55% ± 5%; $P < 0.01$) excretion. The inhibitory effect of 250 ng of PU-15 was smaller than that of 150 ng, but it was still significant for sodium (−62% ± 10%; $P < 0.005$) and volume (−36% ± 8%; $P < 0.025$). In contrast, the dose of 500 ng had the opposite effect: it produced a change, but not significant in the excretion of sodium (+92% ± 32%). In control rats, the excretion of sodium, potassium, and volume after the second bolus of ANP tends to be higher than after the first one, suggesting that the percentage inhibition attained with PU-15 could be somehow greater than that expressed by the numbers given above.

Figure 2 compares the time course of urinary excretion in control rats and rats treated with 150 ng of PU-15. This figure illustrates that the baseline urine excretion was not modified by id injections of PU-15 or vehicle.

Effect of PU-15 Given in the Peritoneal Cavity. The ip injection of 150 ng of PU-15, 40 min before the second bolus of ANP reproduced the trend observed in the experiments with iv and id injections. The result, as depicted in Figure 3, demonstrates a remarkable blunting effect on the three excretory parameters, with reductions of 83% ± 7% for sodium ($P < 0.001$), 66% ± 4% for potassium ($P < 0.005$) and 74% ± 7% for volume ($P < 0.001$). These numbers represent the highest inhibition registered in all the series carried out. Again, PU-15 did not modify baseline excretion, as seen in the period between PU-15 and ANP injections.

Table II. Effects of id Administration of PU-15 or vehicle on ANP Response and MAP

	First bolus ANP before PU-15				Second bolus ANP after PU-15				*n*
	Na (μEq)	K (μEq)	Volume (μl)	MAP (mm Hg)	NA(μEq)	K (μEq)	Volume (μl)	MAP (mm Hg)	
PU-15									
50 ng	10.0 ± 0.5	5.6 ± 0.4	98 ± 5	111 ± 5	11.4 ± 1.3	6.6 ± 0.9	107 ± 8	107 ± 8	6
100 ng	12.7 ± 2.9	4.8 ± 0.6	93 ± 7	100 ± 5	4.7 ± 0.8[a]	3.5 ± 0.6	45 ± 9[b]	106 ± 4	7
150 ng	16.0 ± 2.8	5.0 ± 0.9	176 ± 32	119 ± 5	3.5 ± 0.6[c]	3.1 ± 0.5	70 ± 9[b]	113 ± 5	8
250 ng	8.0 ± 0.7	6.5 ± 1.4	86 ± 5	105 ± 4	3.3 ± 1.0[b]	3.6 ± 0.4	56 ± 8[a]	109 ± 3	6
500 ng	7.2 ± 1.1	4.5 ± 0.8	73 ± 5	96 ± 7	15.0 ± 4.5	5.3 ± 0.4	109 ± 20	89 ± 8	6
Vehicle (isotonic glucose solution)									
0.25 ml	14.2 ± 1.6	6.5 ± 0.9	121 ± 14	113 ± 4	18.2 ± 2.0	7.3 ± 1.2	146 ± 15	117 ± 6	6

Note. Sodium, potassium, and volume excreted in the urine during the 20-min period following the administration of the first and second bolus of ANP (0.5 μg). Forty minutes before the second bolus, a dose of PU-15 in 0.25 ml of isotonic glucose solution was introduced in duodenal lumen. Five different doses of PU-15 were tested in separated groups of rats. In addition, the administration of vehicle was assayed in a control group. Values of MAP in the periods following ANP injections are also shown. Values represent means ± SEM/100 g body wt.

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ versus first ANP.

Effect of HOE-140. Since HOE-140 prevented the anti-ANP effects of BK (10) or the 16 aa kinin from ox kininogen (9), we tested its ability to affect the response to PU-15 in the present bioassay. Figure 4 shows that the inhibition of ANP diuretic effects elicited by 150 ng of PU-15 given iv was completely prevented by administration of HOE-140. The same result was observed when PU-15 was given ip (not shown). HOE-140 alone produced no detectable changes in the excretory responses induced by ANP and no alteration to the blood pressure (Fig. 4). When HOE-140 was given prior to the highest dose of PU-15 (1 μg) the sodium excretion following the first ANP bolus, in a group of five rats was: 10.3 ± 1.6 and 19.2 ± 2.9 after the second one. In the group that received PU-15 alone, the sodium excretion were: 10.8 ± 1.5 and 20.2 ± 3.4, respectively (Table I), but no significant difference in this parameter between both groups was found.

