Cellular effects of gastric electrical stimulation on antral smooth muscle cells in rats

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Li SY, Chen JD. Cellular effects of gastric electrical stimulation on antral smooth muscle cells in rats. Am J Physiol Regul Integr Comp Physiol 298: R1580–R1587, 2010. First published March 31, 2010; doi:10.1152/ajpregu.00024.2010.—The cellular effects of gastric electrical stimulation (GES), which has recently been introduced as a potential therapy for the treatment of gastroparesis and obesity, were investigated in rat antrum smooth muscle cells (SMCs). Effects on cell membrane potentials of single electrical current pulses (pulse width from 0.1 ms to 200 ms) and 2-s pulse train stimuli with different pulse widths (0.1–4 ms), different frequencies (20–200 Hz), and different intensities were studied: 1) the stimulus amplitude had an exponential relationship to the pulse width from 2 ms to 200 ms, along with a rapidly rising strength-duration curve at pulse widths less than 5 ms, and a relatively flat curve at pulse widths greater than 50 ms; 2) when the pulse frequency was at 80 Hz or above, pulse train electrical stimulation, with a pulse width of 2 ms or above but not ≤1 ms, was able to depolarize cell membrane potentials to above −30 mV and/or generate action potentials. Electrical stimulation with a single long pulse and a width of 50 ms or greater is effective in depolarizing cell membrane potentials of SMCs with low amplitude. Pulse train electrical stimulation with a pulse width of ≤1 ms fails to generate action potentials in SMCs, whereas pulse train electrical stimulation with a pulse width of 2–4 ms and a sufficiently high pulse frequency is able to generate action potentials. These cellular findings may be useful in optimizing stimulation parameters of GES.

GASTRIC ELECTRICAL STIMULATION (GES) has recently been introduced as a potential therapy for the treatment of gastroparesis (1, 8, 18, 19) or obesity (2, 4, 8). GES can be classified into three categories based on pulse width: 1) repetitive single electric stimulus with long pulses; 2) repetitive single electric stimulus with short pulses; and 3) trains of short pulses: a series of stimuli of short pulses given as cyclic trains (5).

Short-pulse or trains of short-pulse GES at a frequency several times that of the intrinsic slow waves, such as the Enterra therapy (Medtronic, Minneapolis, MN), has no acute effects on gastric slow waves or gastric emptying, but it is capable of improving symptoms of nausea and vomiting (1, 5). Long-pulse and pulse trains of long pulses have been reported to normalize slow-wave dysrhythmias and/or improve gastric emptying in patients and animals (5, 12, 23, 43). Slow waves, generated by rhythmic depolarization from interstitial cells of Cajal (ICC), will periodically depolarize nearby smooth muscle cells (SMCs) that are close to the “mechanical threshold,” which defines the degree of depolarization at which sufficient Ca2+ entry occurs to accomplish excitation-contraction (21). Slow-wave depolarization sometimes elicits Ca2+ action potentials, which result in brief and intense Ca2+ entry and strong contractions in SMCs (26). All SMCs have ion channels to regenerate a slow wave following depolarization, which can be caused by the ICC pacemaker system (10) or externally applied GES, which can directly entrain gastric slow waves in mutant mice (W/Wv) lacking myenteric ICC in the gastrointestinal tract (41). However, GES with long-pulse or trains of long pulses requires greater energy, and no implantable pulse generators are available to deliver these stimuli. The main challenge for the new generation of devices is to optimize experimental pulse width for the energy-efficient stimulation of SMCs (39, 42).

