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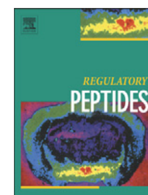

Excitatory regulation of angiotensin II on gastric motility and its mechanism in guinea pig

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ABSTRACT

In the present study, we investigated the effect of Ang II on gastric smooth muscle motility and its mechanism using intracellular recording and whole-cell patch clamp techniques. Ang II dose-dependently increased the tonic contraction and the frequency of spontaneous contraction in the gastric antral circular smooth muscles of guinea pig. ZD7155, an Ang II type 1 receptor (AT_1R) blocker, completely blocked the effect of Ang II on the spontaneous contraction of gastric smooth muscle. In contrast, TTX, a sodium channel blocker, failed to block the effect. Furthermore, nicardipine, a voltage-gated Ca^{2+}-channel antagonist, did not block the effect of Ang II on the tonic contraction of gastric smooth muscle, but external free-calcium almost completely blocked this effect. Both ryanodine, an inhibitor of calcium-induced Ca^{2+} release (CICR) from ryanodine-sensitive calcium stores, and thapsigargin, which depletes calcium in calcium stores, almost completely blocked the effect of Ang II on tonic contraction. However, 2-APB, an inositol trisphosphate (IP_3) receptor blocker, significantly, but not completely, blocked the Ang II effect on tonic contraction. We also determined that Ang II depolarized membrane potential and increased slow wave frequency in a dose-dependent manner. It also inhibited delayed rectifying potassium currents in a dose-dependent manner, but did not affect L-type calcium currents or calcium-activated potassium currents. These results suggest that Ang II plays an excitatory regulation in gastric motility via AT_1R-IP_3 and the CICR signaling pathway. The Ang II-induced inhibition of delayed rectifying potassium currents that depolarize membrane potential is also involved in the potentiation of tonic contraction and the frequency of spontaneous contraction in the gastric smooth muscle of guinea pig.
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1. Introduction

Angiotensin (Ang) II, a main effector peptide in the renin-Ang system (RAS), is known to elicit a wide range of diverse cellular responses, including growth, proliferation, and vascular smooth muscle contraction [1]. Ang II also plays a fundamental role as a vasoconstrictor in controlling cardiovascular function and renal homeostasis [1]. Our understanding of the RAS has remarkably changed over the past two decades. In particular, new functional components [e.g., Ang-(1−7), Ang IV, Ang-(1−12)] and pathways [e.g., Ang converting enzyme (ACE)2] of the system have been described [2–5]. It has also become clear that all bioactive Ang peptides can be generated not only in systemic circulation, but also in several tissues and organs. Thus, local RASs, as well as various biological functions of multiple Ang peptides acting via an autocrine and/or paracrine manner, have been described in the kidney, heart, liver, pancreas, adrenal gland, brain, ovaries, and testes [6–9].

There is relatively scarce information about the formation and actions of Ang peptides in the gastrointestinal tract, especially in the stomach wall. Previous studies have demonstrated that ACE mRNA and protein, and more recently ACE2, are detected in mucous and chief cells and in endothelial cells of the mucosal vasculature [10–12]. There are also several indirect pieces of evidence, originating from using ACE inhibitors and Ang receptor blockers in previous studies, that Ang II may regulate mucosal blood flow and play a role in mucosal damage [13–17]. Early study indicated that AT_1R expressed in guinea pig gastric smooth muscle and Ang II can cause gastric smooth muscle contraction via quite distinct post-receptor mechanisms that may involve, at least in part, a crucial tyrosine kinase signal transduction pathway in guinea pig [18]. Another study demonstrated that Ang II-induced contraction of the lower esophageal sphincter and internal anal sphincter smooth muscle cells of rat was inhibited by genistein, PD-98059 [a specific inhibitor of MAPKs (MEK 1/2)], herbimycin A (a pp60c-src inhibitor), and antibodies to pp60c-src and p120 ras GTPase-activating protein (p120 rasGAP). Furthermore, the Ang II-induced contraction of the tonic smooth muscles was accompanied by an increase in the tyrosine phosphorylation of p120 rasGAP. This result was attenuated by genistein, but not by PD-98059. In addition,

