Effects of endothelin-1 on the membrane potential and slow waves in circular smooth muscle of rat gastric antrum

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Abstract

Electrophysiological effects of endothelin-1 (ET-1) on circular smooth muscle of rat gastric antrum were investigated by using intracellular membrane potential recording techniques. ET-1 (10 nM) caused an initial hyperpolarization of the membrane which was followed by a sustained depolarization. ET-1 also increased the frequency but not the amplitude of slow waves. In the presence of the endothelin type A (ET_A) receptor antagonist, BQ123 (1 μ M), ET-1 (10 nM) depolarized the membrane and increased the frequency of slow waves, but without the initial hyperpolarization. The selective endothelin type B (ET_B) receptor agonist, sarafotoxin S6c (10 nM), also depolarized the membrane and increased the frequency of slow waves. In the presence of the ET_B receptor antagonist, BQ788 (1 μ M), ET-1 (10 nM) hyperpolarized the membrane. However, in the presence of BQ788, ET-1 caused neither the depolarization nor the increase in the frequency of the slow waves. The ET-1-induced hyperpolarization was completely abolished by apamin (0.1 μ M). In the presence of apamin, ET-1 depolarized the membrane and increased the frequency of slow waves. The ET-1-induced depolarization was significantly attenuated by 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS, 0.3 mM). The increase of the frequency by ET-1 was observed both in the presence and absence of DIDS. These results suggest that, ET-1 hyperpolarizes the membrane by the activation of Ca²⁺-activated K⁺ channels via ET_A receptors, and depolarizes the membrane by the activation of Ca²⁺-activated Cl⁻ channels via ET_B receptors. ET-1 also appears to increase the frequency of slow waves via ET_B receptors, however this mechanism would seem to be independent of membrane depolarization.

Key words: gastric smooth muscle, endothelin-1 receptor, slow wave, membrane potential, Ca²⁺-activated K⁺ channel

Introduction

Spontaneous rhythmical depolarizing changes in membrane potential, termed slow waves, are observed in smooth muscle cells of many regions of the gastrointestinal tract (Tomita, 1981). The mechanism of the generation of slow waves has been intensively investigated for decades. Recent studies have revealed that the pacemaker cells, known as interstitial cells of Cajal (ICC), are responsible for the generation of slow waves (Ward *et al.*, 1994; Huizinga *et al.*, 1995; Sanders, 1996; Dickens *et al.*, 1999; Kito and Suzuki, 2003; Takaki, 2003). At least two populations of ICC lie in the wall of the gastrointestinal tract, a population found in the myenteric plexus (ICC-MY) and a second population of ICC distributed through the muscle layer (intramuscular ICC, ICC-IM) (Komuro *et al.*, 1996; Burns *et al.*, 1997). The correlation of the distribution of ICC-MY and the generative region for slow waves has suggested that in the stomach, ICC-MY are more dominant for the initiation of slow waves than ICC-IM (Burns *et al.*, 1997). From studies using genetically modified mice, the inositol trisphosphate (IP₃) pathway is thought to be essential for the generation of slow waves (Suzuki *et al.*, 2000).

Endothelin-1 (ET-1) is originally reported as a potent endogenous vasoconstrictor composed of 21-amino acids (Yanagisawa *et al.*, 1988; Inoue *et al.*, 1989). The functional effects of ET-1 are mediated through at least two distinct subtypes of receptors, the endothelin type A (ET_A) and endothelin type B (ET_B) receptors (Arai *et al.*, 1990; Sakurai *et al.*, 1990). It is generally accepted that in smooth muscle cells, ET-1 increases intracellular free Ca²⁺ by triggering Ca²⁺ influx and Ca²⁺ release from intracellular stores (Rubanyi and Polokoff, 1994). The following studies have reported that ET-1 has diverse effects on vascular and non-vascular smooth muscle. Some studies indicate that ET-1 produces a transient vasodilation through the release of nitric oxide (NO) from endothelial cells (De Nucci *et al.*, 1988; Higashi *et al.*, 1997) or by the activation of adenosine triphosphate (ATP)-sensitive K⁺ (K_{ATP}) channels (Hasunuma *et al.*, 1990; Eddahibi *et al.*, 1993). In several regions of the gastrointestinal tract, ET-1 has biphasic effects on smooth muscle, namely an initial relaxation followed by a sustained contraction (Lin and Lee, 1990; Allcock *et al.*, 1995; Chakder and Rattan, 1999). Activation of Ca²⁺-activated K⁺ (K_{Ca}) channels has been shown to be responsible for the generation of the initial relaxation (Lin and Lee, 1992; Imaeda *et al.*, 2002).