Discussion

The experiments with PU-15 add new evidence that kininogens under enzymatic hydrolysis by pepsin may release kinins which exert anti-ANP properties. These kinins can explain the anti-ANP action of pepsanurin given ip (5, 6) or id (7), and the antidiuretic effect shown by PU on hyperhydrated rats (1, 2). Among kinins likely to be released by pepsin, PU-15 appears to be one of the most potent inhibitors of the ANP action on urinary excretion investigated up to now (9). It is feasible to assume that these kinins, in contact with blood plasma or tissue proteases, may set free BK and hence trigger the activation of BK receptors. The efficiency of HOE-140 in counteracting the effect of BK, PU-15, and other assayed kinins endowed with anti-ANP property (9, 10) indicates that a functional integrity of the kinin B2 receptor is required for the inhibitory action. In our hands, PU-15 was at least 10- to 20-fold less potent than BK in contracting the isolated rat uterus, a tissue rich in BK B2 receptors (not shown). Whereas, the anti-ANP potency of PU-15 was quite comparable to that of BK when tested by the iv route (10). These findings reinforce the concept that *in vivo* transformation of larger kinins into BK is required for the anti-ANP effect to take place.

A remarkable feature of this group of peptides is their ability to induce dual effects according to the range of dose used. With small doses (100–150 ng rat, in the case of BK and PU-15), a striking blockade on ANP diuretic-natriuretic action is induced, whereas greater doses can bring about an opposite effect, facilitating the diuretic-natriuretic response to ANP, at least in the bioassay here employed. A natriuretic effect for years has been considered a typical action of the Kallikrein-Kininogen-Kinin System localized in the kidney (12). With regard to this biphasic effect, it is worth mentioning that angiotensin II, one of the main opponents of BK on diuretic and vascular effects, can also show antagonistic effects on proximal tubular transport. At rather low concentration (10^{-12} to 10^{-10} *M*) angiotensin II stimulates sodium and bicarbonate transport, whereas higher concentrations (10^{-8} to 10^{-6} *M*) inhibit their transport steps (13).

We cannot disregard the B1 BK receptor, which is present along with BK B2 (14), and its possible role in the process that originates the duality of the kinin effects. Nevertheless, since the anti-ANP effect of BK and PU-15 are completely counteracted by HOE-140, which specifically acts upon the BK B2 receptor, we think that PU-15, BK, and the other kinins assayed at low doses produce the inhibition on ANP-mediated natriuresis through the activation of B2 receptors. In this connection, it has recently been proposed that the

