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Natriuretic peptides cause relaxation of human esophageal mucosal muscle

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Abstract

Natriuretic peptides have been demonstrated to cause relaxation of the human gallbladder muscle through interaction with natriuretic peptide receptor-B (NPR-B/NPR2). Effects of natriuretic peptides in the human esophageal muscle were unknown. To investigate the effects of natriuretic peptides in the human esophagus, we measured relaxation of muscularis mucosae strips isolated from the human esophagus caused by C-type natriuretic peptide (CNP), brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP) and des[Gln^{18}, Ser^{19}, Gly^{20}, Leu^{21}, Gly^{22}]ANP(4-23) amide (cANP(4-23)), a selective natriuretic peptide receptor-C (NPR-C) agonist. In endothelin-1 or carbachol-contracted mucosal muscle strips, CNP caused moderate, sustained and concentration-dependent relaxation. BNP caused a very mild relaxation whereas ANP and cANP(4-23) did not cause any relaxation. CNP was much more potent than BNP and ANP in causing relaxation. These suggest the existence of NPR-B mediating relaxation. The CNP-induced relaxation was not affected by tetrodotoxin or atropine in endothelin-1-contracted esophageal strips and not by tetrodotoxin in carbachol-contracted strips, indicating a direct effect of CNP on the human esophageal muscularis mucosae. Taken together, these results demonstrate that natriuretic peptides cause relaxation of the muscularis mucosae of the human esophagus and suggest that the relaxation is through interaction with NPR-B. Natriuretic peptides may play an important role in the control of human esophageal motility.

Keywords: Natriuretic peptide receptor; Esophagus; Motility; Endothelin

1. Introduction

Atrial natriuretic peptide (ANP), the first of a family of peptides with potent natriuretic, diuretic, and vasorelaxant activity, was isolated from rat atrial extracts. The principal natriuretic peptides are ANP, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), which are 28, 32 and 22-amino-acid peptides, respectively. ANP and BNP are synthesized in the heart muscle cells of the atria and in small amounts in the heart ventricles as well as several non-cardiac sites, including the central nervous system, gonads and kidneys. Both ANP and BNP circulate in the plasma, and the concentrations are increased in patients with congestive heart failure. In contrast, negligible amounts of CNP are found in plasma. It is believed that CNP exerts autocrine and paracrine functions. CNP is present in the central nervous system and vascular endothelial cells. The presence of CNP has also been reported in the ileum, colon and kidney [1–4]. In the cardiovascular system, ANP, BNP and CNP dilate both arteries and veins. In the kidneys, ANP dilates afferent arteriolar vessels and has a striking relaxation effect on glomerular mesangial cells [1–3,5,6]. Three receptors for natriuretic peptides, i.e. the natriuretic peptide receptor-A (NPR-A/NPR1), NPR-B (NPR2) and NPR-C (NPR3), have been identified in mammalian tissues [1–3,7–10]. NPR-A is the most abundant subtype in the large vessels, but there is also some NPR-B. NPR-B predominates in the brain. Both NPR-A and NPR-B are present in the adrenal glands and kidney. NPR-C is expressed in the kidneys, adrenals, brain, lungs and vascular walls [1,3]. NPR-A has a high affinity for ANP and BNP but a low affinity for CNP, while NPR-B has a high affinity for CNP but a low affinity for ANP and BNP. NPR-C has a high affinity for ANP and des[Gln^{18}, Ser^{19}, Gly^{20}, Leu^{21}, Gly^{22}]ANP(4-23) amide (cANP(4-23)), which is a selective NPR-C agonist [1–4,10]. In the gastrointestinal and hepatobiliary system, natriuretic peptides have been demonstrated to cause relaxation of the lower esophageal sphincter, gastric muscle, pyloric sphincter, hepatic stellate cells, gallbladder muscle, colon

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muscle and internal anal sphincter [11–18]. In the opossum lower esophageal sphincter, pyloric sphincter and internal anal sphincter strips, ANP causes a fall in the basal tension [11]. In dispersed rabbit gastric smooth muscle cells, ANP inhibits cholecystokinin-induced contraction [12]. In cultured human hepatic stellate cells, ANP reduces endothelin-1 induced contraction [13]. In addition, in human and guinea-pig gallbladder strips, CNP causes relaxation [14]. In rabbit and rat colon strips, CNP inhibits basal tension or phasic contraction [17,18]. Furthermore, in dispersed guinea-pig cecal smooth muscle cells, CNP, BNP and ANP all inhibit cholecystokinin-induced contraction [15,16]. CNP mRNA has been detected in various gastrointestinal tissues, including stomach, small intestine and colon, as well as cecal smooth muscle cells [1,16,17]. Receptors for natriuretic peptide have been identified in various gastrointestinal tissues. NPR-B and NPR-C mRNAs are detected in rabbit gastric muscle cells whereas NPR-A and NPR-B mRNAs, in the rabbit colon muscle [12,17].

