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Atrial natriuretic peptide (ANP) in rat gastrointestinal tract

O. Vuolteenaho, O. Arjamaa, O. Vakkuri, T. Maksniemi, L. Nikkilä, J. Kangas, J. Puurunen*, H. Ruskoaho* and J. Leppäluoto

Departments of Physiology and **Pharmacology, University of Oulu, 90220 Oulu, Finland*

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Acid extracts of rat stomach and small intestine contained 8.6 ± 3.7 and 39 ± 15 ng/g of immunoreactive atrial natriuretic peptide (ANP). When studied by gel filtration and reverse-phase high-performance liquid chromatography, the stomach immunoreactivity consisted of multiple components, whereas the small intestine contained mostly proANP and ANP 1–28-like material. These findings indicate that ANP may have a role in the physiology of the gastrointestinal tract, e.g. in the regulation of water and electrolyte absorption.

Natriuretic peptide; Radioimmunoassay; (Rat, Stomach, Intestine)

1. INTRODUCTION

Atrial natriuretic peptide is a novel hormone involved in the regulation of water and electrolyte homeostasis [1]. Quantitatively the most important source of ANP is the heart atria [2] but ANP has also been extracted from the heart ventricles [3,4], brain [2,5], eyes [6], adrenal gland [7], pituitary, lungs [8] and blood vessels [9].

Radioactive ANP, when given intravenously in rats, is specifically bound by several tissues, including the mucosal layer of the small intestine, indicating the presence of ANP receptors in the tissue [10]. ANP given intracerebroventricularly has been found to stimulate gastric acid secretion [11]. In winter flounder, ANP has been shown to have important effects on the ion transport across the intestinal wall [12]. The gastrointestinal tract has an important role in the water and electrolyte balance of the body, and therefore it would be very interesting to know if ANP is present in the mammalian gastrointestinal tract.

Correspondence address: O. Vuolteenaho, Department of Physiology, University of Oulu, 90220 Oulu, Finland

Abbreviations: ANP, atrial natriuretic peptide; BSA, bovine serum albumin; HPLC, high-performance liquid chromatography; IR, immunoreactive; PMSF, phenylmethylsulphonyl fluoride; RIA, radioimmunoassay; TFA, trifluoroacetic acid; V_o, void volume

2. MATERIALS AND METHODS

2.1. *Preparation of the tissue samples*

Male Sprague-Dawley rats (200–300 g) were killed by decapitation. Stomach and the first 20 cm of the small intestine were dissected, rinsed in 0.9% NaCl, blotted, weighed and frozen in liquid nitrogen. The tissues were kept at −20°C until extraction.

The frozen tissues were weighed and pulverized in liquid nitrogen. The powder was extracted in 10 vols of 0.3 M HCl containing PMSF (10 mg/l) using an Ultra-Turrax (2 min). The homogenates were spun at 28000 × *g* for 15 min at 4°C. The supernatants were diluted with an equal volume of water, aliquoted and lyophilized.

2.2. *Chromatography*

The tissue extracts (solubilized in 30% acetic acid/0.02% 2-mercaptoethanol) were spun at 10000 × *g* for 10 min. A trace of radioiodinated rat ANP 1–28 (1000–2000 cpm) was added to the supernatant to serve as an internal standard, and the samples were chromatographed at room temperature in a 1.5 × 118 cm column of Sephadex-G50SF (Pharmacia) using the above solution for elution. Flow rate was 10–11 ml/h and fractions of 25 min were collected. The fractions were counted in a gamma counter and aliquots were dried in a Savant Speed Vac in the presence of 0.1 mg BSA. Synthetic rat ANP 1–28 coeluted with radioiodinated ANP 1–28 on the column and atrial proANP eluted just after the void volume (K_d = 0.08). The recovery of immunoreactive ANP in the samples was 85–105%.

