Inhibitory effect of C-type natriuretic peptide on spontaneous contraction in gastric antral circular smooth muscle of rat[1]

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ABSTRACT

AIM: To investigate the effect of natriuretic peptides on gastric motility and its possible mechanism. **METHODS:** Spontaneous contraction of gastric antral circular muscle of rats was recorded by four channel physiograph. The concentration of cyclic guanosine monophosphate (cGMP) was measured by radioimmunoassay. The distribution of natriuretic peptide receptors (NPR) was analyzed by autoradiograph. **RESULTS:** NPR existed in different regions of rat stomach and its density was the largest in gastric antrum. Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) all inhibited the spontaneous contraction of gastric antral circular smooth muscle. Among them, the inhibitory effect of CNP on the spontaneous contraction was the most potent and exhibited a dose-dependent manner. CNP-induced inhibition was diminished by LY83583 (a kind of inhibitor of guanylate cyclase) and potentiated by zaprinist (a kind of inhibitor of cGMP sensitive phosphoesterase). CNP markedly enhanced the concentration of cGMP in antral smooth muscle. The inhibitory effect of CNP on spontaneous contraction was also inhibited by tetraethylammonium (a nonselective potassium channel blocker, TEA). **CONCLUSION:** The distribution density of NPR is the most in gastric antrum. CNP significantly inhibits gastric motility in rat gastric antrum. The inhibitory effect occurs via a cGMP dependent pathway and potassium channel is also involved in the relaxation induced by CNP in gastric antrial circular smooth muscle of rat.

INTRODUCTION

Since antrial natriuretic peptide (ANP) was isolated from atrium by de Bold *et al* in 1981, Brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), dendroaspis (DNP), micrurus natriuretic peptide (MNP), and ventricular natriuretic peptide (VNP) were found in succession. Up to now, natriuretic peptides (NP) distributed in all over the body besides heart and exerted natriuretic-diuretic, vasorelaxation, and other functions designed to lower blood pressure and to control electrolyte homeostasis and so on. Recently, natriuretic peptide receptors(NPR) were found in gastrointestinal tract[1] and played many roles in regulating gastrointestinal functions. In gastrointestinal tract, the study about physiologic functions of NP mainly focused on absorption, secretion, and intestinal motility[2-4]. Previous studies indicated NP exerted many physiological function by activating cGMP system[5-7]. However, Carvajal *et al*[8] showed that NP-induced relaxation of

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myometrium in the pregnant guinea pig was not mediated by cGMP, instead, it was mediated by a novel mechanism, indicating that NP regulated physiological functions not only through cGMP pathway. Nowadays, the series studies of NP in regulating gastric smooth muscles motility have not been reported.

In the present study, the distributions of NPR in gastric different regions were observed and the effect of CNP on spontaneous contraction was investigated in gastric antral circular smooth muscle of rat, and the relationship between the effect of CNP on spontaneous contraction and cGMP was analyzed in gastric antral circular smooth muscle of rat.

MATERIALS AND METHODS

Strip preparation SD rats of either sex, bred by Experimental Animal Center of Yanbian University College of Medicine, weighing 300±50 g, were euthanized by lethal dose of pentobarbital sodium (50 mg/kg, iv). The abdomen of each rat was opened along the midline and stomach was removed and placed in pre-oxygenated Tyrode's solution at room temperature. The mucous layer was removed and strips (about 2.0×15.0 mm) of gastric antral circular muscle were prepared. The longer axis of the stomach was cut parallel to the circular muscle fibres. Muscle strips were placed in a chamber. One end of the strip was fixed on lid of the chamber through glass claw, the other end was attached to an isometric force transducer (TD-112S, JAPAN) to record contraction. The chamber (2 mL volume) was constantly perfused with pre-oxygenated Tyrode's solution at 1 mL/min. Temperature was maintained at 37.0±0.5 ºC by a water bath thermostat (WC/09-05, Chongqing, China). The muscle strip was allowed to incubate for at least 40 min before experiments were started.

Autoradiograph of NPR Method was described elsewhere[9].

Radioimmunoassay of cGMP Production of cGMP was measured by a specific radioimmunoassay as described previously[10].

