Rat Atrial Natriuretic Polypeptide Increases Net Water, Sodium and Chloride Absorption Across Rat Small Intestine In Vivo

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Abstract—Localization of binding sites for synthetic rat atrial natriuretic polypeptide (α-rANP) and the effect of the peptide on net water and electrolyte movement in vivo perfused small intestine of the rat were studied. Autoradiographic study demonstrated that specific binding sites were localized on a space between the base of epithelia and lamina propria of the small intestine. Alpha-rANP (0.25 μg/min) infused into the superior mesenteric artery of rats increased net absorption of water (46% increase in comparison with controls), Na (84% increase) and Cl (70% increase) across the small intestinal tract perfused with Ringer's solution. These increases were also observed when glucose-free Ringer's solution was employed. Thus it would be concluded that the Na-glucose cotransport system is not involved in the action of α-rANP. These observations suggest that α-rANP controls circulating water-electrolyte balance through regulating not only renal function but also intestinal water, Na and Cl absorption.

Atrial natriuretic polypeptide (ANP) possesses potent diuretic and natriuretic activities (1-3). Koseki et al. (4, 5) have shown that specific binding sites of α-rANP were in the glomerulus, inner medullary collecting tubule and the vasa recta of rat kidneys. Furthermore, ANP binding sites were localized on extra-renal organs such as the small intestine, adrenal, eye, lung, heart, liver and brain (6), while ANP effects in these organs are not yet clarified.

In this study, we have investigated the localization of specific binding sites for [125I]α-rANP in the small intestine and the effect of α-rANP on net water and electrolyte movement by applying in vivo perfused rat small intestine.

Materials and Methods

Preparation of monoidinated α-rANP: Synthetic α-rANP was radioiodinated by the lactoperoxidase method as described previously (4). The specific activity of [125I]-α-rANP was 0.65 mCi/μg.

Autoradiographic studies: Male Sprague-Dawley rats (350 g) were given 39 ng of [125I]α-rANP either alone or in combination with 39 μg of cold α-rANP and sacrificed at 4 min following intravenous administration. For whole body autoradiography, according to Ullberg (7), 50 μm freeze-dried sections were prepared and placed in contact with X-ray films (No. 150, Fuji Film Co., Ltd., Japan) for 4 weeks at 4°C. For semi-micro autoradiography, 10 μm frozen sections were mounted onto glass slides and dried at room temperature. These sections were apposed tightly to the X-ray films. For light microscopic autoradiography, the rats were perfused with 100 ml of Zamboni's solution via the aorta. To complete the fixation process, the dissected organs were kept for 24 hr in Zamboni's solution which consists of 9 mM picric acid, 20 mg/l paraformaldehyde, 16 mM NaH2PO4 2H2O and 91 mM Na2HPO4 12H2O. Ten μm frozen sections were washed in phosphate-buffered saline (pH 7.2) and dipped into the nuclear track emulsion (NTB-2, Kodack, U.S.A.) and exposed for 2 weeks. After the sections were
developed and fixed, they were stained with haematoxylin-eosin and photographed.

Small intestine perfusion studies: We performed the perfusion experiment of rat small intestine with a modification of the methods of Dharmasthaphorn et al. (8) and Seeber et al. (9). Fasting male Sprague-Dawley rats weighing 200–300 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a tracheotomy was performed. After ligation of bile duct, tubes were cannulated into both the duodenum and distal end of the ileum. The washed small intestine was perfused with warmed Ringer's solution at the rate of 0.5 ml/min, by using a peristatic pump (Model P-1, Pharmacia, Sweden). The effluent was collected for 10 min periods. The Ringer's solution contained 137 mM NaCl, 3.4 mM KCl, 1.4 mM CaCl₂, 0.1 mM MgCl₂, 5 mM glucose and 60 μM ¹⁴C-inulin (10,000 dpm/ml, specific activity 11.4 mCi/mmol, Amersham, England). Glucose-free Ringer’s solution was prepared by replacing the glucose with 5 mM D-mannitol. The osmotic pressure of perfusate was 275 mosmol/l (Model 3W2, Advanced Instruments, U.S.A.). Glucose-free Ringer’s solution was prepared by replacing the glucose with 5 mM D-mannitol. The osmotic pressure of perfusate was 275 mosmol/l (Model 3W2, Advanced Instruments, U.S.A.). The superior mesenteric artery was cannulated from the femoral artery with PE-10 tubes (Clay Adams, U.S.A.). Completing this operation, normal blood circulation in the small intestine was confirmed. Through this tube, saline or α-rANP was infused at the rate of 25 μl/0.25 μg/min with a peristatic pump (Model P-3, Pharmacia, Sweden). Body temperature was maintained at 37°C. Radioactivity in the samples was measured by a liquid scintillation spectrometer (Model LS-7500, Beckman, U.S.A.). Na and K concentrations were determined by use of a flame photometer (Model 750, Hitachi, Japan). CI was measured by potentiometric titration using a chloridimeter (Chloride counter, Hiranuma, Japan). Water and electrolyte flux was expressed as μl or μmoles/min/m of the length of the small intestine. Positive numbers represent net absorption and negative numbers, net secretion. All results were expressed as the mean±S.E. Statistical analysis was performed by Student's t-test.

