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Effect of Sorbin on Duodenal Absorption of Water and Electrolytes in the Rat

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Sorbin is a newly isolated intestinal peptide that has been purified because of its ability to induce water absorption. The effects that sorbin and some synthetic peptides corresponding to its C-terminal sequence have on duodenal absorption of water, chloride, and sodium were studied in comparison with the effects of vasoactive intestinal peptide (VIP), [D-Ala,Met]-enkephalinamide (DAMA), and angiotensin II. The technique of an in situ ligated duodenal loop in the rat was used for all peptides. Under the experimental conditions used, a low basal secretion of water, chloride, and sodium was obtained; VIP induced an increase of the secretion, whereas DAMA induced an absorption, both in the nanomolar dose range. Angiotensin II and sorbin induced an absorption in the picomolar dose range. The most effective doses of sorbin peptides but not of angiotensin induced the lowest final concentrations of Na^+ and Cl^- obtainable without inducing secondary water secretion. All synthetic peptides containing the C-terminal heptapeptide of sorbin were active in the picomolar dose range. Contrary to angiotensin, they had no effect on blood pressure.

Sorbin is an intestinal peptide isolated from porcine small intestine.[1] The purification, based on the physiological effect of this peptide stimulating water absorption in the duodenum[2] and in the gallbladder,[1] led to the isolation of a 153-amino acid peptide. The amino acid sequence of sorbin was fully determined[1] despite a low recovery from the upper part of the intestine (~1 mg per ton).

C-terminal peptides of natural sorbin were synthesized, and their effects on duodenal water and ion movements were evaluated to characterize the minimal active site of sorbin. The validity of our animal model was controlled by measuring the well-known effects of other peptides such as vasoactive intestinal peptide (VIP),[3,4] [D-Ala^2,Met^5]-enkephalinamide (DAMA),[5,6] and angiotensin II.[7] To characterize the mechanism of action of sorbin, the effect on blood pressure was studied in awake rats.

Materials and Methods

Duodenal Water and Ion Transport

Male Sprague-Dawley rats (n = ~400; Iffa Credo, L'Arbresle, France) weighing 200 ± 25 g were fasted for 48 hours with free access to water. They were anesthetized with intraperitoneal pentobarbital (4.2 mg/100 g body wt). A catheter was inserted into the jugular vein, and 0.9% sodium chloride solution was perfused at a rate of 3 mL/h. The saline infusion was continued for 1 hour (controls) or replaced by one of the peptide solutions: VIP (GIH Research Lab., Karolinska Institutet, Stockholm, Sweden) at doses of 0.15, 0.3, 0.6, 1.2, and 2.4 nmol · 100 $g^{-1} \cdot h^{-1}$; DAMA (UCB Bioproducts, Paris, France) at doses of 2, 8, and 32 nmol · 100 $g^{-1} \cdot h^{-1}$; angiotensin II (Asp^1, Ile^5 ANG II; Sigma, La Verpillière, France) at doses of 4, 16, 64, and 256 pmol · 100 $g^{-1} \cdot h^{-1}$; natural sorbin (80% pure) at doses of 12.5, 25, 50, and 100 pmol · 100 $g^{-1} \cdot h^{-1}$; or synthetic sorbin C-terminal fragments (Table 1): C-20 (C-terminal sorbin eicosapeptide amide) at doses of 12.5, 25, 50, 100, and 200 pmol · 100 $g^{-1} \cdot h^{-1}$; C-10 sorbin (C-terminal sorbin decapeptide amide) at doses of 3.125, 6.25, 12.5, 25, and 50 pmol · 100 $g^{-1} \cdot h^{-1}$, and C-7 (C-terminal sorbin heptapeptide amide) at doses of 12.5, 25, 50, and 100 pmol · 100 $g^{-1} \cdot h^{-1}$. The synthetic peptides were synthesized using CRB FMOC amino acid derivatives[8] and a CRB Pepsynthesiser (Cambridge, England). The synthetic peptides were purified by high-performance liquid chromatography and sequenced by the dimethylaminoazobenzene isothiocyanate double-coupling method,[9] using by-products as an aid for thin-layer chromatographic identifications.[10] Their amino acid composition was determined using the Pico-Tag technique (Waters, St Quentin en Yvelines, France). The experiments were randomized for each peptide and its own control.