Drugs Tyrode's solution containing (mmol/L) NaCl 147, KCl 4, $MgCl_2 \cdot 6H_2O$ 1.05, $CaCl_2 \cdot 2H_2O$ 0.42, $Na_2PO_4 \cdot 2H_2O$ 1.81, and glucose 5.5 was used. The pH was adjusted to 7.35 by using NaOH. LY83583 and Zaprinat were obtained from Research Biochemicals International (USA). Other reagents were purchased from Sigma (USA).

Statistics Data were expressed as Mean±SD. Statistical significance was evaluated by *t*-test. Differences were considered significant when *P* value was less than 0.05.

RESULTS

Distribution of natriuretic peptide receptor in rat stomach wall Using radioautograph technique, the distribution of NPR in different regions of rat stomach was detected. NPR existed both in mucosal layer and muscle layer, and the distribution order of NPR in density was antrum>body>fundus in muscle layer (Fig 1, *n*=6).

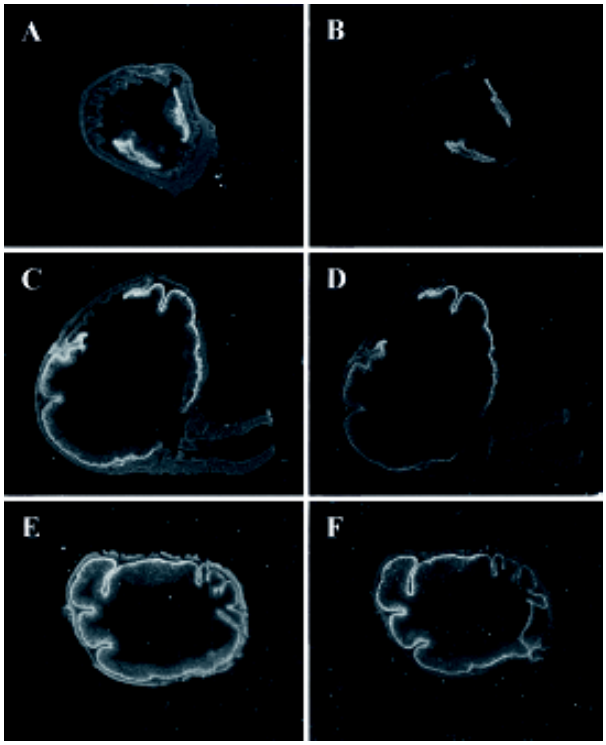


Fig 1. The autoradiograph of NPR in different regions of stomach in rats. A: gastric fundus(total binding); B: gastric fundus(non-specific binding); C: gastric body(total binding); D: gastric body(non-specific binding); E: gastric antrum (total binding); F: gastric antrum(non-specific binding).

Effect of NP on spontaneous contraction in antral circular smooth muscle The spontaneous contraction usually appeared about 40 min after incubating the muscle strips in Tyrode's solution. The effects of 0.1 μmol/L ANP, BNP, and CNP on spontaneous contraction in gastric antral circular smooth muscle of rat were observed. Three kinds of NP all significantly inhibited spontaneous contraction and the inhibition per-

centage of ANP, BNP, and CNP was 14 %±2 %, 26 %± 5 %, and 73 %±13 %, respectively (Fig 2, n=6, P<0.01 *vs* ANP group and P<0.01*vs* BNP group). Based on the above results, the effect of CNP was most potent among them, therefore CNP was selected to observe the effects of NP on gastric motility. Different concentrations of CNP obviously inhibited spontaneous contraction in a concentration-dependent manner and the inhibition percentage was 23 %±7 %, 45 %±8 %, 69 %± 13 %, and 98 %±5 % at 0.01, 0.03, 0.1, and 1 μmol/L, respectively (Fig 3, n=7). High concentration (1 μmol/L) of CNP completely inhibited spontaneous contraction and shifted down the base line.