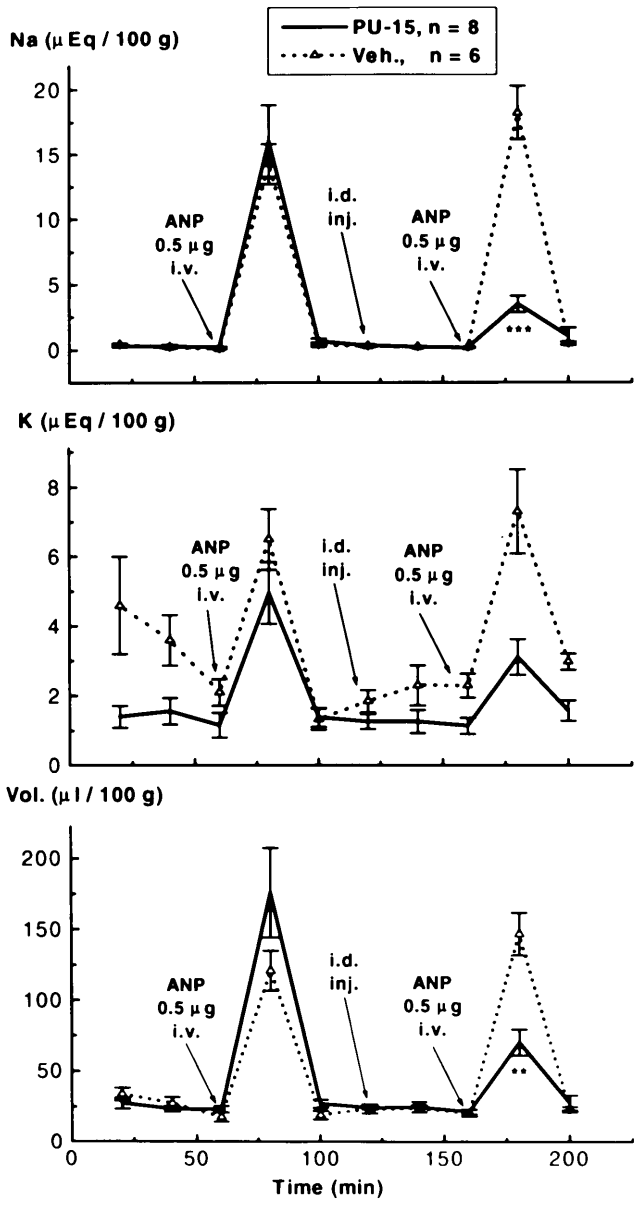

Figure 2. Effect of PU-15 given intraduodenally. Urinary excretion of sodium, potassium, and volume in two groups of rats injected twice with ANP (0.5 μg, iv). One group received 150 ng PU-15 in 0.25 ml of isotonic glucose solution into the duodenum lumen 40 min before the second ANP bolus. The other group received the vehicle (Veh.) at the respective time. $^{**}P < 0.01$; $^{***}P < 0.001$, versus first ANP response.

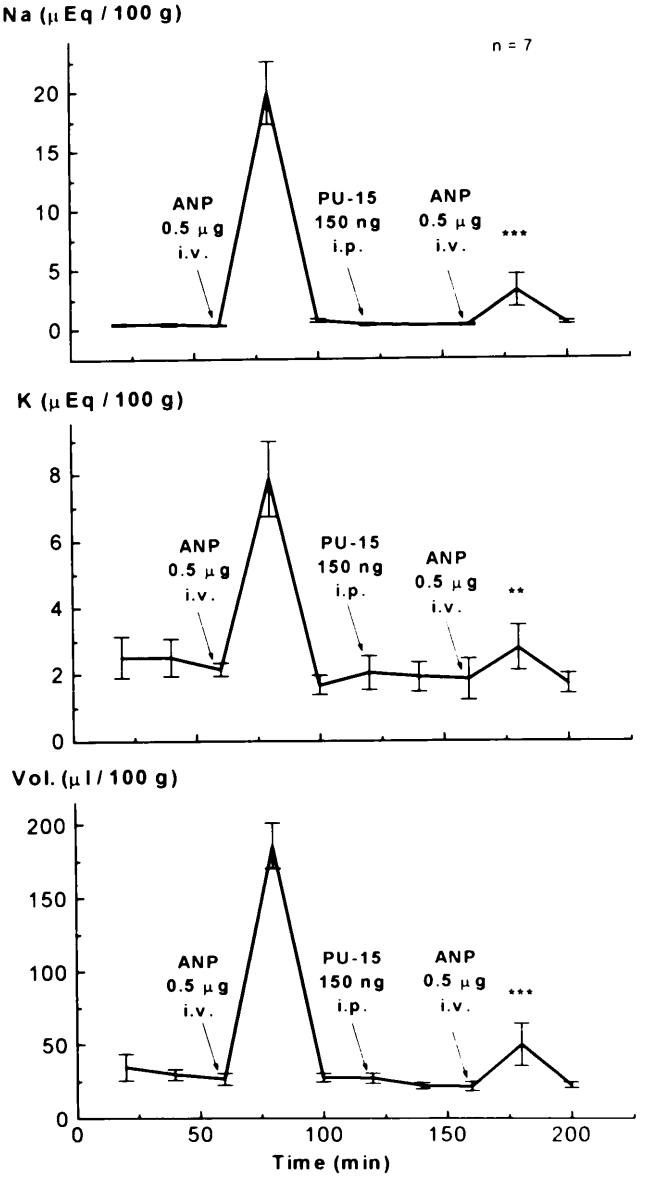

Figure 3. Effect of PU-15 given intraperitoneally. Time course of urinary excretion of sodium, potassium, and volume in response to two iv boluses of 0.5 μg of ANP. given before and after administration of 150 ng of PU-15. The peptide was injected in 0.25 ml of isotonic glucose solution in the peritoneal cavity 40 min before the second ANP bolus. $^{**}P < 0.005$; $^{***}P < 0.001$, versus first ANP response.

relaxation, followed by contraction induced by BK in the isolated rat duodenum, an example of a typical biphasic response to BK, is due to the activation of only the BK B2 receptor, which activates different intracellular mechanisms, depending on the concentration used (15, 16). At present, for us, it remains an open question as to which steps after the activation of BK receptors allow BK and its analogs to act either as anti-ANP or as facilatory agents. Currently we know that the urinary excretion of cGMP, the second messenger for ANP, is blunted in proportion to the inhibition of diuresis induced by BK (10).