The animals were laparotomized and a 10 cm long duodenal loop (representing about 1 g of fresh tissue) was prepared. Ligation (cotton suture 00) began at the pylorus. The

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Table 1. Synthetic Peptides Containing the C-Terminal End of Natural Sorbin

Tyr-Glu-Pro-Gly-Lys-Ser-Ser-Ile-Leu-Gln-His-Glu-Arg-Pro-Val-Thr-Lys-Pro-Gln-Ala-amide
<---------->
C-20 sorbin
<---------->
C-10 sorbin
<---------->
C-7 sorbin

hepatobiliary canal was tied off. One milliliter of the test solution was instilled into the loop with the aid of a calibrated syringe, and an additional ligature was placed on the injection site. The ligated loop was replaced into the abdominal cavity, which was then sutured. The animals were kept in an incubator at 25°C and reanesthetized if necessary. Sixty minutes later, they were killed by an intravenous injection of air. The loops were exteriorized and cut just proximally to the outside of the ligature; the contents of the squeezed loops were then collected and centrifuged, and the volume of the supernatant was measured.

The test solution instilled into the loop contained 70 mmol/L NaCl, 5 mmol/L KCl, 1.2 mmol/L $CaCl_2$, 10 mmol/L $HNaCO_3$, and 136 mmol/L mannitol, pH 8.1. Radioactive [^{36}Cl]H (NEN, Dreieich, Germany) was added at a concentration of 0.05 μCi/mL as an influx marker. Tritiated polyethylene glycol 4000 PEG 4000 (NEN), mixed with 5 g/L of cold PEG 4000, was added at a concentration of 0.05 μCi/mL as a nonabsorbable marker. Only those experiments in which recovery of [^{3}H]PEG 4000 was 88% or greater were analyzed. Recovery of [^{14}C]mannitol from the lumen was controlled in a complementary study and was in excess of 90%.

Na^+ and K^+ contents were measured by flame photometry, Cl^- content by coulometric titration, bicarbonate content by acid alcalimetry, and radioactivity by liquid scintillimetry with a double-counting program. All data are given per 10 cm loop.

Comparisons between controls and experiments are based on mean values derived from 7 to 14 loops for each dose and agent. Net absorption from the lumen is expressed as a negative value and net secretion as a positive value. Statistical analysis of differences was performed using variance analysis. A regression was calculated for the responses to peptide doses.

Effect on Blood Pressure

Twenty male Sprague–Dawley rats (Iffa Credo) weighing 200 ± 25 g were fasted 24 hours with free access to water. They were anesthetized with pentobarbital (3.5 mg/100 g body wt). A catheter (OD, 0.96 mm; ID, 0.58 mm) was inserted into the jugular vein and perfused with 0.9% sodium chloride solution at a rate of 3 mL/h. Between the experiments the catheter was filled with heparinized saline (1 mg/mL) and closed. A catheter (OD, 1.27 mm; ID, 0.30 mm) filled with heparinized saline was inserted into the carotid artery, closed, and exteriorized through the nape. The catheter was carefully attached to the skin and enclosed in a plastic bag fixed on the back. During the experiments, the animal was placed in a Bollman cage. The catheter was connected to a pressure transducer (Statham, Gould Instruments, Hato Rey, PR), to an amplifier, and to a recorder. Dose response to angiotensin II was determined using doses of 5–100 ng/100 g body wt (5–100 pmol), given in rapid intravenous injections or with a continuous intravenous injection of 3 ng/100 g body wt per minute. VIP was given at doses of 2.5 and 5 μg/100 g body wt (0.75 and 1.5 nmol/100 g). C-10 sorbin was injected at doses of 3.125, 6.25, 12.5 pmol/100 g body wt in rapid intravenous injections.

Results

Effect on Duodenal Transport

Duodenal water output. During control basal conditions, there was a small secretion of water (0.22 ± 0.03 mL/h; N = 98). There was no significant difference between control groups ($F_{91}^{6} = 0.8$). VIP increased the secretion of water ($F_{44}^{5} = 22.1$, $P < 0.01$) in relation to its nanomolar dose ($r = 0.799$, N = 36, $P < 0.01$). DAMA induced a decrease in secretion and an absorption in a nanomolar range in relation to the doses ($F_{27}^{5} = 9$, $P < 0.01$; $r = -0.442$, $P < 0.05$) (Figure 1).

