Physiological function and molecular composition of ATP-sensitive K⁺ channels in human gastric smooth muscle

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Abstract

Gastric motility is controlled by slow waves. In general, the activation of the ATP-sensitive K⁺ (K⁺ATP) channels in the smooth muscle opposes the membrane excitability and produces relaxation. Since metabolic inhibition and/or diabetes mellitus are accompanied by dysfunctions of gastric smooth muscle, we examined the possible roles of K⁺ATP channels in human gastric motility. We used human gastric corpus and antrum smooth muscle preparations and recorded the mechanical activities with a conventional contractile measur-
ing system. We also identified the subunits of the K<sub>ATP</sub> channels using Western blot. Pinacidil (10 μM), a K<sub>ATP</sub> channel opener, suppressed contractions to 30% (basal tone to −0.2 g) of the control. The inhibitory effect of pinacidil on contraction was reversed to 59% of the control by glibenclamide (20 μM), a K<sub>ATP</sub> channel blocker. The relaxation by pinacidil was not affected by a pretreatment with L-arginine methyl ester, tetraethylammonium, or 4-aminopyridine. Pinacidil also inhibited the acetylcholine (ACh)-induced tonic and phasic contractions in a glibenclamide-sensitive manner (42% and 6% of the control, respectively). Other K<sub>ATP</sub> channel openers such as diazoxide, cromakalim and nicorandil also inhibited the spontaneous and ACh-induced contractions. Calcitonin gene-related peptide (CGRP), a gastric neuropeptide, induced muscle relaxation by the activation of K<sub>ATP</sub> channels in human gastric smooth muscle. Finally, we have found with Western blot studies, that human gastric smooth muscle expressed K<sub>ATP</sub> channels which were composed of Kir 6.2 and SUR2B subunits.

**Key words:** human stomach, ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channel, CGRP, gastric antrum and corpus

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**Introduction**

ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels, which open in response to metabolic changes, were firstly characterized in cardiac myocytes (1). To date, K<sub>ATP</sub> channels have been reported to perform important roles in many cells, such as in pancreatic and gastric cells, as well as in vascular smooth muscle cells (2, 3). Since the opening of K<sub>ATP</sub> channels is coupled to intracellular energy metabolism and the ratio of ATP/ADP levels, the probability of K<sub>ATP</sub> channels being open is increased by a decline in the intracellular ATP and decreased by an increase in intracellular ATP levels (1, 2). On the molecular level, K<sub>ATP</sub> channels are composed of two subunits: the K<sup>+</sup>-permeable pore-forming subunit such as Kir6.1 or Kir6.2, and the sulfonylurea receptor subunits, such as SUR1 or SUR2, which have drug-sensitive binding structures. The combination of the two components forms the molecular cassette structure of a K<sub>ATP</sub> channel. There are several types of K<sub>ATP</sub> channels throughout the body. They may be distinguished by differences in structure, electrophysiological characteristics, and pharmacological sensitivities (1, 4–6). In human gastric corpus smooth muscle, Lee et al. found that the Kir 6.2 transcript was expressed rather than Kir 6.1 (7). The effects of K<sup>+</sup> channel openers (KCO), such as pinacidil and cromakalim, and blockers, such as glibenclamide, on smooth muscle have been reported (4–6).

The gastrointestinal (GI) tract plays an important role in food digestion and nutrient ingestion. The digestive motility process starts at the esophagus, which moves down food boluses into the gastric fundus, where the food is stored by receptive relaxation (8). A mix of the food and gastric juice moves down to the gastric antrum where muscle thickness and contractility are much bigger than those in the fundal area (9). Finally, the chyme formed in the stomach reaches the small and then the large intestine (8). This entire process is regulated by intrinsic and phasic contractions called peristalsis, with spontaneous contractions and relaxations of the gut (8). The peristalsis of the gut is produced by slow waves originating from the interstitial cells of Cajal in the GI tract (10, 11). Smooth muscle contractility results from an increased excitability generated by membrane depolarization and Ca<sup>2+</sup> influx during the generation and propagation of slow waves. A decreased excitability as a result of repolarization of the slow waves decreases smooth muscle contractility (12). In the case of regulation of gastric motility by intrinsic factor, many hormones and neuropeptides are known to regulate gastric motility. Among them, calcitonin gene-related peptide (CGRP) which is located in the visceral sensory nerve fibers (12–15), has been reported to produce muscle relaxation and vasodilation (16, 17).
In general, many types of ion channels are responsible for the contraction and relaxation of smooth muscle cells in the GI tract. Excitable neurotransmitters, such as acetylcholine (ACh), produce contractions by increasing excitability through the activation of nonselective cationic channels (NSCC). The activation of NSCCs leads to membrane depolarization then the activation of the voltage-dependent Ca\textsuperscript{2+} channels. From these responses, increased intracellular Ca\textsuperscript{2+} produces contractions (12). In the GI tract, smooth muscle contractions can be blocked by agents such as tetraethylammonium (TEA) and 4-aminopyridine (4-AP), which are known to block Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels and voltage-dependent K\textsuperscript{+} channels, respectively (18, 19). Therefore, each channel associated with excitability and contractility in the GI tract should be examined to elucidate its main physiological action of channel in GI tract. In the guinea pig stomach, K\textsubscript{ATP} channels were isolated that had 37 pS conductance (6). Since K\textsubscript{ATP} channels are known to open under low concentrations of intracellular ATP, a malfunction in metabolism and processes related to the lack of glucose control in diabetic patients could cause the modulation of K\textsubscript{ATP} channels in the stomach. However, the regulatory effects of KCOs and blockers in the human gastric motility have not been studied in detail yet. In addition, the exact mechanism of delayed gastric emptying, one of the common problems caused by hormone and neuropeptides in diabetic patients, is still not understood (8). Therefore, it is crucial to determine the characteristics of K\textsubscript{ATP} channels and their physiological function in the human stomach.