Although numerous studies have investigated the effects of ET-1 on the relaxation and contraction of gastrointestinal smooth muscle, little is known about the effects of ET-1 on gastric spontaneous activity. Since ET-1 acts through the G-protein-IP₃ signaling pathway, ET-1 may have a close relationship with gastric spontaneous electric activity. In the present study, we

Abbreviations

ATP, adenosine triphosphate; BK_{Ca} channel, large conductance Ca²⁺-activated K⁺ channel; Cl_{Ca} channel, Ca²⁺-activated Cl⁻ channel; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid; DMSO, dimethyl sulfoxide; ET, endothelin; ET-1, endothelin-1; ET_A receptor, endothelin type A receptor; ET_B receptor, endothelin type B receptor; IP₃, inositol 1,4,5-trisphosphate; ICC, interstitial cells of Cajal; ICC-MY, interstitial cells of Cajal in myenteric plexus; ICC-IM, intramuscular interstitial cells of Cajal; K_{ATP} channel, ATP-sensitive K⁺ channel; K_{Ca} channel, Ca²⁺-activated K⁺ channel; NO, nitric oxide; SK_{Ca} channel, small conductance Ca²⁺-activated K⁺ channel; TTX, tetrodotoxin

have electrophysiologically investigated the effects of ET-1 on the circular smooth muscle of the rat gastric antrum. The results indicated that ET-1 affected the frequency and membrane potential through the different types of ET receptors and ion channels.

Methods

Tissue preparation

Male rats, weighing 250–400 g, were anaesthetized with Cevofren and then cervically dislocated, a procedure approved by the Animal Ethics Committee of the Nagoya City University Graduate School of Medical Sciences. The stomach was quickly excised and placed in Krebs solution at room temperature. The stomach was cut open in the longitudinal direction along the lesser curvature and pinned flat with the mucosal side up. The mucosa was gently removed with micro-scissors. Full thickness strips (1–2 mm wide and 8–10 mm long) were cut from the area of the antrum, just to the oral side of the thickened prepyloric area. A strip usually included a few bundles of smooth muscle cells.

Intracellular recording

Preparations were pinned onto the transparent silicon rubber in the base of a small recording chamber (volume, approximately 2 mL). The chamber was mounted on an inverted compound microscope (TMD-100, Nikon, Tokyo, Japan), and the preparation continuously superfused with warmed (37°C) Krebs solution bubbled with 95% O₂–5% CO₂ to pH 7.4 at a rate of 2 mL min⁻¹. The ionic composition of the Krebs solution was (mM): NaCl, 118.4; NaHCO₃, 25.0; NaH₂PO₄, 1.13; CaCl₂, 2.4; KCl, 4.7; MgCl₂, 1.3; glucose, 11.1. Conventional intracellular recording techniques were used to measure changes in the membrane potential of circular smooth muscle cells. Changes in membrane potential were recorded using glass micro-electrodes (outer diameter 1.5 mm, filled with 0.5 M KCl, resistance 150–250 MΩ) connected to the high input impedance headstage of an Axoclamp-2B (Axon Instruments, CA, U.S.A.) and displayed on a cathode-ray oscilloscope (SS-9622, Iwatsu, Tokyo, Japan). After low-pass filtering (cut off frequency, 10 Hz), potential changes were digitized and stored with a Digidata 1200A data acquisition system (Axon Instruments, CA, U.S.A.) for subsequent analysis. The criteria for an acceptable impalement of a smooth muscle cell were a rapid change in potential upon impalement and withdrawal, and a stable recording for at least 3 minutes prior to each experimental procedure.

Drugs

Drugs used were apamin, 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS), endothelin-1 (ET-1), nifedipine, sarafotoxin S6c, tetrodotoxin (TTX) (all from Sigma), BQ123, BQ788 (RBI). Individual drugs were dissolved in distilled water or dimethyl sulfoxide (DMSO; Sigma), at concentrations at least 1,000 times higher than used in the experiments and serially diluted in Krebs solution to the required final bath concentration.

Statistical analysis

Measured values were expressed as the mean $\pm$ s.e.mean, unless stated otherwise. The *n* value refers to the number of preparations. Statistical significance was determined using a paired or unpaired Student's *t* test. Probabilities of less than 5% ($P < 0.05$) were considered to be significant.