Angiotensin II reversed basal secretion to an absorption ($F_{34}^{4} = 1.6$, $P > 0.05$) as did C-10 sorbin ($F_{105}^{5} = 3.5$, $P < 0.01$; the regression was calculated without the supramaximal doses: $r = -0.384$, N = 87, $P < 0.01$). Angiotensin II and C-10 sorbin curves were of the same type in a picomolar range, with supramaximal doses inducing an inhibition of maximal duodenal absorption. Sorbin synthetic peptides including at least the C-terminal heptapeptide (Table 1) induced an absorption, with doses ranging from 12.5 to 50 pmol · 100 g^{-1} · h^{-1} (Figure 2). The most active doses differed from control values ($F_{39}^{4} = 5.6$, $P < 0.01$). All the maximal water net fluxes observed with the different peptides were similar (−0.18 mL/h for DAMA, −0.18 mL/h for C-10 sorbin, and −0.17 mL/h for C-7 sorbin).

Duodenal chloride output. In basal conditions, there was a secretion of chloride (16.3 ± 4.7 μEq/h, N = 98). There were no differences between the different groups of control loops ($F_{91}^{6} = 1.3$, $P > 0.05$). VIP induced a secretion of chloride ions ($F_{44}^{5} = 18.2$, $P < 0.01$; $r = 0.761$, $P < 0.01$) and DAMA an

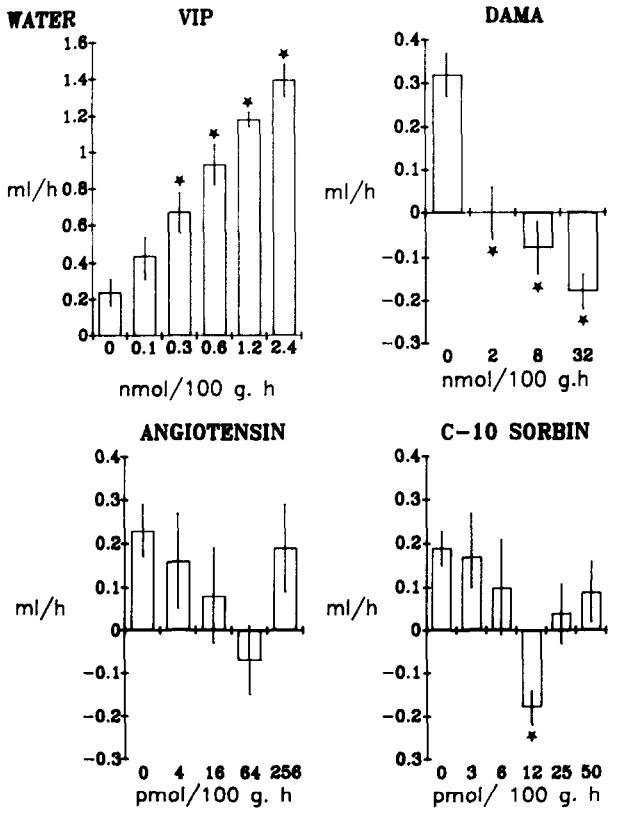

Figure 1. Net water fluxes in the duodenal loop after 1 hour (means ± SEM), positive for secretion and negative for absorption, in response to VIP (N = 50), DAMA (N = 33), angiotensin II (N = 39), and C-10 sorbin (N = 66). The control values (0) for all the treatments did not differ significantly (variance analysis: $F_{91}^{6} = 0.8$, $P > 0.05$). *Significant variation between control and each dose (variance analysis).

absorption ($F_{27}^{5} = 6.7$, $P < 0.01$; $r = -0.424$, $P < 0.05$) (Figure 3). Angiotensin II ($F_{34}^{4} = 1.4$, $P > 0.05$) and C-10 sorbin ($F_{105}^{5} = 3.3$, $P < 0.01$; the regression was calculated without the supramaximal doses: $r = -0.381$, $P < 0.01$) induced a decrease of basal chloride secretion, then an absorption according to the doses. All the synthetic peptides including the C-terminal sorbin heptapeptide induced chloride absorption (Figure 2). Values in all groups differed from control values ($F_{39}^{4} = 5.8$, $P < 0.01$).