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The fractions coeluting with radioiodinated ANP 1–28 in gel filtration were pooled and concentrated with Sep-Pak C18 extraction [13,14]. The eluate was dried in a Speed Vac and redissolved in 0.4 ml of aqueous 0.1% TFA, passed through a 0.45 μm Millipore filter and subjected to reverse-phase HPLC. The HPLC instrument was a Varian model 5020 liquid chromatograph connected to a Knauer 220 nm UV detector and a Shimadzu model C-R3A integrator/plotter. The Vydac C18 column (4.6 × 250 mm, 5 μm, 30 nm) was eluted with a linear 30 min gradient from 12 to 48% acetonitrile in water containing 0.1% TFA with a flow rate of 1 ml/min. Fractions of 1 ml were collected and dried in the Speed Vac (with 0.1 mg BSA) for use in ANP radioimmunoassay. The recovery of immunoreactivity through Sep-Pak and HPLC was 80–95%. Injection of 0.1% TFA without sample and running the program as above yielded no detectable immunoreactive ANP in the fractions. The column was calibrated with synthetic rat ANP 3–28 (a gift from Dr N. Ling, Salk Institute) and rat ANP 1–28 (Sigma).

2.3. *ANP radioimmunoassay*

The dried tissue extracts and column fractions were dissolved in RIA buffer. The tissue extracts were assayed in at least three serial dilutions and the column fractions in one dilution in ANP-RIA [15]. Synthetic rat ANP 1–28 was used as standard.

3. RESULTS

Acid extracts of rat stomach and small intestine contained 8.6 ± 3.7 and 39 ± 15 (mean ± SD, n = 7) ng rat ANP 1–28 equivalents of ANP immunoreactivity/g of wet wt, respectively. The tissue extracts showed parallel displacement curves to those produced by synthetic ANP (fig.1). The gel filtration pattern of the stomach extracts was complex, with the majority of the immunoreactive material eluting in the low molecular mass region (fig.2A). The small intestine elution pattern was much simpler with two major peaks eluting at the positions of proANP and ANP 1–28 (fig.2B).

In order to examine more closely whether the tissues contain ANP 1–28, the circulating form of ANP, the gel filtration fractions at and around the elution position of radioiodinated ANP 1–28 (stomach) or those coeluting with it (small intestine) were pooled and analyzed in reverse-phase HPLC after having been concentrated with Sep-Pak C18 extraction. The material from stomach again gave a very complex elution pattern with a minor portion of the immunoreactivity coeluting with ANP 1–28 (fig.2C). Most of the small intestine immunoreactivity coeluted with ANP 1–28, with a small shoulder on the ascending site of the peak indicating the presence of a small amount of less hydrophobic material, possibly ANP 3–28 (fig.2D).

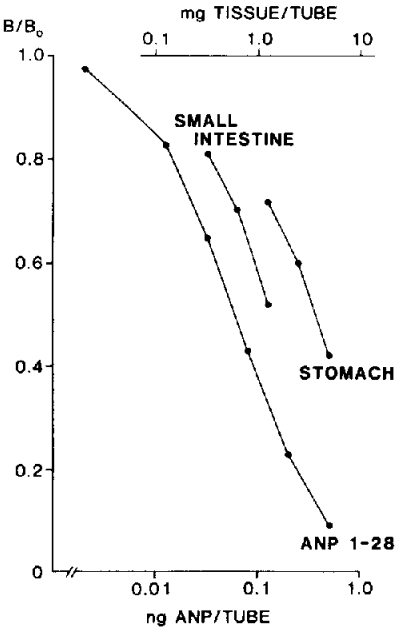

Fig.1. Dose-response curves showing the displacement of rat radioiodinated ANP 1–28 from binding to the ANP antiserum '7-44' by rat ANP 1–28 (standard) and acid extracts of rat stomach and small intestine.