Results

Electrophysiological properties of circular smooth muscle cells in the rat gastric antrum

At rest, circular smooth muscle cells in the rat gastric antrum maintained a resting membrane potential of between -47 and -62 mV (mean $\pm$ s.d.: -57.3 ± 3.3 mV, $n=42$) and showed spontaneous oscillatory depolarizing changes (slow waves). Spike potentials were often observed on the top of these slow waves. Frequencies of the slow waves ranged between 1.6 and 3.0 min $^{-1}$ and averaged 2.3 ± 0.6 min $^{-1}$ (mean $\pm$ S.D., $n=5$). These electrical activities were not altered by the application of TTX (0.1 μ M), indicating that they were not neurogenic. The spike potentials on top of the slow waves were nifedipine sensitive (1 μ M; $n=5$), and the amplitude of the body of the slow waves was decreased (control, 22.8 ± 1.2 mV; nifedipine, 18.4 ± 1.0 mV, $n=5$, $P < 0.05$). The frequency of the slow waves also decreased from 2.3 ± 0.3 min $^{-1}$ ($n=5$) to 1.8 ± 0.3 min $^{-1}$ ($n=5$, $P < 0.05$).

Effects of ET-1 on smooth muscle cells of the rat antrum

ET-1 is a potent constrictor of gastrointestinal smooth muscle as a result of the influx of Ca^{2+} via voltage-dependent Ca^{2+} channels (Lin and Lee, 1990; Imaeda *et al.*, 2002). Intracellular recordings were performed in the presence of nifedipine (1 μ M) to stabilize the recordings made in the presence of ET-1. ET-1 (10 nM) initially induced a transient small hyperpolarization, followed by a sustained depolarization (Fig. 1). ET-1 also increased the frequency of slow waves (control, 1.6 ± 0.2 min $^{-1}$; ET-1, 4.0 ± 0.5 min $^{-1}$, $n=6$, $P < 0.05$, Fig. 1A). The effects of ET-1 continued after the cessation of ET-1 application, with up to an hour of washing required for the recovery of the frequency of the slow waves and the membrane potential to resting levels (Fig. 1A, B).

Effects of ET receptor antagonists on the ET-1-induced membrane potential changes

In the presence of BQ123 (1 μ M), a selective antagonist of ET_A receptors, the agonistic actions of ET-1 were observed on the ET_B receptors. BQ123 (1 μ M) did not alter the resting membrane potential (control, -62.0 ± 0.7 mV; BQ123, -61.4 ± 0.8 mV, $n=5$) nor the frequency of slow waves (control 2.3 ± 0.2 waves min $^{-1}$; BQ123, 2.4 ± 0.2 waves min $^{-1}$, $n=5$). In the presence of BQ123 (1 μ M), ET-1 (10 nM) induced a sustained depolarization (control, -63.0 ± 0.7 mV; ET-1, -56.8 ± 1.7 mV, $n=5$, $P < 0.05$) and increased the frequency of slow waves (control, 2.2 ± 0.2 waves min $^{-1}$; ET-1, 4.6 ± 0.6 waves min $^{-1}$, $n=4$, $P < 0.05$), with no associated initial hyperpolarization (Fig. 2A). Sarafotoxin S6c (S6c, 10 nM), a selective agonist of ET_B receptors, also induced a sustained depolarization (control, -62.0 ± 0.5 mV; S6c, -57.6 ± 0.4 mV, $n=5$, $P < 0.05$) and increased the frequency of slow waves (control, 2.0 ± 0.3 min $^{-1}$; S6c, 5.2 ± 0.3 min $^{-1}$, $n=5$, $P < 0.05$, Fig. 2B).

Fig. 1. Effects of ET-1 on slow waves in the circular smooth muscle of the rat gastric antrum. The upper trace (A) is representative of the effect of ET-1 (10 nM) on slow waves recorded from the circular smooth muscle of the rat gastric antrum. Nifedipine (1 μ M) was present throughout the recording. The traces in the lower panel (a, b, c and d) show parts of the upper trace (A) with a different time base. 'd' was observed 60 min after washout of ET-1. The resting membrane potential was -63 mV.

Fig. 2. Effects of ET-1 on ET_B receptors on the circular smooth muscle of rat gastric antrum. The upper trace (A) is representative of the effects of ET-1 in the presence of BQ123, an inhibitor of ET_A receptors. BQ123 (1 μ M) was applied prior to the application of ET-1 for 15 min. The resting membrane potential was -56 mV. Nifedipine (1 μ M) was present throughout the recording. The lower trace (B) shows the representative of the effect of S6c, a selective agonist of ET_B receptor. Nifedipine (1 μ M) was present throughout the recording. The resting membrane potential was -63 mV.