A crucial question is the physiological role which can be assigned to kinins acting as inhibitors of ANP in low concentrations. Pertinent to this point, recent data show that the kinin content measured in renal medullary interstitial space is small in rats under a high sodium intake, whereas a considerable rise of BK in the renal cortex is observed in rats on a low-sodium diet (17), suggesting that intrarenal kinins do not participate as natriuretic agents. Theoretically, it is possible to infer that a modulation to restrain the action of ANP on renal excretion would be needed in physiological episodes that require a reduction in the loss of water and sodium through the kidneys, for instance during

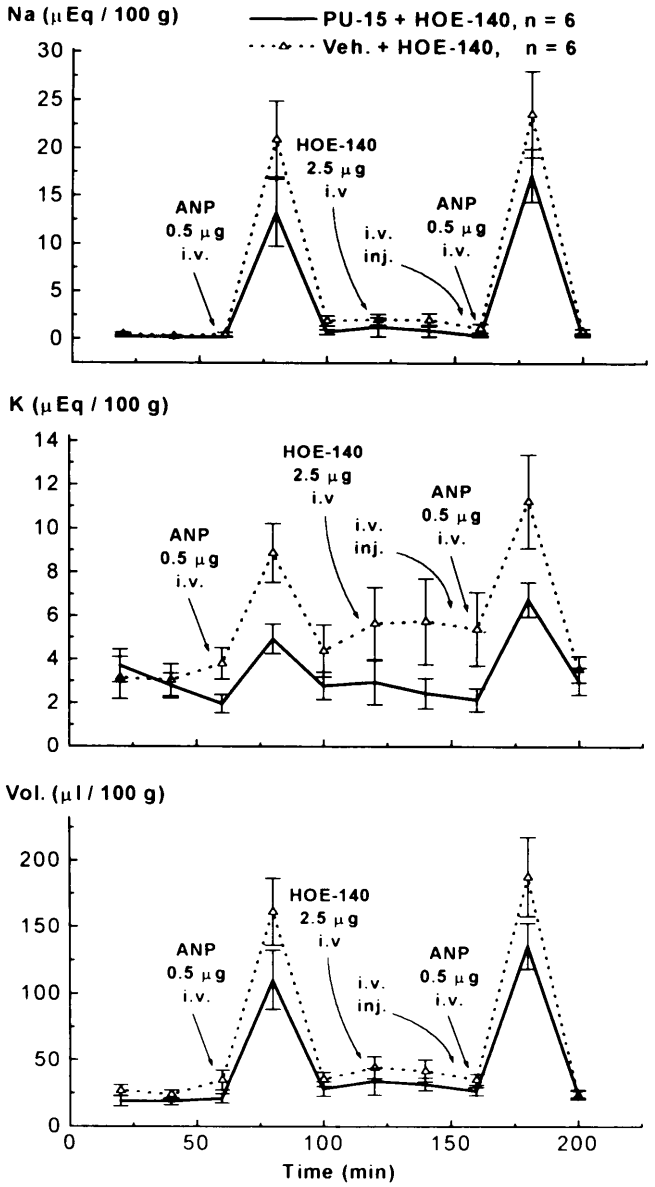

Figure 4. Effect of HOE-140 on PU-15 anti-ANP action. Urinary excretion of sodium, potassium, and volume in two groups of rats receiving two doses of 0.5 μg ANP boluses. The experimental group was given with HOE-140 (2.5 μg) and PU-15 (150 ng) both by iv route, 45 and 3 min prior to the second ANP bolus, respectively. The control group received HOE-140 and the PU-15 vehicle. No significant differences in the three parameters between the first and second ANP response were observed. Note that after HOE-140, PU-15 did not block the urinary response to the atrial hormone, compared with its effect when given alone, shown in Figure 1.

intense muscle activity or during prandial periods. In feeding cycles, a regulation based on the inhibition of ANP release by the atria does not fit with the digestive tract requirements, since the atrial hormone appears to be important to facilitate the production of digestive juices and to keep the fluidity of the intestinal content (18). Therefore, an inhibitory action upon ANP renal excretory effect could be expected during prandial periods. On these grounds, we have proposed that agents, such as BK or prokinins, that might be originated in the digestive tract could participate as inhibitors of ANP renal action (9). Kallikrein is highly represented in the intestinal tract (19), and the occurrence of pepsin, or a pepsin-like enzyme, can set free some kinins resistant to protease that can exert their anti-ANP activity. This concept is supported by the demonstration that BK analogs such as PU-16 and PU-18 (9), and particularly PU-15 here reported, are resistant to proteases in the duodenal mucosa and can induce anti-ANP effects, probably after being absorbed and reaching the kidneys, with similar potency as when administered iv. At present time, we cannot conclude whether or not kinins released in the intestinal mucosa during digestion might also activate the liberation of some intestinal hormone able to counteract ANP action in the kidneys. Investigations to explore these assumptions are still in progress.

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