While a number of studies have been performed to investigate the efficacy, as well as the gastric and extragastric mechanisms of GES in the treatment of gastroparesis and obesity, the underlying mechanisms of GES, especially on the cellular level of SMCs, remain poorly understood. The methodologies for various GES applications have not been optimized. Depolarized cell membrane potential measured by the patch-clamp technique provides a simple reduced method to optimize the effect of GES with various parameters on modulating SMCs function. One of the fundamental characteristics of single-pulse stimulation is the strength-duration relationship, or the effect of pulse width on the efficacy of current amplitude in eliciting a response such as an action potential. The threshold amplitude of stimulated tissue varies according to the duration of the stimulus current, and the current intensity necessary to trigger a response is inversely proportional to its pulse width. The strength-duration relationship was explored in other excitable tissues, such as nerves (20, 27) and cardiac myocytes (36).

Pulse trains with long pulses have been reported to increase antral contractions and accelerate gastric emptying with little uniformity in the parameters. GES with 20 Hz, 2–10 ms directly stimulates smooth muscle to accelerate gastric emptying in vagotomized dog, while GES with 40 Hz, 2 ms enhances gastric motility mainly through the cholinergic pathway (7, 43). This relationship of GES with varied pulse width and frequency on cell membrane potential at the cellular level could better our understanding of electrical properties of SMCs and could be used to design effective stimulus protocol in its final application.

The aims of this study were 1) to investigate the strength-duration relationship in the effect of GES on gastric antral SMCs in rats using the patch-clamp techniques; and 2) to investigate the effects of pulse train GES with varied pulse width, frequency, and amplitude on membrane potentials of antral SMCs in rats. The long-term goal of this study is to derive the best stimulation parameters for various applications.
of GES, so they can be effectively and efficiently used for the treatment of gastroparesis or obesity.

MATERIALS AND METHODS

Isolation of Gastric Antrum SMCs

The experimental study was approved by the Institutional Animal Care and Use Committee at the Veterans Affairs Medical Center, Oklahoma City, OK, and the International Guiding Principles for Biomedical Research Involving Animals were followed. SMCs were isolated using modified procedures, as previously described (15). Male rats, weighing 300–450 g, were killed by cervical dislocation under anesthesia. The gastric antrum was quickly removed and placed in low-calcium physiological saline (in mM): 10 HEPES, 135 NaCl, 6 KCl, 2 CaCl₂, 1.2 MgCl₂, and 10 glucose (pH 7.4 with NaOH). The conventional whole-cell mode of the voltage-clamp technique was used to measure changes in membrane potentials from antrum circular SMCs at room temperature (22–24°C). The bath solution contained (in mM): 10 HEPES, 135 NaCl, 5 KCl, 2 CaCl₂, 1.2 MgCl₂, 10 glucose (pH 7.4 with NaOH). The longitudinal muscle layer was peeled off using fine forceps, and the remaining circular muscle strips were removed by sharp dissection under anatomical microscope. The muscle strips were incubated in a low-calcium physiological solution for 30 min at 4°C and were then transferred to fresh low calcium physiological solution containing enzymes (in mg/ml: 1 collagenase IA; 1 papain; 2 BSA; 1 soybean trypsin inhibitor) for 15 min at 35°C. Then, the muscle pieces were washed several times in an enzyme-free low-calcium physiological solution and gently agitated to create a cell suspension. Isolated cells were stored at 4°C and used within 6 h. All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO).

Whole Cell Current-Clamp Recording

Current-clamp technique was used to measure changes in membrane potentials from antrum circular SMCs at room temperature (22–24°C). The bath solution contained (in mM): 10 HEPES, 135 NaCl, 5 KCl, 2 CaCl₂, 1.2 MgCl₂, 10 glucose (pH 7.4 with NaOH). Patch pipettes (borosilicate glass; World Precision Instruments, Sarasota, FL) were pulled with a Flaming Brown P-97 micropipette puller (Sutter Instruments, Novato, CA, USA), and the tips were fire polished with MF-83 microforge (Narishige, Japan). The resistance of the patch pipettes was between 4–6 MΩ when filled with an intracellular solution containing enzymes (in mg/ml: 1 collagenase IA; 1 papain; 2 BSA; 1 soybean trypsin inhibitor) for 15 min at 35°C. Then, the muscle pieces were washed several times in an enzyme-free low-calcium physiological solution and gently agitated to create a cell suspension. Isolated cells were stored at 4°C and used within 6 h. All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO).