Fig. 3. Effects of ET-1 on ET_A receptors on the circular smooth muscle of rat gastric antrum. The upper (A) is representative of the effects of ET-1 in the presence of BQ788, an inhibitor of ET_B receptors. BQ788 (1 μM) was applied prior to the application of ET-1 for 10 min. Nifedipine (1 μM) was present throughout the recording. The resting membrane potential was -64 mV. The lower trace (B) is representative of the effect of apamin on ET-1-induced hyperpolarization. Nifedipine (1 μM) was present throughout the recording. The resting membrane potential was -66 mV.

Attempts were made to stimulate only the ET_A receptors with the application of ET-1 in the presence of BQ788 (1 μM), a selective antagonist of ET_B receptors. BQ788 (1 μM) did not alter the resting membrane potential (control, -59.5 ± 1.2 mV, BQ788, -59.5 ± 0.9 mV, $n=4$) nor the frequency of slow waves (control, 2.3 ± 0.8 min⁻¹; BQ788, 2.2 ± 0.8 min⁻¹, $n=4$). However, in the presence of BQ788 (1 μM), ET-1 (10 nM) induced a sustained hyperpolarization (control, -57.0 ± 1.1 mV; ET-1, -61.5 ± 1.2 mV, $n=4$, $P<0.05$), and the frequency of slow waves tended to be decreased, although the change was not significant (control, 1.7 ± 0.3 min⁻¹; ET-1, 1.4 ± 0.3 min⁻¹, $n=4$; Fig. 3A). Removal of ET-1 and BQ788 immediately increased the frequency of the slow waves. The excitatory effects of ET-1 appeared to remain for up to an hour but the effects of BQ788 disappeared very rapidly (Fig. 3A).

Electrophysiological properties of the ET-1-induced hyperpolarization

To investigate the type of channel(s) involved in the ET-1-induced hyperpolarization produced during activation of the ET_A receptors, we tested the effects of apamin, an inhibitor of small conductance Ca²⁺-activated K⁺ (SK_{Ca}) channels. Apamin depolarized the membrane from -58.4 ± 1.9 mV ($n=5$) to -55.8 ± 1.8 mV ($n=5$, $P<0.05$) and increased the frequency of slow waves from 2.1 ± 0.3 min⁻¹ ($n=5$) to 2.9 ± 0.4 min⁻¹ ($n=5$, $P<0.05$). In the presence of apamin, ET-1-induced hyperpolarization was completely abolished and ET-1 now depolarized the membrane (ET-1, -62.2 ± 2.8 mV, ET-1 plus apamin, -46.0 ± 2.5 mV, $n=3$, $P<0.05$), with increasing the frequency of slow waves (Fig. 3B).

Fig. 4. Properties of ET-1-induced depolarization in the circular smooth muscle of rat gastric antrum. The upper (A) trace is representative of the effect of ET-1 in the presence of apamin ($0.1 \mu\text{M}$). Apamin was applied prior to the application of ET-1 for 10 min. Nifedipine ($1 \mu\text{M}$) was present throughout the recording. The resting membrane potential was -60 mV . The lower trace (B) is representative of the effects of ET-1 and S6c in the presence of apamin ($0.1 \mu\text{M}$) and DIDS (0.3 mM). Apamin and DIDS were applied prior to the application of ET-1 for 10 min. Nifedipine ($1 \mu\text{M}$) was present throughout the recording. The resting membrane potential was -60 mV .

Electrophysiological properties of the ET-1-induced depolarization

To investigate the ET-1-induced depolarization via ET_B receptors, ET-1 was applied in the presence of apamin ($0.1 \mu\text{M}$). ET-1 depolarized the membrane from $-54.4 \pm 1.5 \text{ mV}$ ($n=5$) to $-41.6 \pm 1.6 \text{ mV}$ ($n=5$, $P<0.05$) while increasing the frequency of slow waves (apamin, $2.7 \pm 0.3 \text{ min}^{-1}$, apamin plus ET-1, $5.7 \pm 0.7 \text{ min}^{-1}$, $n=5$, $P<0.05$) in the presence of apamin (Fig. 4A).

To investigate the channel(s) responsible for the ET-1-induced depolarization, DIDS (0.3 mM), an inhibitor of Ca^{2+} -activated Cl^- (Cl_Ca) channels, was applied. In the presence of apamin, DIDS decreased the frequency of slow waves (apamin, $2.7 \pm 0.2 \text{ min}^{-1}$; apamin plus DIDS, $1.7 \pm 0.3 \text{ min}^{-1}$, $n=3$, $P<0.05$), however did not alter the membrane potential (apamin, $-58.3 \pm 1.2 \text{ mV}$; apamin plus DIDS, $-59.0 \pm 2.6 \text{ mV}$, $n=3$). In the presence of both apamin and DIDS, ET-1-induced depolarization was significantly smaller than that in the presence of apamin alone (apamin, $13.0 \pm 2.6 \text{ mV}$, apamin plus DIDS, $2.3 \pm 1.2 \text{ mV}$, $n=3$, $P<0.05$, Fig. 4B). Although the ET-1-induced depolarization was significantly reduced by the application of DIDS, the ET-1-induced increase of the frequency of slow waves was not altered by the application of DIDS (apamin, $5.2 \pm 0.6 \text{ min}^{-1}$, apamin plus DIDS, $4.8 \pm 0.5 \text{ min}^{-1}$, $n=3$, Fig 4B).

