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Dendroaspis natriuretic peptide is the most potent natriuretic peptide to cause relaxation of lower esophageal sphincter

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ABSTRACT

Atrial natriuretic peptide (ANP) causes relaxation in the opossum lower esophageal sphincter. The effects of *dendroaspis* natriuretic peptide (DNP) and other natriuretic peptides in the lower esophageal sphincter were not known. We measured the relaxation of transverse strips from the guinea pig lower esophageal sphincter caused by DNP, ANP, brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and a natriuretic peptide receptor-C agonist des[Gln^{18}, Ser^{19}, Gly^{20}, Leu^{21}, Gly^{22}]ANP(4–23) amide (cANF(4–23)) in vitro. In resting strips of the guinea pig lower esophageal sphincter DNP and BNP caused marked relaxations. Furthermore, in both sarafotoxin S6c and carbachol-contracted lower esophageal sphincter strips, DNP caused marked and BNP caused moderate, concentration-dependent relaxations. ANP as well as CNP caused mild relaxations. In contrast, cANF(4–23) did not cause relaxation. The relative potencies for natriuretic peptides to cause relaxation were DNP>BNP>ANP>=CNP in both sarafotoxin S6c and carbachol-contracted lower esophageal sphincter strips. The DNP and BNP-induced relaxations were not affected by tetrodotoxin or atropine, suggesting that the natriuretic peptide-induced response was not neutrally mediated. In conclusion, these results demonstrate that natriuretic peptides cause the relaxation of the guinea pig lower esophageal sphincter. DNP is the most potent natriuretic peptide to cause lower esophageal sphincter relaxation, which might be mediated by natriuretic peptide receptor-A or a novel DNP-selective natriuretic peptide receptor.

1. Introduction

Natriuretic peptides are a family of peptides with similar 17-residue disulfide ring structure and natriuretic, diuretic as well as vasorelaxant activity. The family includes atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and D-type natriuretic peptide (DNP), which are 28, 32, 22 and 38-amino-acid peptides, respectively [1–5]. ANP and BNP are synthesized in the heart, brain, gonad and kidney. Both ANP and BNP are released to the circulation and plasma concentrations are increased in patients with congestive heart failure. CNP is expressed in vessels, brain, chondrocytes, ileum, colon and kidney [1,3–5]. In contrast to ANP and BNP, negligible amounts of CNP are found in plasma. DNP, isolated in 1992 from the venom of a green mamba snake, is a new member of the natriuretic peptide family [2,6]. It has been detected in human plasma, heart, vessels and rat colon [7–9].

Three receptors for natriuretic peptides, i.e. the natriuretic peptide receptor-A (NPR-A), NPR-B and NPR-C, have been identified in mammalian tissues [1–5]. NPR-A is abundant in the large vessels whereas NPR-B predominates in the brain. NPR-C is expressed in various tissues including the kidney, brain, lung and vascular walls. 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 (cANF(4–23)), which is a selective NPR-C agonist. DNP binds with high affinity to NPR-A and NPR-C but not NPR-B [2,10,11].

In the gastrointestinal system, natriuretic peptides influence motility. They cause relaxation of the esophagus, stomach, gallbladder and colon. Specifically, in the esophagus CNP causes relaxation [12] and in the lower esophageal sphincter ANP causes relaxation [13]. In the stomach, ANP inhibits cholecystokinin-induced contraction [14]. In the colon, DNP and CNP inhibit basal tension or spontaneous contraction [8,15]. Furthermore, in the cecum, ANP, BNP and CNP all inhibit cholecystokinin-induced contraction [16,17]. In the internal anal sphincter, ANP causes relaxation [13]. In addition, in the gallbladder CNP causes relaxation and in the liver, ANP reduces endothelin-induced contraction of stellate cells [18,19]. The receptors for natriuretic peptides have been identified in various gastrointestinal tissues. NPR-B and NPR-C are detected in the rabbit gastric muscle whereas NPR-A and NPR-B, in the rabbit colon muscle [14,15]. Previous studies showed that natriuretic peptides cause relaxation in many gastrointestinal smooth muscles and, in some muscle tissues, through NPR-B or NPR-C. NPR-B mediates natriuretic peptide-induced relaxation in the human and guinea pig gallbladder as well as human esophageal muscularis mucosae whereas NPR-C mediates relaxation in the rabbit stomach [12,15,19]. ANP causes relaxation of the opossum lower esophageal

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sphincter [13]. However, the effects of DNP and other natriuretic peptides in the lower esophageal sphincter were not known. The aim of the present study was to investigate the effects of DNP in the guinea pig lower esophageal sphincter in vitro and compare effects of DNP to other natriuretic peptides.

2. Materials and methods

Male Hartley guinea pigs were obtained from the Animal Center, National Science Council, Taiwan. ANP, BNP, CNP, cANF(4–23) and sarafotoxin S6c were purchased from American Peptide Company, Sunnyvale, CA, USA. DNP was purchased from Bachem, Bubendorf, Switzerland. Carbachol, atropine, and buffer reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA. Tetrodotoxin was obtained from Tocris Cookson Inc., Avonmouth Bristol, UK.

2.1. Measurement of relaxation of isolated lower esophageal sphincter strips

The Institutional Animal Care and Use Committee of Buddhist Tzu Chi General Hospital, Hualien, approved the protocol for this study. Male guinea pigs, weighing 350–400 g, were sacrificed with CO_2. The stomach, including a portion of the esophagus, was quickly removed and placed in the oxygenated standard incubation solution (see below). The esophagus and stomach were cut open in the longitudinal direction along the greater curvature and pinned flat with the mucosal side up. The mucosa was removed with micro-scissors. A transverse strip (2 mm wide and 10 mm long) was cut from the area of the lower esophageal sphincter, which was easily identified as a thickened region of muscle between the esophagus and the stomach [20,21].