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Pulse train electrical stimulation on membrane potentials. This experiment was designed to investigate the effects of pulse train electrical stimulation with various pulse widths and frequencies on the membrane potentials of the gastric antrum circular SMCs in rats. The stimuli were delivered as 2-s repetitive rectangular pulses with variable pulse widths (0.1, 0.3, 0.5, 1, 2, and 4 ms), frequencies (20, 40, 80, 160, and 200 Hz), and amplitudes (low: 50 pA, medium: 100 pA, and high: 250 pA). The mean cell membrane potential during each 2-s stimulation was calculated by Clampfit 9.0 and used as an index of cell membrane potential depolarization.

Whole Cell Voltage-Clamp Recording of Voltage-Gated L-Type Calcium Channel Current

The conventional whole-cell mode of the voltage-clamp technique was used to measure the change in membrane currents from antrum circular SMCs at room temperature (22–24°C). TEA 6 mM was added to the bath solution, and potassium was substituted with cesium in the pipette solution to block potassium currents. The bath solution contained (in mM): 10 HEPES, 135 NaCl, 6 TEA-Cl, 2 CaCl₂, 1.2 MgCl₂, and 10 glucose (pH 7.4 with NaOH). The intracellular solution contained (in mM): 10 HEPES, 130 CsCl, 10 EGTA, 1 TEA, 1 Na₂ATP, 1 Na₃GTP, and 1 MgCl₂; pH was adjusted with CsOH to 7.2. The membrane currents were recorded using a Multiclamp 700B amplifier (Axon Instruments, Foster City, CA) and digitized on-line at 2 kHz. Data acquisition and analysis were performed using pClamp 9.0 and Clampfit 9.0 software (version 9.0; Axon Instruments).

Voltage Stimulus Protocols

Two kinds of pulse protocols were used. One protocol included a depolarizing pulse with a pulse width of 100 ms from holding potentials of −80 mV to test potentials between −80 mV and +60 mV in 10-mV increments. The interval between two pulses was 10 s, which allowed for recovery of calcium channels. The other protocol was a double-pulse protocol with the membrane potential stepped to conditioning voltages between −80 and +20 mV in 10-mV increment for 1 s and then pulsed to +10 mV for 100 ms. The interval between two stimulation sweeps was set to 20 s. Macroscopic current values were normalized for cell capacitance as whole cell current densities (pA/pF).

Statistical Analysis

The amplitude of the action potential was calculated as the difference between the threshold and peak amplitude. Data are presented as means ± SE. Significant differences between two groups of means were assessed by Student’s t-test, and P value was set at the 0.05 level. The differences in the data among three or more groups of means were evaluated by one-way ANOVA analysis followed with post hoc
Current-Voltage Relationship of L-type Ca\(^{2+}\) Channels in Gastric Circular SMCs

Fig. 2 shows that depolarizing pulses activated inward L-type Ca\(^{2+}\) channels' currents (I\(_{\text{Ca,L}}\)), and the amplitude of these currents displayed a U-shaped dependence on membrane potentials between -80 and +60 mV. The inward currents were activated around -30 mV, peaked around 0 mV, and reversed around 60 mV. The peak current density at the 0 mV test pulse is 6.3 \(\pm\) 0.5 pA/pF (n = 13) with average cell capacitance of 47.4 \(\pm\) 2.6 pF (n = 13).

Fig. 1. External tetraethylammonium chloride (TEA) induced repetitive action potentials in rat gastric antral circular smooth muscle cells (SMCs). A: cell membrane potentials in response to 10–40 pA depolarizing pulses in the absence of TEA; B: cell membrane potentials in response to 10–40 pA depolarizing pulses in the presence of TEA; C: repetitive action potentials from the same cell were generated when the smooth muscle cell membrane potential was held at -50 mV.
current between −30 mV and 0 mV, according to the activation and inactivation curves.