The maximal net Cl^- fluxes induced by the different peptides were similar (−37 μEq/h for DAMA, −42 μEq/h for C-10 sorbin, and −39 μEq/h for C-7 sorbin).

^{36}Cl influx was 86% the instilled ^{36}Cl in controls. VIP induced a dose-dependent decrease to 51%. DAMA, angiotensin II, and C-10 sorbin induced an increase to 93%, 94%, and 97%, respectively. The final chloride concentration was lower than the control value for each of the peptides (Table 2).

Duodenal sodium output. In basal condition, there was a secretion (41.2 ± 5.1 μEq/h, N = 98). There was no difference between the different control groups ($F_{91}^{6} = 0.9$, $P > 0.05$). VIP induced a secretion of sodium ($F_{44}^{5} = 23$, $P < 0.01$; $r = 0.808$, $P < 0.01$) and DAMA an absorption ($F_{27}^{5} = 9$, $P < 0.01$; $r = -0.339$, $P > 0.05$) (Figure 4). Both angiotensin II ($F_{34}^{4} = 1.4$, $P > 0.05$) and C-10 sorbin ($F_{105}^{5} = 3.0$, $P < 0.05$; the regression was calculated without the supramaximal doses: $r = -0.352$, $P < 0.01$) induced an absorption in the picomolar dose range. All C-terminal sorbin synthetic peptides induced either a significant decrease of sodium secretion or a significant increase of sodium absorption in the duodenum ($F_{39}^{4} = 5.5$, $P < 0.01$) (Figure 2), as did sorbin itself. The net sodium fluxes induced by the different peptides were comparable (−19 μEq/h for DAMA, −17 μEq/h for C-10 sorbin, and −18 μEq/h for C-7 sorbin). The final sodium concentration was lower than the control value for each of the peptides (Table 2).

Duodenal bicarbonate output. In basal conditions, there was a bicarbonate secretion of 15.2 ± 1.1 μEq/h (N = 57). No differences between the control groups were observed ($F_{53}^{4} = 0.2$, $P > 0.05$). VIP induced a stimulation of bicarbonate secretion ($F_{44}^{5} = 27.4$, $P < 0.01$) and DAMA an inhibition ($F_{29}^{3} = 8.8$, $P < 0.01$). The decrease of bicarbonate secretion produced by angiotensin II and C-10 sorbin did not reach the level of significance ($F_{34}^{4} = 1.0$, $P > 0.05$; $F_{35}^{5} = 0.7$, $P > 0.05$).

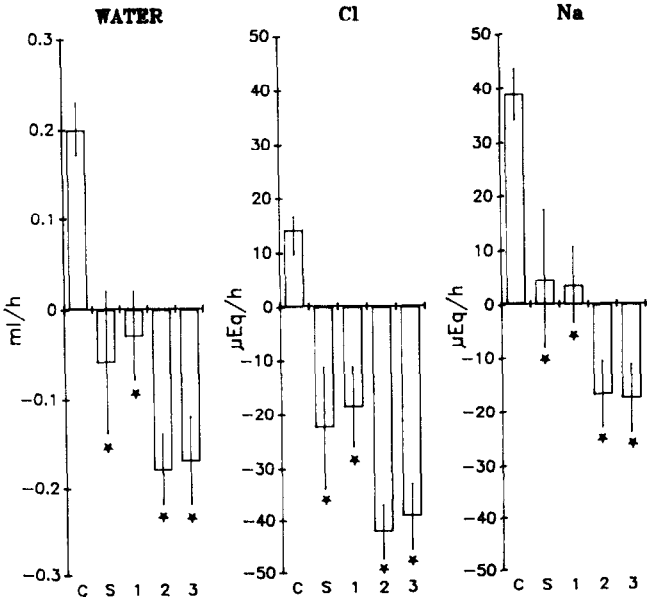

Figure 2. Water, chloride, and sodium fluxes in the duodenal loop after 1 hour (means ± SEM), positive for secretion and negative for absorption, in response to the maximal (most active) dose of 25 pmol · 100 g^{-1} · h^{-1} sorbin (S; N = 5) and synthetic peptides including the C-terminal part of the molecule: 50 pmol · 100 g^{-1} · h^{-1} C-20 (1; N = 16), 12.5 pmol · 100 g^{-1} · h^{-1} C-10 sorbin (2; N = 13), 50 pmol · 100 g^{-1} · h^{-1} C-7 sorbin (3; N = 5), and controls (C; N = 62). *Significant variation between control and each peptide.