Discussion

In the present study, we have shown that ET-1 has diverse effects on membrane activities in

circular smooth muscle cells of the rat gastric antrum. ET-1 initially caused a transient small hyperpolarization which was followed by a sustained depolarization, accompanied by an increase in the frequency of slow waves. Application of selective antagonists to each subtype of ET receptor allowed us to observe the specific actions produced by activation of each receptor type. In the presence of BQ123, a selective antagonist of ET_A receptors, ET-1 depolarized the membrane and increased the frequency of slow waves. These actions are produced by the activation of ET_B receptors. These results were confirmed by the production of similar activity using S6c, a selective agonist of ET_B receptors. The ET-1-induced depolarization was inhibited by DIDS, indicating that Cl_{Ca} channels were responsible for this depolarization. In the presence of DIDS, ET-1 increased the frequency of slow waves with no associated depolarization, indicating that the increase in the frequency by ET-1 was not due to the depolarization of the membrane. Selective stimulation of the pacemaker mechanism was considered. In the presence of BQ788, a selective antagonist of ET_B receptors, ET-1 hyperpolarized the membrane. The ET-1-induced hyperpolarization was completely abolished by the application of apamin, an inhibitor of SK_{Ca} channels. These results suggest that ET-1-induced hyperpolarization is mediated by ET_A receptors and SK_{Ca} channels are responsible for this hyperpolarization.

Accelerative effect of ET-1 on frequency of slow waves

Intensive investigations have been undertaken in the last decade to clarify the mechanism for the generation of gastrointestinal slow waves. Studies using *W/W^v* mutant mice have revealed that the lack of ICC-MY results in the absence of slow waves in the small intestine (Ward *et al.*, 1994; Huizinga *et al.*, 1995; Burns *et al.*, 1996), thus ICC-MY are likely to be essential for the generation of slow waves. Direct intracellular recording from ICC-MY has shown that large amplitude driving potentials are generated in ICC-MY, and that the driving potentials spread passively to both the longitudinal and circular muscle layers (Dickens *et al.*, 1999; Hirst and Edward, 2001). Slow waves recorded in gastric circular smooth muscle cells consist of two components, namely the initial phase of depolarization produced by the driving potential and regenerative potentials which are triggered by the initial depolarization (Suzuki and Hirst, 1999; Kito and Suzuki, 2003). In *W/W^v* mutant mice, ICC-MY are reported to be absent in the intestine (Ward *et al.*, 1994; Huizinga *et al.*, 1995), while later reports indicate that in some regions of the stomach ICC-MY are retained and ICC-IM are absent (Burn *et al.*, 1996; Ward *et al.*, 1998). In the antrum of *W/W^v* mutant mice, slow waves which lack the secondary regenerative component can be recorded (Dickens *et al.*, 2001). These results indicate that ICC-IM are responsible for the generation of the regenerative potential. On the other hand, a number of morphological studies have revealed that in the stomach ICC-IM are closely apposed between nerve terminals and smooth muscle cells with gap junctions (Komuro *et al.*, 1999). Recent physiological studies have demonstrated the functional significance of ICC-IM in inhibitory (Burns *et al.*, 1996; Ward *et al.*, 1998) and excitatory (Ward *et al.*, 2000) neurotransmission. Thus, ICC-IM are considered to play an important role in not only pacemaking but also in neurotransmission in the stomach. The accelerative effect of ET-1 on the frequency of slow waves indicates that ET-1 acts on ICC-MY and/or ICC-IM through ET_B receptors. The mechanism underlying is still under discussion. Previous studies have shown

that cholinergic stimulation increases the frequency of slow waves (Szurszewski, 1975; Sara and Daniel, 1975; Hirst *et al.*, 2002), and that the IP₃ pathway is involved in this acceleration (Kim *et al.*, 2003). Animals lacking type 1 IP₃ receptors are incapable of generating slow waves, suggesting that this subtype of IP₃ receptor is essential for the pacemaking mechanism (Suzuki *et al.*, 2000). In pulmonary arterial smooth muscle cells, it has been demonstrated that ET-1 increases the frequency of Ca²⁺ sparks via the ET_A-IP₃ signaling pathway (Zhang *et al.*, 2003). Taken together, these results suggest that ET-1 activates the IP₃-pathway of ICC-MY in the rat gastric antrum, resulting in an increased frequency of gastric slow waves.