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Ang II-induced increase in the phosphorylation of p44/42 MAPKs and caldesmon was attenuated by both genistein and PD-98059 [19]. These data suggest that pp60c-src and p44/42 MAPKs play an important role in the Ang II-induced contraction of lower esophageal sphincter and internal anal sphincter smooth muscles. However, although Ang II is a potent endogenous activator of vascular smooth muscle contraction, its actions on the gastrointestinal wall musculature have not been thoroughly investigated. There are only a few reports about the regulation of Ang II on motility in gastrointestinal smooth muscle.

Since a number of evidences have demonstrated that Ang is generated from gastric mucosa [20] and AT_1R is expressed in guinea pig gastric smooth muscle [18], Ang II may play an important role in the regulation of gastric smooth muscle motility. The purpose of this investigation was to identify critical events in intracellular calcium signal transduction, changes in membrane potential, and an ion channel mechanism in the Ang II-induced contraction of gastric smooth muscle of guinea pig.

2. Materials and methods

2.1. Preparation of muscle strips and isometric tension measurement

EWG/B guinea pigs (provided by the Experimental Animal Center of Chinese Academy of Sciences in Shanghai) of either sex weighing 300 ± 50 g were euthanized by a lethal dose of intravenous pentobarbital sodium (50 mg/kg). The whole stomachs of guinea pigs were excised and placed in a pre-oxygenated phosphate-buffered Tyrode's solution at room temperature. Corporal regions were obtained and cut in the longitudinal direction along the lesser curvature. After the contents of each stomach were removed, patches of the muscle coat were obtained by removing the mucosal layer in Tyrode's solution. Muscle strips (approximately 2 mm × 10 mm) of gastric antral and body circular smooth muscle were prepared by cutting along the vertical direction of the longer axis of each stomach. All experimental protocols included in this manuscript were approved by our local animal care committee and conform to the Guide for the Care and Use of Laboratory Animals published by the Science and Technology Commission of the P. R. China (STCC Publication No. 2, revised 1988).

Isometric contraction measurements of muscles were recorded using a vertical chamber with a capacity of 2.5 mL, which was designed to perfuse continuously. To record the contraction of muscle strips, one end of each strip was fixed in the lid of the chamber with a glass claw and the other end was attached to an isometric force transducer (RM6240C, Chengdu, China). The chamber was constantly perfused with CO_2/bicarbonate-buffered Tyrode's solution at 1 mL/min. Temperature was maintained at 37.0 ± 0.5 °C by a water bath thermostat (WC/09-05, Chongqing, China).

2.2. Cell preparation

Gastric myocytes were isolated using collagenase as described previously [21]. Each stomach was rapidly cut and the mucosal layer was separated from the muscle layers in Ca^{2+}-free physiological salt solution (Ca^{2+}-free PSS). The circular muscle layer was dissected from the longitudinal layer using fine scissors and cut into small segments (2 mm × 3 mm). These segments were incubated in medium that was somewhat modified (recipe in Section 2.5) from Kraft-Bruhe (K-B) medium at 4 °C for 30 min. Samples were then incubated in Ca^{2+}-free PSS digestion medium (recipe in Section 2.5) containing 0.1% collagenase II, 0.1% dithioerythritol, 0.1% trypsin inhibitor, and 0.2% bovine serum albumin at 36 °C for 20−30 min. After digestion, the supernatant was discarded and the softened muscle segments were again transferred into modified K-B medium, and single cells were dispersed by gentle trituration with a wide-bore, fire-polished, glass pipette. Isolated gastric myocytes were kept in modified K-B medium at 4 °C until use.