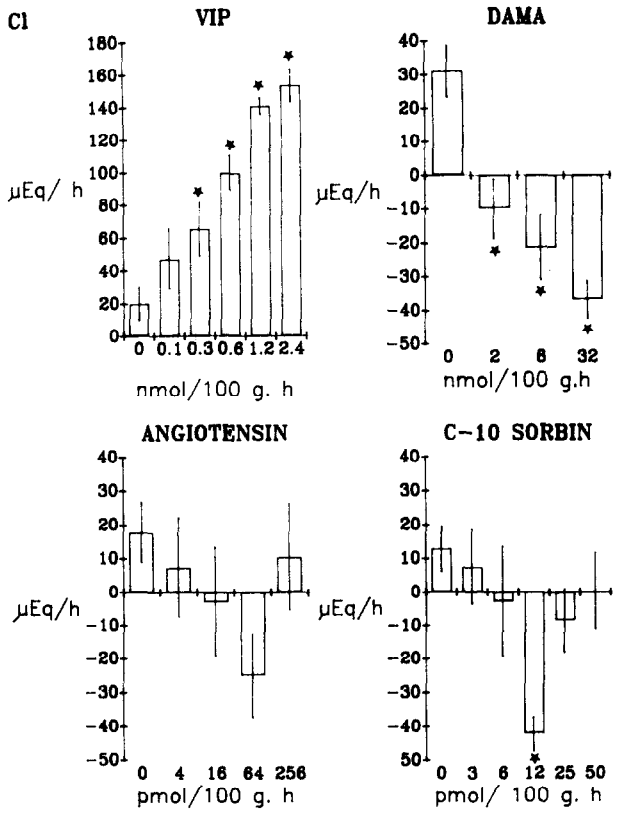

Figure 3. Chloride fluxes in the duodenal loop after 1 hour (means ± SEM), positive for secretion and negative for absorption, in response to VIP, DAMA, angiotensin II, and C-10 sorbin. The control values (0) for all the treatments did not differ significantly (variance analysis: $F_{91}^{6} = 1.3$, $P > 0.05$). *Significant variation between control and each dose.

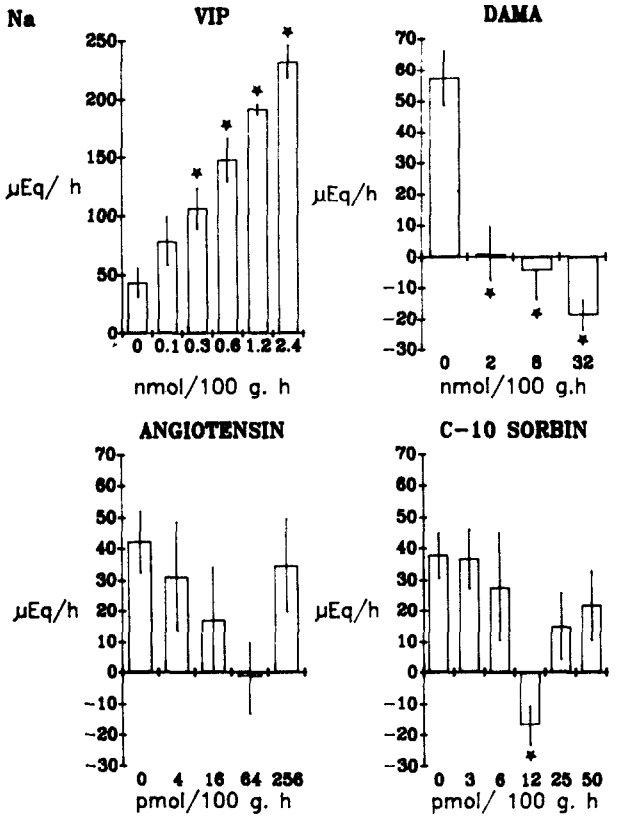

Figure 4. Sodium fluxes in the duodenal loop after 1 hour (means ± SEM), positive for secretion and negative for absorption, in response to VIP, DAMA, angiotensin II, and C-10 sorbin. The control values (0) for all the treatments did not differ significantly (variance analysis: $F_{91}^{6} = 0.9$, $P > 0.05$). *Significant variation between control and each dose.