Strength-Duration Relationship of Single-Pulse Electrical Stimulation

The effect of electrical stimulation with varying stimulation pulse widths on the threshold level of generating action potentials was studied in rat gastric antrum circular SMCs (n = 5–11 cells in different pulse widths). Fig. 4A shows that stimulation currents with pulse durations from 0.1 to 1 ms and pulse amplitude of 1 nA failed to elicit action potentials in SMCs. Most cells exhibited a single action potential in response to depolarizing current pulses of durations from 2 ms to 200 ms. The stimulation amplitude had an exponential relationship to the pulse width between 2 ms and 200 ms, with a rapidly rising strength-duration curve at pulse durations less than 5 ms and a relatively flat curve at pulse durations greater than 50 ms (see Fig. 4A). Beyond 50 ms, a wider pulse yielded only a small reduction in current amplitude amplitude. As shown in Fig. 4A, an estimate of the rheobase (~30 pA) was given by the intercept of this line with the ordinate, and from this, the chronaxie was calculated to be −55 ms, which was the minimum duration of a current pulse at twice the rheobase strength required to excite the cell. In Fig. 4B, the energy consumption of electrical stimulation with pulse width from 2 ms to 200 ms was calculated and compared. The single-pulse electrical stimulation with a pulse width from 50 to 200 ms required stimulation energy around $1 \times 10^5$ ms.pA$, whereas electrical stimulation with a pulse width below 20 ms required stimulation energy that was more than 10 times higher.

Effects of Pulse Train Electrical Stimulation on Cell Membrane Potentials

The effects of pulse train electrical stimulation on cell membrane potentials are presented in Fig. 5. For electrical stimulation with pulse trains, in general, longer pulse width, higher pulse frequency, and greater amplitude led to more
potent effects of stimulation on depolarizing cell membrane potentials. Effects of pulse train electrical stimulation using parameters similar to Enterra therapy. Electrical stimulation with the Enterra therapy parameters (pulse width below 1 ms and frequency below 40 Hz) failed to activate SMCs. Pulse train electrical stimulations with pulse width of 0.1 ms (n = 9), 0.3 (n = 9), or 0.5 ms (n = 5) and a frequency of 20 or 40 Hz were unable to significantly depolarize the cell membrane potentials (P > 0.05, Fig. 5, A–C, E), even when the current amplitude was increased to 250 pA, which was far above the threshold current of ≤40 pA observed in the single electrical long-pulse stimulation study (Fig. 5, C and E). With a pulse width of 0.5 ms or below, electrical stimulation was able to solicit a graded membrane potential increase (P < 0.05, n = 5–9 vs. baseline) at a higher frequency (80–200 Hz) and amplitude (100 pA or 250 pA), but it was unable to evoke action potentials or depolarize mean cell membrane potentials above the mechanical threshold in antrum SMCs.

Effects of pulse train electrical stimulation with increased pulse width. As shown in Fig. 5, pulse train electrical stimulations with a pulse width of 1 ms (n = 7), 2 ms (n = 8), or 4 ms (n = 8), and a frequency of 20 or 40 Hz were able to depolarize the cell membrane potentials significantly but failed to generate action potentials. Only when stimulated with 250 pA high-intensity current and pulse widths of 2 and 4 ms could the cell membrane potentials be depolarized near −30 mV (Fig. 5F). However, action potentials were evoked, and cell membrane potentials were depolarized above −30 mV (P < 0.05, n = 7–8 vs. membrane potentials in response to pulse stimulation of 20 or 40 Hz) when the pulse frequency was 80 Hz or above or the amplitudes were high.

