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Natriuretic peptides cause relaxation of human and guinea-pig gallbladder muscle through interaction with natriuretic peptide receptor-B

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Abstract

Atrial natriuretic peptide (ANP) binding sites have been demonstrated in the guinea-pig gallbladder muscle with unclear function. To investigate effects of natriuretic peptides in the gallbladder, we measured relaxation of isolated human and guinea-pig gallbladder strips caused by natriuretic peptides, including C-type natriuretic peptide (CNP), brain natriuretic peptide (BNP) and ANP, as well as 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. Results in the human gallbladder were similar to those in the guinea-pig gallbladder. CNP, BNP, ANP and cANP(4-23) alone did not cause contraction or relaxation in resting gallbladder strips. However, in carbachol or endothelin-1-contracted strips, CNP caused moderate, sustained and concentration-dependent relaxation. The relaxation was not affected by tetrodotoxin or atropine in endothelin-1-contracted gallbladder strips and not by tetrodotoxin in carbachol-contracted strips. These indicate a direct effect of CNP on the gallbladder muscle. The relative potencies for natriuretic peptides to cause relaxation were CNP>>BNP≥ANP. cANP(4-23) did not cause relaxation. These indicate the existence of the natriuretic peptide receptor-B (NPR-B) mediating the relaxation. Taken together, these results demonstrate that natriuretic peptides cause relaxation of human and guinea-pig gallbladder muscle through interaction with the natriuretic peptide receptor-B.

Keywords: C-type natriuretic peptide; Brain natriuretic peptide; Atrial natriuretic peptide; Endothelin; Carbachol; Motility

1. Introduction

Atrial natriuretic peptide (ANP), the first of a family of peptides with potent natriuretic, diuretic, and vasorelaxant activity, was isolated in 1982 [1]. 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. All natriuretic peptides share moderate structural homology, particularly in the 17-amino-acid disulfide loop region. While ANP and BNP have 5- and 6-amino acid residues attached to the C-terminal tail, respectively, CNP completely lacks this tail. ANP and BNP are produced primarily in the cardiac atria and ventricles, respectively. CNP is produced in the brain, the kidney and vascular endothelium. 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 [2–4]. In the cardiovascular system, ANP and BNP dilate both arteries and veins [2,5,6]. In the kidneys, ANP dilates afferent arteriolar vessels, increases glomerular filtration and has a striking relaxation effect on glomerular mesangial cells. CNP is more potent than ANP in eliciting smooth muscle relaxation but is a less potent inducer of diuresis and natriuresis [2].

Three receptors for natriuretic peptides, i.e. the natriuretic peptide receptor-A (NPR-A), the natriuretic peptide receptor-B (NPR-B) and the natriuretic peptide receptor-C (NPR-C), have been identified in mammalian tissues [2,7–10]. 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

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but a low affinity for ANP and BNP. NPR-C has a high affinity for ANP, BNP, CNP 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 [2–4,10]. 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 the kidney. NPR-C is expressed in the kidneys, adrenals, brain, lungs and vascular walls [2].

In the gastrointestinal and hepatobiliary system, natriuretic peptides have been demonstrated to cause relaxation of the lower esophageal sphincter, gastric smooth muscle, pyloric sphincter, hepatic stellate cells, colon smooth muscle and internal anal sphincter [11–17]. In 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 markedly reduces endothelin-1 induced cell contraction [13]. In dispersed guinea-pig cecal circular smooth muscle cells, CNP, BNP and ANP all potently inhibit cholecystokinin-induced contraction [14,15]. Furthermore, CNP inhibits basal tension of rabbit colon strips and phasic contraction of rat colon strips [16,17]. CNP immunoreactivity is detected in smooth muscle cells, myenteric and submucosal neurons of the guinea-pig cecum [15]. On the other hand, NPR-B and NPR-C mRNAs are detected in rabbit gastric muscle cells whereas NPR-A and NPR-B mRNAs are in detected in the rabbit colon muscle [12,16].

ANP binding sites have been demonstrated in the muscle layer of guinea-pig gallbladder [18]. However, at the present time, no data are available on effects of natriuretic peptides in the gallbladder. The aim of this study was to investigate the effects of natriuretic peptides on the human and guinea-pig gallbladder muscle and to characterize any natriuretic peptide receptors that mediate any effect.

2. Materials and methods

Male Hartley guinea-pigs (200–300 g) were obtained from the Animal Center, National Science Council, Taiwan. 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, Sunnyvale, CA; carbachol and atropine were obtained from Sigma Chemical, St. Louis, MO, USA. Tetrodotoxin was obtained from Tocris Cookson, Avonmouth Bristol, UK.

The protocol for this work was approved by the Research Review Board of the Tzu Chi General Hospital. Human specimens, 12 diseased gallbladders with the pathological diagnosis of chronic cholecystitis and one healthy, histologically normal gallbladder, were obtained from 13 patients (5 male and 8 female, aged 38–74 years) undergoing surgery for gallstones, hepatocellular carcinoma, cholangiocarcinoma or colon cancer with hepatic metastases. Informed consents were obtained. Immediately after surgical removal of the gallbladder, a 3×5 cm area was excised from the identical middle portion of each gallbladder corpus and placed in oxygenated standard incubation solution (see below) for transportation to the laboratory, where the contraction experiment was promptly initiated. The period of anoxia, after ligation of the cystic artery, was always less than 20 min [19].

2.1. Measurement of contraction and relaxation of muscle strips isolated from human and guinea-pig gallbladders

Measurements of contraction and relaxation of muscle strips from human and guinea-pig gallbladders were performed according to the procedure published previously with minor modification [19–21]. In brief, the guinea-pig was sacrificed with CO_2 and the gallbladder removed. The guinea-pig or human gallbladder, obtained as mentioned above, was 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 guinea-pig gallbladder was cut longitudinally, washed and finally cut to make four strips. The middle portion of the human gallbladder, dissected free of fat tissue and serosa, was washed and finally cut along the longitudinal axis into 1.0×0.3 cm muscle strips. Gallbladder muscle strips were attached to an organ bath using a surgical silk suture and incubated at 37 °C in the standard incubation solution continuously gassed with 95% O_2-5% CO_2. The strips were connected to an isometric transducer (Grass FT.03), which was connected to an integrated amplifier and recorder (Gould). The basal tension of the muscle strips was adjusted to 0.5 g for guinea-pig and 1.0 g for human gallbladder strips. Experiments were started after a 45-min equilibration period. Natriuretic peptides and cANP(4-23) were added to baths in a non-cumulative fashion, i.e. with single dose administration. For measurements of relaxation in contracted strips, natriuretic peptides and cANP(4-23) were added to carbachol or endothelin-1-contracted muscle strips 15 min after the addition of carbachol or endothelin-1. Carbachol or endothelin-1-induced tone before the addition of natriuretic peptides and cANP(4-23) was used as a reference to express relaxation to natriuretic peptides and cANP(4-23). All experiments with natriuretic peptides and cANP(4-23) were performed with single dose administration because preliminary experiments had shown that single dose administration of natriuretic peptides induced a stronger relaxation in the carbachol and endothelin-1-contracted guinea-pig gallbladder than cumulative administration. Only one single dose response, with or without atropine or tetrodotoxin, was studied with each preparation. For studies using atropine and tetrodotoxin, the muscle strips were exposed to the indicated concentrations of these