As shown in Fig. 1A, ET-1-induced depolarization is accompanied by a rise in the frequency of slow waves. It has been shown that depolarization increases the frequency of slow waves in isolated circular smooth muscle preparations (Huang *et al.*, 1999). However, in the presence of DIDS, although depolarization was blocked, facilitation of the frequency of slow waves was observed (Fig. 4B). This observation suggests that the effect of ET-1 on frequency is not due to depolarization, but rather that it occurs by the stimulation of the ongoing pacemaker mechanism.

Depolarization and Hyperpolarization

ET-1 hyperpolarized the membrane by the activation of SK_{Ca} channels through ET_A receptors in smooth muscle cells of rat gastric antrum (Fig 3A, B). A similar observation has been reported in the guinea pig lower esophageal sphincter (Imaeda *et al.*, 2002). The mechanism involved is thought to be that the stimulation of ET_A receptors on smooth muscle cells activates G-proteins, which then trigger the release of Ca²⁺ from intracellular Ca²⁺ stores, and results in opening of the SK_{Ca} channels. It has been reported that ICC-IM are rich in SK_{Ca} (Fujita *et al.*, 2001), and that they are involved in inhibitory neurotransmission (Burns *et al.*, 1996; Ward *et al.*, 1998). So it is also likely that ET-1 acts on ICC-IM through ET_A receptors, which activate SK_{Ca} channels resulting in hyperpolarization of the membrane of smooth muscle cells.

On the other hand, ET-1 depolarized the membrane by the activation of Cl_{Ca} channels through ET_B receptors in the rat gastric antrum (Fig 4A, B). Cl⁻ channels have been reported to be involved in the generation of pacemaker potentials (Huizinga *et al.*, 2002). DIDS, an inhibitor of Cl_{Ca} channels, abolished the plateau phase of the driving potential in ICC-MY of the guinea pig antrum (Kito *et al.*, 2002; Kito and Suzuki, 2003). These results suggest that ET-1 acts on ET_B receptors in ICC-MY, then releases Ca²⁺ from intracellular Ca²⁺ stores through the activation of the G-protein-IP₃ pathway. Released Ca²⁺ may activate Cl_{Ca} channels, resulting in depolarization. However, it is still unclear why Ca²⁺ released by the stimulation of ET_A receptors activates K_{Ca} channels while Ca²⁺ released by the stimulation of ET_B receptors activates Cl_{Ca} channels. Actually, it has been reported that the oscillating inward current is caused by the activation of Cl_{Ca} via ET_A receptors in pig detrusor smooth muscle cells (Kajioka *et al.*, 2004). One hypothesis is that the distribution of subtypes of ET receptors and ion channels are different between interstitial cell types. ICC-MY may be rich in ET_B receptors and Cl_{Ca} channels, because ICC-MY generate the driving potential which is mainly produced by the activation of Cl_{Ca} channels. On the other hand, ICC-IM may be rich in ET_A receptors and SK_{Ca} channels,

because ICC-IM intermediate the inhibitory neurotransmission between nerve terminals and smooth muscle cells and inhibitory junction potentials are mainly produced by the activation of SK_{Ca} channels in several regions of the gastrointestinal tract. However, Fujita *et al.* (2003) have reported the localization of SK_{Ca} channels in fibroblast-like cells forming gap junctions with smooth muscle cells, but not in ICC in the rat and mouse intestine. Further morphological and electrophysiological investigation is needed to clarify this discrepancy.

In summary, ET-1 affects both the frequency of slow waves and the membrane potential through different types of ET receptors and ion channels. ET-1 accelerates the frequency of slow waves and depolarizes the membrane through the ET_B receptor. It seems likely that Cl_{Ca} channels are responsible for the ET-1-induced depolarization. The acceleration of slow waves caused by ET-1 is thought to be due to the action on the pacemaking mechanism, but is not linked to membrane depolarization. On the other hand, ET-1 hyperpolarizes the membrane through ET_A receptors. ET-1-induced hyperpolarization is produced by the activation of apamin-sensitive SK_{Ca} channels. The different effects of ET-1 are speculated to be due to a different distribution of ET receptor subtypes and ion channels on ICC-MY and ICC-IM.