DISCUSSION

In this study, we found that stimulus amplitude had an exponential relationship with the pulse width between 2 ms and 200 ms and that low-amplitude long-pulse electrical stimulation with a width of above 50 ms generated action potentials in SMCs. Pulse train electrical stimulation with parameters similar to that of the Enterra therapy (pulse width below 1 ms, frequency below 40 Hz) was unable to depolarize cell membrane potentials above the mechanical threshold (−30 mV). Pulse train electrical stimulation with a pulse width of 2 ms or above elicited action potentials when the pulse frequency was at 80 Hz or above.

Threshold to Activate SMC

According to activation and inactivation curves, there was a window current between −30 mV and 0 mV, which meant that some calcium channels were not inactivated in this voltage range and would contribute to the maintenance of a plateau depolarization and sustained calcium influx. The voltage dependence of the calcium channels of rat gastric SMCs in our study was in agreement with previous studies in guinea pigs and canines. Long-duration depolarization of isolated colonic and gastric cells results in sustained entry of Ca^{2+} due to incomplete inactivation of Ca^{2+} current in the range of potentials achieved during the plateau potential around −40 to −30 mV (34). We chose −30 mV as the threshold for successful activation of SMCs for GES studies, based on the work of Ozaki et al. (21), which showed that slow waves must exceed a level of depolarization, termed the mechanical threshold, for excitation-contraction coupling to occur. GES-induced depolarization above this threshold is thought to achieve significant Ca^{2+} entry, and therefore initiates contractions.

Strength-Duration Relationship

The strength-duration curve, an approximately exponential relationship between the threshold current and the stimulus duration, is a measure of the membrane properties of excitable tissue. Chronaxie is proportional to the product of membrane resistance (R) and capacitance (C) (i.e., the
membrane time constant $\tau = R \cdot C$ (25). The myelinated axon has a smaller chronaxie ($50–200 \mu s$) than the unmyelinated fibers ($200–700 \mu s$) (20). Although there is limited data regarding the chronaxie of neurons in gastrointestinal organs, the gastric SMCs in our study may have a much larger chronaxie value (about 55 ms) than neurons in the stomach, motor neurons (0.17 ms) and ventricular myocardium (1.82 ms) (36) due to the lack of sodium channel on rat gastric SMCs membrane and the relatively large cell size. Smooth muscle bundle in guinea pig antrum has been reported to have a time constant of 260 ms (9), and this is in agreement with the findings of this study: a pulse width of 50 ms or higher is required for electrical stimulation to solicit action potentials. The measured differences in the chronaxie of myocardium and myelinated nerve fibers have been used to design stimulus waveforms that selectively stimulate motor neurons without exciting myocardium (36).

Action Potentials Evoked by Single Long-Pulse Stimulation

In our study, the strength-duration relationship showed that single pulses with amplitude smaller than 50 pA and width up to 100–200 ms could reliably depolarize membrane potential and evoke action potentials in rat gastric circular SMCs with minimum energy consumption. This was consistent with previous reports in which GES with long pulses was reported to improve gastric emptying in a canine model of gastroparesis (3) and in patients with gastroparesis (19). GES with long pulses has also been shown to normalize gastric dysrhythmia or entrain gastric slow waves (5, 12, 13, 23). Slow waves are generated by interstitial cells of ICC, and these events spread actively within ICC networks and conduct passively to nearby SMCs (6, 11, 26, 37). Voltage-dependent calcium conductance is required for active propagation of slow waves in the gastrointestinal smooth muscle, as propagation of slow waves gen-
erated by electrical field stimulation can be blocked or reduced by reducing extracellular Ca\(^{2+}\) and inhibitors of voltage-dependent Ca\(^{2+}\) channels (14, 38). Repetitive single electrical stimulation with long pulses delivered near the physiological frequency of gastric slow waves may entrain slow waves and enhance gastric motility by depolarizing SMCs membrane potentials periodically and activating voltage-dependent calcium channels to initiate contraction.