2.3. Intracellular recording

Excised stomachs were opened by cutting along their small curvatures in CO_2/bicarbonate-buffered Tyrode's solution. The mucosal layer of each was removed by cutting with fine scissors and smooth muscle tissues were isolated from the antral region. Under a dissecting microscope, the circular muscle layer was carefully removed and a segment (~1.5 mm wide × 3 mm long) of smooth muscle was prepared. Tissue was pinned down on a silicon rubber plate and fixed at the bottom of an organ bath (8 mm wide × 8 mm deep × 20 mm long), with the circular muscle border uppermost. Tissue was superfused with warmed (37 °C) and oxygenated CO_2/bicarbonate-buffered Tyrode's solution, at a constant flow rate of about 2 mL/min. Experiments were typically carried out in the presence of 1 μM nifedipine, so as to minimize muscle movement. However, some experiments were carried out in the absence of nifedipine. A conventional microelectrode technique was used to record the intracellular electrical responses of smooth muscle cells. Briefly, glass capillary microelectrodes (1.2 mm outer diameter and 0.6 mm inner diameter; Hilgenberg, Germany) filled with 0.5 M KCl, with tip resistances ranging between ~50 and 80 MΩ, were inserted into cells. Electrical responses were recorded and amplified through a high input impedance amplifier (SYS-773 Duo 773 Electrometer, WPI, Sarasota, FL, USA).

2.4. Whole-cell patch clamp experiment

Isolated cells were transferred to a 0.1 mL chamber on the stage of an inverted microscope (IX-70 Olympus, Tokyo, Japan) and allowed to settle for 10−15 min. Cells were then continuously perfused with a physiologic salt solution at a rate of 0.9−1.0 mL/min. An 8-channel perfusion system (L/M-sps-8, List Electronics, Darmstadt, Germany) was used to exchange the solution. Patch-clamp pipettes were manufactured from borosilicate glass capillaries (GC 150 T-7.5, Clark Electromedical Instruments, London, UK) using a two-stage puller (PP-83, Narishige, Tokyo, Japan). The resistance of the patch pipettes was 3–5 MΩ when filled with pipette solution. Whole-cell currents were recorded with an EPC-10-HEAKA amplifier (Heaka Instrument, Berlin, Germany). All experiments were performed at room temperature (20–25 °C).

2.5. Solutions and drugs

Phosphate-buffered Tyrode's solution contained NaCl (145 mM), KCl (5.4 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1.5 mM), NaH_2PO_4 (0.42 mM), Na_2HPO_4 (1.81 mM), and glucose (5.5 mM), and had a pH of 7.4. CO_2/bicarbonate-buffered Tyrode's solution contained NaCl (116 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1.5 mM), $NaHCO_3$ (24 mM), and glucose (5 mM). This solution had a pH of 7.3−7.4 and was bubbled with 5% CO_2 and 95% O_2. Ca^{2+}-free PSS contained NaCl (135 mM), KCl (5 mM), $MgCl_2$ (1 mM), glucose (5 mM), and HEPES (N-[2-hydroxyethyl] piperazine-N-[2-ethanesulphonic acid]) (10 mM). The pH of this solution was adjusted to pH 7.4 with Tris [hydroxymethyl] aminomethane (TRIZMA). Modified K-B solution contained l-glutamate (50 mM), KCl (50 mM), taurine (20 mM), $MgCl_2$ (3 mM), KH_2PO_4 (20 mM), glucose (10 mM), HEPES (10 mM), and EGTA (ethyleneglycol-bis(β-aminoethyl ether)-N, N, N , N -tetraacetic acid) (0.5 mM) and was adjusted to a pH of 7.4 with KOH. PSS medium contained NaCl (135 mM), KCl (5 mM), $MgCl_2$ (1 mM), glucose (5 mM), and HEPES (10 mM) and the pH was adjusted to pH 7.4 with Tris.

Ang II and ryanodine were purchased from Tocris Bioscience (Ellisville, MO, USA). Thapsigargin, 2-Aminoethoxydiphenyl borane (2-APB), and ryanodine were dissolved in dimethyl sulfoxide to

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