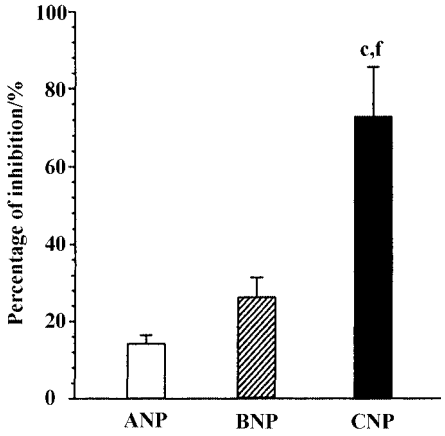


Fig 2. Effect of ANP, BNP, and CNP 0.1 μmol/L on spontaneous contraction of gastric circular muscle in rats. n=6. Mean±SD. ^{c}P<0.01 *vs* ANP group. ^{f}P<0.01 *vs* BNP group.

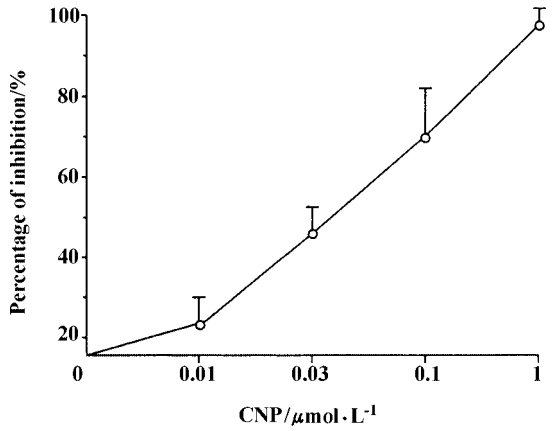


Fig 3. Concentration response of CNP on spontaneous contraction of gastric circular muscle in rats. n=7. Mean±SD.

Effect of LY83583 and zaparinast on the CNP-induced inhibition To further analyze the mechanism by which CNP inhibited the gastric motility in rat, the effect of CNP 0.1 μmol/L on gastric motility was observed in the condition of administering LY83583, a kind of inhibitor of guanylate cyclase, and zaparinast as a phosphoesterase inhibitor to change production of cGMP. LY83583 (0.1 μmol/L) markedly diminished the inhibitory effect of CNP on spontaneous contraction, but could not completely abolish the inhibitory effect (Fig 4, n=8, P<0.01). Zaparinast (1 μmol/L) potentiated the inhibitory effect of CNP on spontaneous contraction (Fig 5, n=8, P<0.01).

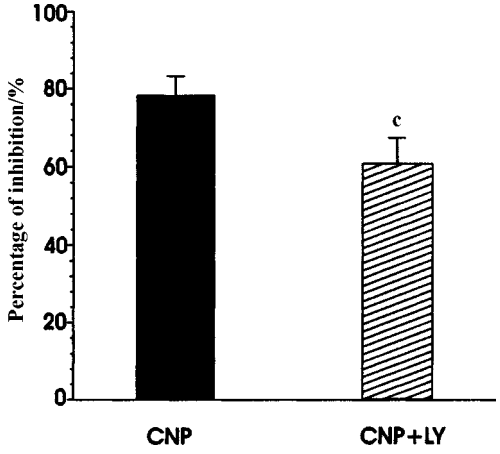


Fig 4. Effect of LY83583 0.1 μmol/L on CNP 0.1 μmol/L-induced inhibition. n=8. Mean±SD. ^{c}P<0.01 *vs* CNP group.

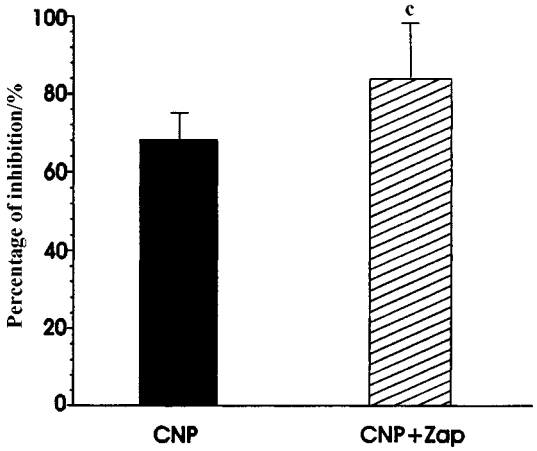


Fig 5. Effect of zaparinast 1 μmol/L on CNP 0.1 μmol/L-induced inhibition. n=8. Mean±SD. ^{c}P<0.01 *vs* CNP group.