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References

- Allcock, G.H., Battistini, B., Fournier, A., Warner, T.D. and Vane, J.R. (1995). Characterization of endothelin receptors mediating mechanical responses to the endothelins in the isolated stomach strip of the rat. *J. Pharmacol. Exp. Ther.* **275**: 120–126.
- Arai, H., Hori, S., Aramori, I., Ohkubo, H. and Nakanishi, S. (1990). Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* **348**: 730–732.
- Burns, A.J., Lomax, A.E.J., Torihashi, S., Sanders, K.M. and Ward, S.M. (1996). Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 12008–12013.
- Burns, A.J., Herbert, T.M., Ward, S.M. and Sanders, K.M. (1997). Interstitial cells of Cajal in the guinea-pig gastrointestinal tract as revealed by c-Kit immunohistochemistry. *Cell Tissue Res.* **290**: 11–20.
- Chakder, S. and Rattan, S. (1999). Mechanisms and sites of action of endothelins 1 and 2 on the opossum internal anal sphincter smooth muscle tone in vitro. *J. Pharmacol. Exp. Ther.* **288**: 239–246.
- De Nucci, G., Thomas, R., D'Orleans-Juste, P., Antunes, E., Walder, C., Warner, T.D. and Vane, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. U.S.A.* **85**: 9797–9800.
- Dickens, E.J., Hirst, G.D.S. and Tomita, T. (1999). Identification of rhythmically active cells in guinea-pig stomach. *J. Physiol. (Lond.)* **514**: 515–531.
- Dickens, E.J., Edwards, F.R. and Hirst, G.D.S. (2001). Selective knockout of intramuscular interstitial cells reveals their role in the generation of slow waves in mouse stomach. *J. Physiol. (Lond.)* **531**: 827–833.

- Eddahibi, S., Springall, D., Mannan, M., Carville, C., Chabrier, P.-E., Levame, M., Raffestin, B., Polak, J. and Adnot, S. (1993). Dilator effect of endothelins in pulmonary circulation: changes associated with chronic hypoxia. *Am. J. Physiol.* **265**: L571–L580.
- Fujita, A., Takeuchi, T., Saitoh, N., Hanai, J. and Hata, F. (2001). Expression of Ca^{2+} -activated K^{+} channels, SK3, in the interstitial cells of Cajal in the gastrointestinal tract. *Am. J. Physiol.* **281**: C1727–C1733.
- Fujita, A., Takeuchi, T., Hanai, J. and Hata, F. (2003). Localization of Ca^{2+} -activated K^{+} channel, SK3, in fibroblast-like cells forming gap junctions with smooth muscle cells in the mouse small intestine. *J. Pharmacol. Sci.* **92**: 35–42.
- Hasunuma, K., Rodman, D.M., O'Brien, R.F. and McMurtry, I.F. (1990). Endothelin 1 causes pulmonary vasodilation in rats. *Am. J. Physiol.* **259**: H48–H54.
- Higashi, T., Ishizaki, T., Shigemori, K., Yamamura, T. and Nakai, T. (1997). Pharmacological characterization of endothelin-induced rat pulmonary arterial dilatation. *Br. J. Pharmacol.* **121**: 782–786.
- Hirst, G.D.S. and Edwards, F.R. (2001). Generation of slow waves in the antral region of guinea-pig stomach - a stochastic process. *J. Physiol. (Lond.)* **535**: 165–180.
- Hirst, G.D.S., Dickens, E.J. and Edwards, F.R. (2002). Pacemaker shift in the gastric antrum of guinea-pigs produced by excitatory vagal stimulation involves intramuscular interstitial cells. *J. Physiol. (Lond.)* **541**: 917–928.
- Huang, S.-M., Nakayama, S., Iino, S. and Tomita, T. (1999). Voltage sensitivity of slow wave frequency in isolated circular muscle strips from guinea pig gastric antrum. *Am. J. Physiol.* **276**: G518–G528.
- Huizinga, J. D., Thuneberg, L., Klüppel, M., Malysz, J., Mikkelsen, H. B. and Bernstein, A. (1995). W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* **373**: 347–349.
- Huizinga, J.D., Zhu, Y., Ye, J. and Molleman, A. (2002). High-conductance chloride channels generate pacemaker currents in interstitial cells of Cajal. *Gastroenterol.* **123**: 1627–1636.
- Imaeda, K., Trout, S.J. and Cunnane, T.C. (2002). Mechanical and electrophysiological effects of endothelin-1 on guinea-pig isolated lower oesophageal sphincter circular smooth muscle. *Br. J. Pharmacol.* **135**: 197–205.
- Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K. and Masaki, T. (1989). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. U.S.A.* **86**: 2863–2867.
- Kajioka, S., Nakayama, S., McCoy, R., McMurray, G., Abe, K. and Brading, A.F. (2004). Inward current oscillation underlying tonic contraction caused via ET_A receptors in pig detrusor smooth muscle. *Am. J. Physiol.* **286**: F77–F85.
- Kito, Y., Fukuta, H. and Suzuki, H. (2002). Components of pacemaker potentials recorded from the guinea pig stomach antrum. *Pflugers Arch.* **445**: 202–217.
- Kito, Y. and Suzuki, H. (2003). Electrophysiological properties of gastric pacemaker potentials. *J. Smooth Muscle Res.* **39**: 163–173.
- Kim, T.W., Koh, S.D., Ordog, T., Ward, S.M. and Sanders, K.M. (2003). Muscarinic regulation of pacemaker frequency in murine gastric interstitial cells of Cajal. *J. Physiol. (Lond.)* **546**: 415–425.
- Komuro, T., Tokui, K. and Zhou, D.S. (1996) Identification of the interstitial cells of Cajal. *Histol. Histopathol.* **11**: 769–786.
- Komuro, T., Seki, K. and Horiguchi, K. (1999). Ultrastructural characterization of the interstitial cells of Cajal. *Arch. Histol. Cytol.* **62**: 295–316.
- Lin, W.W. and Lee, C.Y. (1990). Biphasic effects of endothelin in the guinea-pig ileum. *Eur. J. Pharmacol.* **176**: 57–62.
- Lin, W.W. and Lee, C.Y. (1992). Intestinal relaxation by endothelin isopeptides: involvement of Ca^{2+} -