Results

Localization of specific binding sites for [¹²⁵I]α-rANP: When rats were given 39 ng of [¹²⁵I]α-rANP intravenously and sacrificed at 4 min, high density radioactivities were localized on the kidney and the lung (Fig. 1). On the other organs, radioactivities were shown in the small intestine, liver, adrenal,
To determine whether the binding in the small intestine was specific or nonspecific, 39 ng of [125I]α-rANP was injected either alone (Fig. 2, A and C) or in combination with 39 μg of cold α-rANP (Fig. 2B). Dense grains were observed along the villus (Fig. 2A) and were displaced with an excess of α-rANP (Fig. 2B). Figure 2C shows an obtained autoradiogram superimposed on a histological image of the villus. The grains were distributed on the space between the base of the epithelia and the lamina propria.

**Effects of α-rANP on net water and electrolyte movement:** When the small intestine of rats was perfused with Ringer’s solution in vivo, there was net water absorption across the small intestine and this absorption was nearly constant from 60 to 180 min after the start of perfusion in control animals that received saline (Fig. 3A, open circle). Increase in net water flux across the intestinal tract was demonstrated when 0.25 μg/min of α-rANP was infused into the superior mesenteric artery during perfusion (Fig. 3A, closed circle). Increases in the net fluxes of Na, K and Cl were also observed as compared with the controls (Fig. 3, B–D).

When the small intestine was perfused with a modified Ringer’s solution, in which glucose was displaced with D-mannitol, basal net water absorption was lowered to 53% of that from the original Ringer’s solution. Similar change was also noted in Na and Cl. Even under these conditions, when α-rANP was infused, significant increases were observed in the net absorption of water, Na and Cl as compared to the controls (47%, 85% and 57% increase, respectively).

**Discussion**

It has been demonstrated that autoradiography provides a useful tool to identify the distribution of ANP receptors in the kidney.
Fig. 3. Time course of net water (A), Na (B), K (C) and Cl (D) flux across the wall of rat intestinal tracts perfused with Ringer’s solution. Alpha-rANP (●) or saline (○) (0.25 µg/25 µl/min) was infused into the superior mesenteric artery during the perfusion of the small intestine. Each point represents the mean±S.E. of 6 determinations.

(4–6, 10), brain (11, 12), eye (6, 11) and other organs (6). It has been also reported that the receptors for ANP exist in the basolateral membranes isolated from rat kidney cortex (13) and vascular systems (14–16). Our autoradiographic study was of the area between the base of the epithelia and the lamina propria (Figs. 1 and 2). However, the obtained autoradiogram had insufficient resolution for determining if specific binding sites for α-rANP exist in the basolateral membrane of the epithelia or the capillaries in the lamina propria. Although the observed specific binding has not been well characterized, they suggest that α-rANP receptors are present in the small intestine and may play a role in water and electrolyte handling in the small intestine.