We have demonstrated that natriuretic peptides cause relaxation of the human gallbladder muscle through interaction with NPR-B [14]. Effects of natriuretic peptides in other human gastrointestinal muscles, including esophageal muscles, were unclear. We have also demonstrated that the muscularis mucosae of the human esophagus possesses endothelin receptors which mediate muscle contraction [19]. The muscularis mucosae contains smooth muscle fibers arranged longitudinally and may contribute to the genesis of intraluminal pressure and influence the secretory function of the mucosa. It is more accessible to the direct action of drugs when it has been isolated from the muscularis externa, which is composed partially and almost entirely of striated muscle in the human and guinea-pig esophagus, respectively [20,21]. We hypothesized that natriuretic peptides may have relaxant effects on the smooth muscle of the human esophagus. Thus, the aim of this study was to investigate the effects of natriuretic peptides on the muscularis mucosae of the human esophagus.

2. Materials and methods

ANP, BNP, CNP, des[Gln^{18}, Ser^{19}, Gly^{20}, Leu^{21}, Gly^{22}]ANP (4-23) amide (cANP(4-23)) and endothelin-1 were obtained from American Peptide Company, Sunnyvale, CA, USA. Carbachol, atropine and buffer reagents were obtained from Sigma Chemical, St. Louis, MO, USA. Tetrodotoxin was obtained from Tocris Cookson Inc., Avonmouth Bristol, UK.

The study was performed according to the Declaration of Helsinki and the protocol for this work was approved by the Institutional Review Board of the Buddhist Tzu Chi General Hospital, Hualien. Human specimens were obtained from 14 patients (11 male and 3 female, median age 54.5 years, range 44–83) undergoing esophagectomy for esophageal cancer. Informed consent was obtained. Immediately after surgical removal of the esophagus, two 2 × 1-cm areas, at least 2 cm from the cancer, were excised from the distal third of each esophagus and placed in oxygenated standard incubation solution (see below) for transportation to the laboratory, where the relaxation experiment was promptly initiated. The period of anoxia was less than 30 min [19].

2.1. Measurement of relaxation of muscle strips isolated from human esophageal muscularis mucosae

Measurements of relaxation of isolated muscularis mucosae strips from the human esophagus were performed according to the procedure described previously with minor modification [14,19,22,23]. In brief, separated from the muscularis externa (muscularis propria) under a dissecting microscope, the isolated human esophageal muscularis mucosae strips containing the muscularis mucosae, lamina propria and squamous epithelium were placed in standard incubation solution, containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 14 mM glucose, 1.2 mM NaH_2PO_4, 1.8 mM $CaCl_2$, gassed with 95% O_2–5% CO_2. The final pH at 37 °C is 7.40±0.05. The esophageal muscularis mucosae strip was cut longitudinally and washed, and finally cut to make eight small, 1.0 × 0.3 cm, strips. The isolated muscularis mucosae strips were attached to eight organ baths using surgical silk sutures and incubated at 37 °C in the standard incubation solution continuously gassed with 95% O_2–5% CO_2. The strips were connected to isometric transducers (Grass FT.03), which were connected to an amplifier (Gould) and a computer recording system (BIOPAC systems, Santa Barbara, CA, USA). The basal

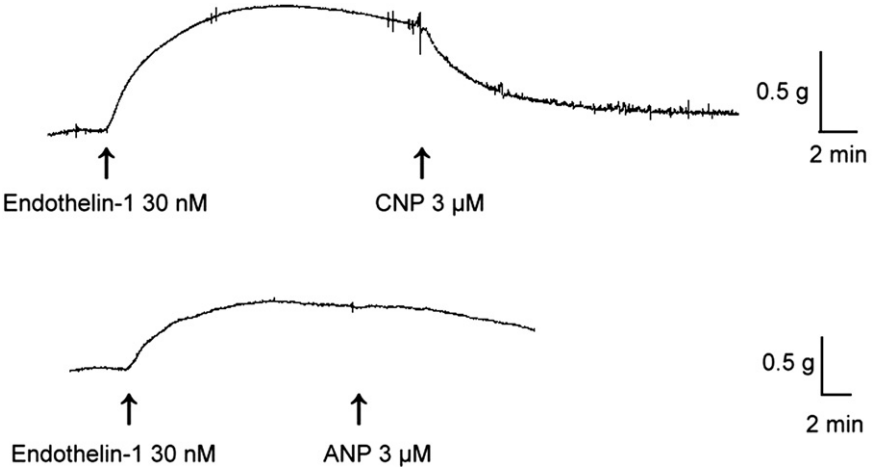

Fig. 1. A typical tracing showing the relaxation of endothelin-1-contracted human esophageal muscularis mucosae by CNP, 3 μM.