- activated K⁺ channels. *Eur. J. Pharmacol.* **219**: 355–360.
- Rubanyi, G.M. and Polokoff, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.* **46**: 325–415.
- Sakurai, T., Yanagisawa, M., Takawa, Y., Miyazaki, H., Kimura, S., Goto, K. and Masaki, T. (1990). Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* **348**: 732–735.
- Sanders, K.M. (1996). A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterol.* **111**: 492–515.
- Sarna, S.K. and Daniel, E.E. (1975). Vagal control of gastric electrical control activity and motility. *Gastroenterol.* **68**: 301–308.
- Suzuki, H., Takano, H., Yamamoto, Y., Komuro, T., Saito, M., Kato, K. and Mikoshiba, K. (2000). Properties of gastric smooth muscles obtained from mice which lack inositol trisphosphate receptor. *J. Physiol. (Lond.)* **525**: 105–111.
- Suzuki, H. and Hirst, G.D.S. (1999). Regenerative potentials evoked in circular smooth muscle of the antral region of guinea-pig stomach. *J. Physiol. (Lond.)* **517**: 563–573.
- Szurszewski, J.H. (1975). Mechanism of action of pentagastrin and acetylcholine on the longitudinal muscle of the canine antrum. *J. Physiol. (Lond.)* **252**: 335–361.
- Takaki, M. (2003). Gut pacemaker cells: the interstitial cells of Cajal (ICC). *J. Smooth Muscle Res.* **39**: 137–161.
- Tomita, T. (1981). Electrical activity (spike and slow wave) in gastrointestinal smooth muscle. In: *Smooth Muscle: An Assessment of Current Knowledge*, ed. by Bülbring, E., Brading, A.F., Jones, A.W. and Tomita, T., Edward Arnold, Londo, pp. 127–156.
- Ward, S.M., Burns, A.J., Torihashi, S. and Sanders, K.M. (1994). Mutation of the proto-oncogene *c-kit* blocks development of interstitial cells and electrical rhythmicity in murine intestine. *J. Physiol. (Lond.)* **480**: 91–97.
- Ward, S.M., Morris, G., Reese, L., Wang, X.-Y. and Sanders, K.M. (1998). Interstitial cells of Cajal mediate enteric inhibitory neurotransmission in the lower esophageal and pyloric sphincters. *Gastroenterol.* **115**: 314–329.
- Ward, S.M., Beckett, E.A.H., Wang, X.-Y., Baker, F., Khoyi, M. and Sanders, K.M. (2000). Interstitial cells of Cajal mediate cholinergic neurotransmission from enteric motor neurons. *J. Neuroscience.* **20**: 1393–1403.
- Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332**: 411–415.
- Zhang, W.M., Yip, K.P., Lin, M.J., Shimoda, L.A., Li, W.H. and Sham, J.S.K. (2003). ET-1 activates Ca²⁺ sparks in PASMC: local Ca²⁺ signaling between inositol trisphosphate and ryanodine receptors. *Am. J. Physiol.* **285**: L680–L690.

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