Measurements of relaxation of the isolated lower esophageal sphincter strips were performed according to the procedure described previously [20,21]. In brief, the isolated guinea pig lower esophageal sphincter strips 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 was 7.40±0.05. The lower esophageal sphincter strips were attached to 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 (FT.03; Grass Technologies, West Warwick, RI, USA), which were connected to an amplifier (Gould Instrument Systems, Valley View, OH, USA) and a computer recording system (BIOPAC Systems, Santa Barbara, CA, USA). The basal tension of the muscle strips was adjusted to 1.0 g [20,21]. Experiments were started after a 45 min equilibration period. For measurements of relaxation in resting lower esophageal sphincter, natriuretic peptides were added to muscle baths in a non-cumulative fashion, i.e. with single dose administration [12,19,20]. The relaxation responses were represented as a percentage (% papaverine) of the relaxation to 100 μM papaverine. For measurements of the relaxation in carbachol or sarafotoxin-contracted strips, natriuretic peptides were added to carbachol or sarafotoxin-contracted muscle strips 15 min after the addition of carbachol or sarafotoxin S6c in a non-cumulative fashion. Sarafotoxin S6c, an endothelin B receptor agonist, was used to stimulate lower esophageal sphincter contraction because endothelin 1 did not stimulate contraction of the guinea pig lower esophageal sphincter [20]. Carbachol or sarafotoxin S6c-induced tone before the addition of natriuretic peptides was used as a reference (% sarafotoxin S6c or carbachol-induced tone) to express relaxation. For studies using atropine and tetrodotoxin, the muscle strips were exposed to the indicated concentration of these agents for 6 and 15 min respectively, and then to the natriuretic peptide [12,19–22]. Only one single dose response, with or without atropine or tetrodotoxin, was studied with each preparation.

3. Analysis of data

Results were expressed as means ± SEM. Statistical evaluation was performed using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's test. $P<0.05$ was considered statistically significant.

4. Results

4.1. Effects of natriuretic peptides on resting guinea pig lower esophageal sphincter strips

In the resting strips of the guinea pig lower esophageal sphincter, papaverine (100 μM) decreased the force of the muscle strips by 0.4 ± 0.1 g. Adding 1 μM DNP and BNP to the resting guinea pig lower esophageal sphincter strips caused 52 ± 2% and 42 ± 5% papaverine (100 μM)-induced relaxation, respectively. In contrast, cANF(4–23) (1 μM) caused 0 ± 0% relaxation, i.e. did not cause relaxation.

4.2. Effects of natriuretic peptides on sarafotoxin S6c-contracted lower esophageal sphincter strips

Sarafotoxin S6c (300 nM), an endothelin receptor B agonist, increased the force of the guinea pig lower esophageal sphincter strips by 0.9 ± 0.1 g and this contraction reached a plateau within 15 min (Fig. 1). Adding DNP to the sarafotoxin S6c-contracted muscle strips at the plateau caused a marked, sustained and concentration-dependent relaxation (Figs. 1, 2). DNP caused detectable relaxation of the sarafotoxin S6c-contracted lower esophageal sphincter strips at 10 nM and maximal relaxation at 1 μM, which caused 78 ± 11% relaxation of the sarafotoxin S6c-contracted lower esophageal sphincter. The highest tested concentration (3 μM) of DNP produced a 76 ± 10% relaxation of the sarafotoxin S6c-contracted lower esophageal sphincter. Similarly, BNP caused moderate relaxation. The highest tested concentration (3 μM) of BNP produced 59 ± 5% relaxation of the sarafotoxin S6c-contracted lower esophageal sphincter (Fig. 2). ANP and CNP caused mild relaxations of the sarafotoxin S6c-contracted lower esophageal sphincter strips. The highest tested concentration (3 μM) of ANP and CNP produced 44 ± 9% and 28 ± 9% relaxations, respectively, of the sarafotoxin S6c-contracted lower esophageal sphincter (Fig. 2). In

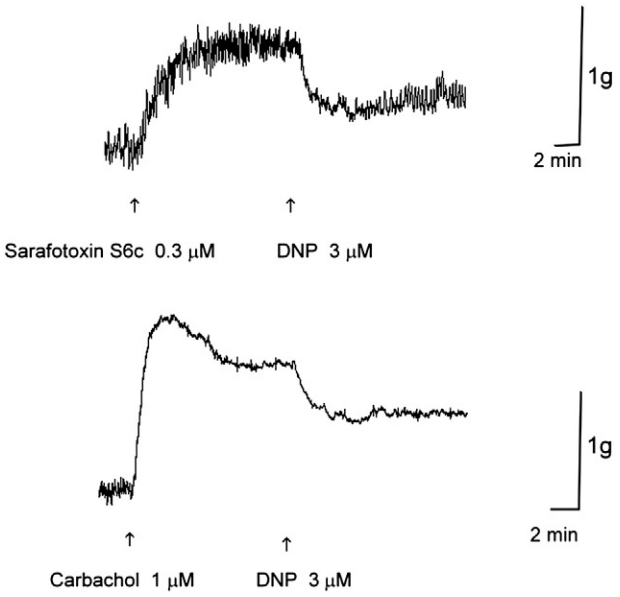

Fig. 1. Typical tracings showing the relaxation of sarafotoxin S6c (upper panel) and carbachol-contracted (lower panel) guinea pig lower esophageal sphincters by DNP, 3 μM.