Effect on Blood Pressure

VIP induced a decrease in blood pressure (Figure 5), whereas angiotensin II showed a strong hypertensive effect proportional to the log of the doses ($r = 0.922$, $N = 12$ in 3 rats, $P < 0.01$). Conversely, C-10 sorbin did not have any effect on blood pressure at any of the doses tested. Administered during a continuous intravenous infusion of angiotensin II of 3 pmol/100 g per minute, which induced an increase in blood pressure of 10 mm Hg, C-10 sorbin (0.3 pmol/100 g rat per minute) did not modify the angiotensin II–induced blood pressure increase.

Table 2. Duodenal Luminal Final Concentration After 1 Hour

	N	$[Na^+]$ (mmol/L)	$[Cl^-]$ (mmol/L)	$[HCO_3^-]$ (mmol/L)
Initial test solution		80	77	10
Controls	109	95.6 ± 1.5	72.2 ± 1.9	20.3 ± 0.6
32 nmol DAMA	6	74 ± 2.5	47 ± 3.9	18.4 ± 1.2
64 pmol angiotensin	6	82 ± 5	53 ± 8.7	22.4 ± 1.8
25 pmol Sorbin	5	88 ± 6	55.9 ± 6.8	—
50 pmol C-20 sorbin	16	84.8 ± 2.9	60.4 ± 4.5	15.4 ± 1.2
12.5 pmol C-10 sorbin	13	75 ± 3.7	40.4 ± 4.3	23.1 ± 1.1
50 pmol C-7 sorbin	4	74.3 ± 3.3	44.6 ± 4.9	—

Discussion

Using in situ ligated duodenum in anesthetized rats, we showed that VIP induces stimulation of water and electrolyte secretion and that DAMA induces stimulation of water and electrolyte absorption at nanomolar doses. With the same animal preparation, angiotensin II induced a slight absorption and C-10 sorbin a significant stimulation of water and electrolyte absorption. Synthetic peptides containing the C-terminal end of sorbin from 7–20 residues, like sorbin itself, also induced a significant absorption. The shortest active site of sorbin is the heptapeptide C terminus; all fragments obtained by several enzymatic cleavages and devoid of this sequence were inactive.

The mechanism of sorbin action is still unknown, but no blood pressure change could be detected in conscious rats at variance with angiotensin. For all sorbin peptides, dose-response curves showed su-