**Incapability of GES with Short Pulses in Evoking Action Potentials or Depolarizing Cell Membrane Potential Above the Mechanical Threshold**

Short-pulse GES at a frequency several times that of the intrinsic slow waves has been reported to be ineffective in altering gastric slow waves but capable of improving symptoms of nausea and vomiting in patients with gastroparesis (1, 5). Most commercially available cardiac pacemakers or nerve stimulators are capable of generating short pulses. Particularly, GES delivered with a train-on time of 0.1 s and train-off time of 5 s, pulse width of 0.33 ms, and frequency of 14 Hz, called the Enterra therapy (Medtronic), was reported to improve nausea and vomiting in patients with medication-refractory gastroparesis (1, 17). Pulse train GES with parameters slightly different from the Enterra therapy (pulse width 0.1–0.3 ms, frequency 14–40 Hz) was found to reduce vasopressin-induced emetic responses without entrainment of intrinsic slow waves in dogs (28). Recent animal studies have indicated the need for a new implantable device capable of generating wider pulses or long pulses for treating obesity (40). In the present study, the strength-duration relationship showed that electrical stimulation with the Enterra therapy parameters (pulse width below 1 ms and frequency below 40 Hz) failed to activate SMCs. These findings may rule out the cellular mechanism involving SMCs as the anti-emetic effect of the Enterra therapy. Similarly, the stimulation parameters used in the initial clinical studies on obesity were also inadequate according to the findings of the present study. Central and peripheral neural mechanisms have been suggested for the beneficial effects of the Enterra therapy on nausea and vomiting and the antiobesity effect of GES for patients with obesity (5, 24, 31).

**Capability of Pulse Train GES with Wider Pulses in Evoking Action Potentials**

In this study, it was found that SMCs could be activated by depolarizing the cell membrane potential above \(-30\) mV and eliciting action potentials with electrical stimulation with a pulse width of 2–4 ms, a pulse frequency above 80 Hz, and/or high-stimulation amplitude. This finding was in agreement with previous in vivo physiological studies. Synchronized GES delivered upon the detection of intrinsic slow-wave peaks with pulse train parameters (train duration of 0.5–0.8 ms, pulse frequency of 40 Hz, pulse width of 2 ms, and amplitude of 4 mA) accelerated glucagon-induced delayed gastric emptying and induced or enhanced antral contractions in dogs (43). In other studies, GES with similar parameters synchronized with intrinsic antral contractions was reported to enhance antral contractions and vagal afferent activity (22).

**Perspectives and Significance**

Normal gastric motility involves the interaction of many electrical events at the cellular and tissue level, including the coordination of myogenic, neuronal, and hormonal factors (10, 32). Extensive afferent and efferent communications exist between the central nervous system and the gut, linking the central nervous system with the enteric nervous system, as well as providing direct contact with smooth muscle and/or ICC, which are generally accepted as the origin of slow waves (32). Slow waves generated by ICC conduct into SMCs and depolarize the membrane, causing a transient contraction due to mechanically produced Ca\(^{2+}\) entry via voltage-gated (L-type) Ca\(^{2+}\) channels. Because slow wave activity and neuronal and smooth muscle action potentials can be triggered by depolarizing currents, the external application of electrical current is able to modulate gastrointestinal motility; however, appropriate stimulation parameters are needed to alter muscle functions as shown in this study. Because of differences in cell capacitance, components of ion channels, excitability, and others, GES with different parameters may modulate gastric motility through different pathways, such as myogenic or neuronal pathways.

In conclusion, GES with single long pulses of above 50 ms is able to generate action potentials in SMCs; the stimulation amplitude required to evoke action potentials is exponentially related to the pulse width in the range of 2 ms to 100 ms. Pulse train GES failed to evoke action potentials in SMCs with a pulse width of \(\leq 1\) ms, whereas GES with a pulse width of 2–4 ms and a sufficiently high pulse frequency was able to evoke action potentials. These cellular findings may be useful in optimizing stimulation parameters in electrical therapies for obesity and gastroparesis.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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