The complex chromatographic profiles of immunoreactive ANP in stomach extracts (fig.2A and C) may be the result of artifactual degradation of the peptides during extraction or chromatography. The most likely explanation for the degradation would be the presence of large amounts of pepsin in the gastric tissue. We attempted to solve the problem by boiling the tissue in water for 4 min before extraction and then proceeding with the extraction in the usual way (an equal volume of 0.6 M HCl, 20 mg/l PMSF was added to the cooled tissue) but there was no significant change in the elution profile. Nor did the addition of pepstatin A (10 mg/l) to the extraction solution alter the elution profile (not shown). Acid acetone extraction [17] gave a very clean gel filtration profile with most of the immunoreactivity coeluting with radioiodinated ANP 1–28 (not shown), but the yield of immunoreactive ANP from stomach was only 1.47 ± 0.11 ng/g (n = 5) (the yield from small intestine with acid acetone was 1.64 ± 0.42 ng/g).

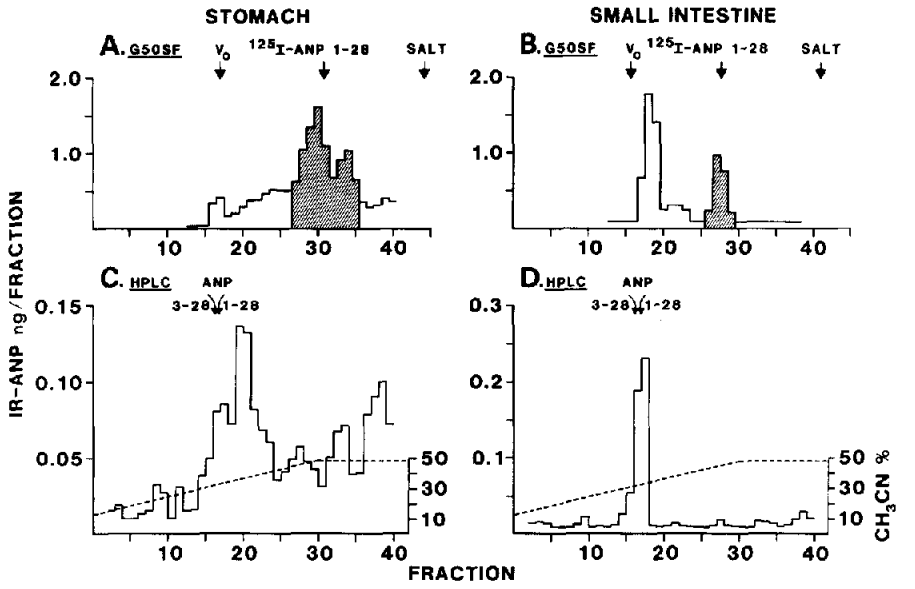

Fig.2. (A and B) Sephadex G50SF gel filtration of rat stomach (A) and small intestine (B) extracts. (C and D) Reverse-phase HPLC of the pooled shaded fractions from gel filtration. (C) Stomach; (D) small intestine. For details see section 2.

4. DISCUSSION

The present results show for the first time the presence of immunoreactive ANP in the gastrointestinal tissues. The concentrations of ANP in rat stomach and small intestine are similar as in the brain [2] but much lower than in the heart atria [15]. Considering the large size of the tissues, however, the gastric and small intestinal tissues together contain a considerable amount of ANP. The relatively high concentration of immunoreactivity and the gel filtration elution patterns (fig.2) would argue against the possibility of blood contamination or binding of the peptide from blood as explanations for the presence of ANP in the tissues. 1 g of blood would contain about 0.05 ng of immunoreactive ANP, and practically all of it would be processed ANP, mostly ANP 1–28 [16].

Intravenously given radioactive ANP binds specifically, among other tissues, to the epithelial cells of the villi of the small intestine [10]. ANP inhibits Na-K-Cl cotransport across the intestinal wall in the flounder [12]. A large part of electrolyte and water absorption in the gastrointestinal tract takes place in the small intestine [18]. These findings, together with our present results suggest that ANP may have a role in the regulation of water and salt balance in the gastrointestinal tract.

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