Effect of CNP on cGMP production To confirm whether cGMP was involved in the CNP-induced inhibition, the content of cGMP in the smooth muscle tissue and in perfusion solution was measured by radioimmunoassay in control group and CNP group (0.1 μmol/L). The result indicated that cGMP in the smooth muscle and perfusion solution was markedly increased

after addition of CNP 0.1 μmol/L (Fig 6, 7, n=8, P<0.01 *vs* control group). When LY83583 0.1 μmol/L was pretreated, the content of cGMP induced by CNP 0.1 μmol/L was significantly diminished (Fig 6, 7, n=8, P<0.01 *vs* LY group; P<0.01 *vs* CNP group).

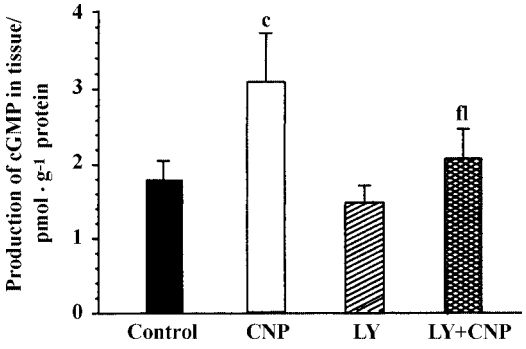
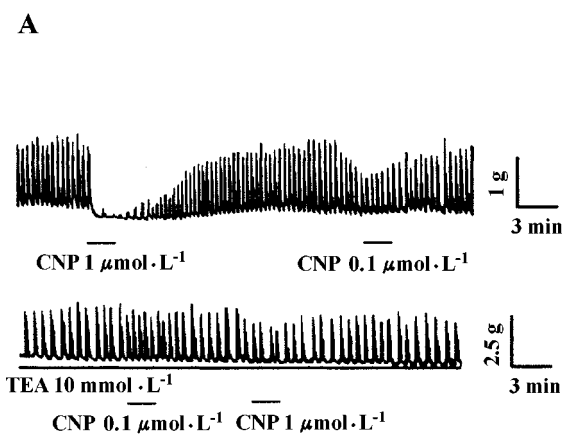


Fig 6. Effect of CNP 0.1 μmol/L on cGMP production in tissue. n=8. Mean±SD. [c]P<0.01 *vs* control group. [f]P<0.01 *vs* LY group. [i]P<0.01 *vs* CNP group.

Effect of CNP on spontaneous contraction in the presence of TEA It is well known that NO and NP are cGMP generation system in the body. Our previous study demonstrated that sodium nitroprusside, a nitric oxide donor, inhibited spontaneous contraction of gastric smooth muscle via increasing calcium activated potassium current[11,12]. So in the study presented here, the effect of TEA, a nonselective potassium channel blocker, on CNP-induced inhibitory effect on gastic motility was observed. After muscle strips were pretreated with TEA (10 mmol/L), the spontaneous contraction was markedly potentiated (Fig 8A). When the contraction reached to steady state, CNP was added and the inhibitory effect of CNP on spontaneous contraction was significantly diminished (Fig 8B, n=7, P<0.01 *vs* CNP 1μmol/L group; P<0.01 *vs* CNP 0.1 μmol/L group).



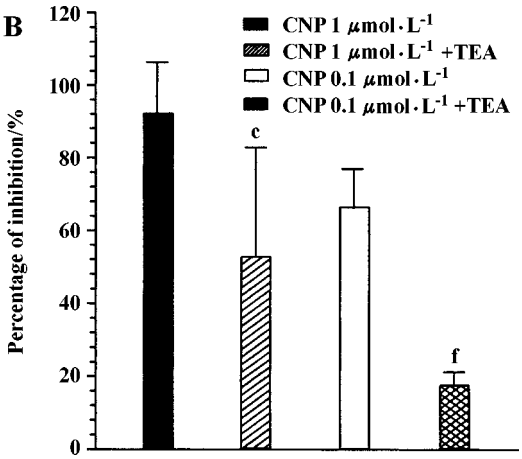


Fig 8. Effect of TEA 10 mmol/L on CNP-induced inhibition. n=7. Mean±SD. [c]P<0.01 *vs* CNP 1 μmol/L group. [f]P<0.01 *vs* CNP 0.1 μmol/L group.