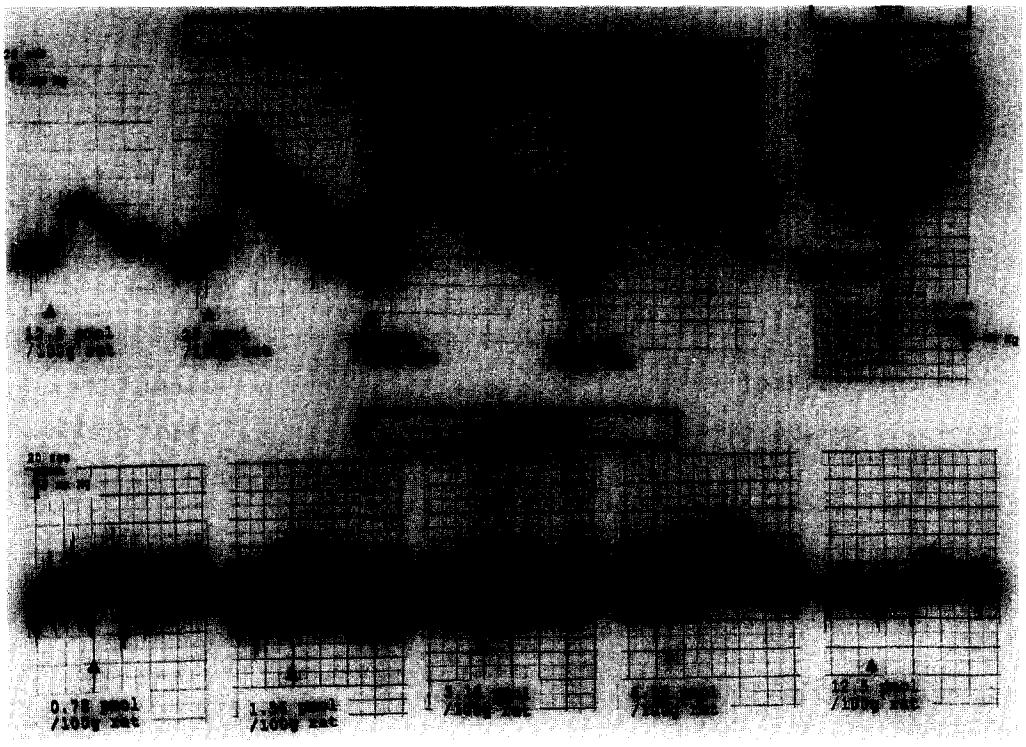

Figure 5. Blood pressure variations in response to angiotensin II, VIP, and C-10 sorbin in one of the awake rats. C-10 sorbin did not induce any change in the rat, which was highly sensitive to the hypertensive action of angiotensin II and the hypotensive action of VIP.

pramaximal inhibition in a similar picomolar range of doses. Maximal effective doses of sorbin peptides induced a strong decrease of Cl^- and Na^+ final concentrations of the same order as that induced by DAMA. These final concentrations of 75 mmol/L sodium and 40 mmol/L chloride are the lowest that can be maintained during any stimulation. Furthermore, we have already verified in a separate study (unpublished) that lowering the concentration of the solution test below this level induced a large secondary movement of water and NaCl from serosa to mucosa, which increases Na^+ and Cl^- concentrations. This secondary induced efflux is responsible for the apparent inhibition observed with supramaximal doses of sorbin peptides. In contrast, for angiotensin, which did not decrease the final concentration to its minimal level, the reverse effect is related to blood pressure increase or enteric prostaglandin production.[7] The net effect of all sorbin peptides was a coupled Na^+ and Cl^- absorption against the chemical gradient with a duodenal absorption of more ions than water. Only bicarbonate absorption followed water absorption without any significant change in concentration.

The basal secretion of water, chloride, sodium, and bicarbonate, and predominantly so for sodium bicarbonate, could be secondary to a passive equilibrium between the luminal contents and the extracellular electrolyte concentration in spite of the presence of an osmotically active substrate such as mannitol. The secretion may be the result of a vagal stimulation induced by the distention of the loop with 1 mL placed into it.[11,12] However, a basal secretion of bicarbonate has been described in duodenal mucosa of amphibians,[13] cats,[12] humans,[14] and rats with HCO_3^--free luminal solution or with a 22 mmol/L HCO_3^--containing solution,[15,16] secreted by both the surface epithelium[15] and Brunner's glands.[12,17] A cephalic phase of duodenal alkaline secretion has been proposed.[18]

The finding of VIP stimulation of secretion of water and electrolytes agrees with many findings in rats,[3,4,11,17] cats,[12] and humans[19] in duodenum and the rest of the intestine.[4,20] The effect of DAMA on duodenal absorption of water, Na^+, and Cl^- is in keeping with what has been found in duodenum,[13] jejunum, or ileum.[5,6,21,22] As far as bicarbonate transport is concerned, our data in rats agree with prior data obtained in humans in whom morphine inhibited bicarbonate secretion.[14] They are at variance with data also obtained in rats in one study that reported that morphine, β-endorphin, and picomolar doses of methionine-enkephalin increased mucosal bicarbonate secretion.[15]

Angiotensin II has been shown to stimulate absorption at low doses by a release of norepinephrine from enteric sympathetic nerves. In our study, the duodenum was less sensitive to angiotensin II than the other parts of the intestine.[7]

Among the numerous molecules that function as absorptive messengers in the gastrointestinal tract,[21] sorbin, which was isolated from the upper part of the small intestine,[1] may play a role by a mechanism that remains to be shown.

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