In the present study, 0.25 µg/min of α-rANP infused into the superior mesenteric artery increased net absorption of water, Na and Cl across the small intestine perfused with Ringer’s solution in vivo. The mechanisms by which α-rANP increased water and solute absorption in the small intestine are unknown at present. However, the following
three possibilities deserve to be considered: 1) α-rANP, acting on submucosal capillaries, might increase water, Na and Cl absorption by decreasing intracapillary hydrostatic pressure which regulates fluid absorption through the paracellular shunt pathway. 2) α-rANP might stimulate active Na transport. However, this possibility is unlikely because it has been reported that ANP has no effect on Na-K-ATPase activity (17). 3) α-rANP might increase the entry of Na and Cl across the brushborder membrane. Since enhanced fluid absorption was observed in either the presence or absence of glucose to the same extent, it is likely that the Na-glucose cotransport system is not involved in the stimulation of fluid absorption by α-rANP. In view of the general effect of ANP on the vascular system (14–16), the first possibility is most likely.

Our finding is seemingly contrary to the effect expected from the natriuretic action of ANP. Recently, Seeber et al. (9) reported that rat atrial extract suppressed the Na-glucose cotransport across the rat intestine perfused with glucose-free Ringer’s solution. Alpha-rANP (■) or saline (□) (0.25 μg/25 μl/min) was infused into the superior mesenteric artery during perfusion of the small intestine. Each point represents the mean ± S.E. of 6 determinations.

Fig. 4. Time course of net water (A), Na (B), K (C) and Cl (D) flux across the wall of rat intestinal tracts perfused with glucose-free Ringer’s solution. Alpha-rANP (■) or saline (□) (0.25 μg/25 μl/min) was infused into the superior mesenteric artery during perfusion of the small intestine. Each point represents the mean ± S.E. of 6 determinations.
Table 1. Effect of \(\alpha\)-rANP on net fluxes of water, Na, K and Cl across the rat small intestine

|          | Water (\(\mu l/min/m\)) | Na (\(\mu moles/min/m\)) | K (\(\mu moles/min/m\)) | Cl (\(\mu moles/min/m\)) |
|----------|-------------------------|--------------------------|--------------------------|--------------------------|
| Ringer's solution control (n=6) | 126±7                  | 11.0±1.7                 | -0.029±0.043             | 14.7±1.8                 |
| \(\alpha\)-rANP (n=6)              | 183±8**                | 20.2±1.8**               | 0.259±0.077**            | 24.9±1.7**               |
| Glucose free Ringer's solution control (n=6) | 66±7                   | 6.4±1.2                  | -0.009±0.038             | 9.7±1.2                  |
| \(\alpha\)-rANP (n=6)              | 97±3**                 | 11.9±1.5*                | -0.024±0.087             | 15.2±1.1**               |

\(\alpha\)-rANP (0.25 \(\mu g/25 \mu l/min\)) or saline was infused through the superior mesenteric artery. Glucose-free Ringer's solution contains D-mannitol instead of glucose. Net absorption of water, Na, K and Cl was calculated from the data (from 60 min to 180 min) in Figs. 3 and 4. Each value represents the mean±S.E. Significant difference from the controls, \(P<0.05 (\ast), P<0.01 (\ast\ast)\).

in vivo. However, this work is difficult to evaluate for the following reasons: 1) They used crude extract instead of synthetic \(\alpha\)-rANP, which has been identified as the native circulating ANP in rats (18). 2) In view of the extremely short biological half life of ANP in the rat (\(t_{1/2}=26.5\) sec) (19), the duration of collection period seems to be too long to detect the acute effect of ANP.

O'Grady et al. (20) reported that atriopeptin III inhibited Na-K-2Cl cotransport in short circulated teleost intestine. However, it is questionable whether this is also applicable to mammals. It has been reported in rabbits that ANP did not affect Cl transport across the thick ascending limb of Henle's loop, where Na-K-2Cl cotransport is also operating (21).

Since we administered \(\alpha\)-rANP directly into the mesenteric artery, it is possible that the vascular effect is exaggerated by this maneuver. Although our autoradiographic study showed that the specific binding sites for \(\alpha\)-rANP were localized on the space between the base of the epithelia and the lamina propria, we can not exclude a possibility that ANP directly acts, on the epithelia, leading to a different result depending on the experimental conditions.

Our observations reported here suggest that ANP has a distinct target on the small intestine and may modulate fluid and salt balance, although the underlying mechanisms which account for the action of \(\alpha\)-rANP on the small intestine remain to be elucidated.

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