DISCUSSION

Our study clearly showed that natriuretic peptide receptors (NPR) existed in the stomach of rats and its density was the largest in antrum. Natriuretic peptides (NP) inhibited the motility of gastric antral smooth muscle and CNP-induced inhibition was the most

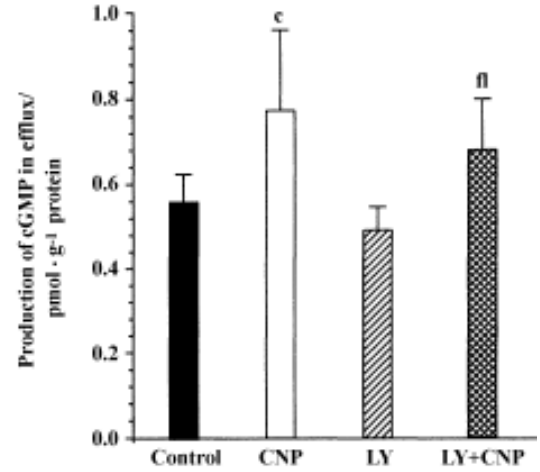


Fig 7. Effect of CNP 0.1μmol/L on cGMP production in efflux. n=8. Mean±SD. [c]P<0.01 *vs* Control group. [f]P<0.01 *vs* LY group. [i]P<0.01 *vs* CNP group.

obvious. CNP-induced inhibitory effect was diminished by LY83583, a kind of inhibitor of guanylate cyclase, and potentiated by zaparinast, a phosphoesterase inhibitor. The production of cGMP in the smooth muscle tissue was significantly enhanced by CNP. However, TEA, a nonselective potassium channel blocker, also suppressed the CNP-induced inhibitory effect.

NPR extensively distributed in many tissues, for example, in the bladder of the toad, in bullfrog brain, in rabbit oviduct, in fetal bovine pulmonary vascular and in porcine coronary artery. In the present study, we confirmed that NPR also existed in the stomach and the density was largest in gastric antrum in rat. As for smooth muscle, many experiments showed that NP relaxed smooth muscle, such as in swine arterial smooth muscle, in vascular smooth muscle of mouse, in bovine tracheal smooth muscle. In the present study, NP also significantly inhibited spontaneous contraction of gastric antral circular smooth muscles.

NP is similar to NO, which is a cGMP generation system in living body and their physiological functions are very important in life science. In regulating the functions of smooth muscle, NP revealed inhibitory effect on the motility via cGMP pathway, for example, CNP mediated coronary vasodilation in dog[13] and tracheal smooth muscle relaxation in guinea-pig[14]. The key point of this study was to determine the relationship between CNP-induced inhibition and cGMP in gastric antral circular smooth muscle. In the present study, CNP-induced inhibition was diminished by LY83583, a kind of inhibitor of guanylate cyclase and potentiated by zaparinast, a phosphoesterase inhibitor, and the production of cGMP was enhanced by CNP. It indicated that CNP-induced inhibitory effect on spontaneous contraction of gastric antral smooth muscle was mediated by cGMP in rat.

As a second messenger, cGMP regulates physiological function by the following pathways: 1) Modulating ion channel; 2) Adjusting activity of phosphodiesterase (PDE); 3) Activating cGMP-dependent protein kinase (GPK); 4) Causing cross reaction with cAMP. Our previous study indicated that NO-induced inhibition of gastric motility in antral circular smooth muscle of guinea-pig was mediated by activating calcium-sensitive potassium channel[12,13] via cGMP pathway. In the study, TEA, a nonselective potassium channel blocker, significantly suppressed CNP-induced inhibitory effect on spontaneous contraction of gastric smooth muscle in rat. It is presumed that the effect of CNP-induced inhibition maybe enhance cGMP generation to activate K^+ channel and hyperpolarize membrane potential. In next study, it is needed to further determine whether CNP potentiates potassium channel activity.

In summary, our study further confirmed that NP system existed in stomach of rat and the density of NPR was the highest in antrum. NP played an inhibitory role in gastric motility of circular smooth muscle and among them, the effect of CNP was the most potent in rat. CNP-induced inhibition was related to enhancing cGMP production and potassium channel was involved in this process.

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