To date, the subtypes of K\textsubscript{ATP} channel and their roles in the human stomach have not been completely identified. Therefore, this study also aimed to characterize the molecular isoforms and the physiological functions of K\textsubscript{ATP} channels in the human gastric smooth muscle of the antrum and corpus.

**Materials and Methods**

*Tissue preparation for isometric contraction*

The experimental protocol for using human stomach was approved by the Institutional Review Board for Clinical Research of Chungbuk National University (CBNU IRB 2008-U01 and 2014-12-012-001). Written informed consent was obtained from all patients who donated their gastric tissue. Human gastric tissues from the greater curvature (tissue samples from corpus and/or antrum) were obtained from 243 patients who underwent gastrectomies between 2011 and 2020 (10, 11, 18–21). Specimens of macroscopically normal tissue in the neoplastic areas were removed immediately after the surgical resection of the stomach. The specimens were placed in Krebs (KRB) solution and pinned down on a Sylgard plate. After the removal of the mucosa and submucosa, muscle strips (0.5 × 2 cm, 0.5 cm thickness) were prepared in a circular muscle direction and mounted in the organ bath (25 ml and 75 ml) of an isometric contractile measuring system. A pathologist identified the smooth muscle cells of the stomach using hematoxylin and eosin staining. In a vertical chamber, one end of the smooth muscle strip was tied tightly to the holder and the other end was linked to a force transducer by a hook-type holder (Harvard, USA). The force transducer was connected to a PowerLab-Data Acquisition System, which was linked to an IBM-compatible computer operated by Charter v5.5 software (ADinstruments, Colorado Springs, CO, USA) to measure isometric contractions. Each strip was stretched passively to resting tension after 1.5–2 h equilibration. Then, the contractile responses of the strips to high K\textsuperscript{+} (50 mM, 10 min) was repeated two or three times until the responses were reproducible.

*Solution and drugs*

KRB solution (CO\textsubscript{2}/bicarbonate-buffered Tyrode) contained the following: 122 mM NaCl, 4.7 mM KCl, 1 mM MgCl\textsubscript{2}, 2 mM CaCl\textsubscript{2}, 15 mM NaHCO\textsubscript{3}, 0.93 mM KH\textsubscript{2}PO\textsubscript{4}, and 11 mM glucose (pH 7.3–7.4, bubbled
with 5% CO2/95% O2. Equimolar Na+ was replaced with K+ to produce a high K+ (50 mM) solution. The external solution was changed every 10 min to a fresh one that had been bubbled with 5% CO2/95% O2, 36 °C before application. A pretreatment with various blockers, such as 4-AP and TEA, was performed for 12–15 min before the treatment with the main agonist. All drugs used in this study were purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA).

**Western blots**

The tissues were fresh-frozen in liquid nitrogen until all samples were collected, and then were homogenized in buffer containing 0.01% (v/v) protease inhibitor cocktail (Sigma-Aldrich, Co.). The tissue homogenates were then centrifuged at 6,000 g at 4 °C for 10 min. The protein concentrations were measured by the Bradford method (Bio-Rad Laboratories, Richmond, CA, USA) using bovine serum albumin as a standard. Equal amounts (20–40 μg) of soluble proteins were separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis at 100 V for 90 min and transferred to polyvinylidene fluoride membranes at 0.25 A for 2 h in a Mini Trans-Blot Cell apparatus (Bio-Rad, Hercules, CA, USA) at 4 °C. The membranes were blocked with 5% skim milk in TBS buffer solution (25 mM Tris-HCl (pH7.4) and 150 mM NaCl) overnight at 4 °C with gentle shaking, followed by incubation with Kir 6.1, Kir 6.2, SUR1, SUR2A, or SUR2B (Santa Cruz Biotechnology, Inc., CA, USA; Millipore; Stress Marq;) antibodies diluted 1:500–1:200 in TBS buffer containing 1% skim milk at room temperature for another hour. After three washes with TBS buffer containing 0.1% Tween-20 (TBST), the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:5,000) diluted in TBS containing 1% skim milk at room temperature for 1 h, followed by three washes with TBST. β-actin antibody (Abfrontier, 1:2,000) and goat anti-rabbit IgG secondary antibody (Santa Cruz Biotechnology, Inc.) were used as a relative loading control. To detect the reactions, the membranes were further treated with ECL (ELPIS) reagent for 1 min and subsequently imaged using a Lass 3000 (FUJIFILM).

**Statistics**

The data are expressed as means ± standard errors of the mean (SEM). The ANOVA, Wilcoxon rank-sum test and Mann-Whitney test were used to measure the statistical significance. *P*-values less than 0.05 were regarded to be statistically significant.

**Results**

**Isometric contractions of human gastric corpus smooth muscle**

Smooth muscle of human gastric smooth muscle from the greater curvature of the corpus produced spontaneous contractions of 0.5 ± 0.09 g (2.0 ± 0.19 cycles/min) (Fig. 1Aa and Ab; *n* =51 and 53, respectively). High K+ (50 mM) produced tonic contraction (Fig. 1B). Figure 1C shows that Bay K 8644, an activator of the voltage-dependent L-type Ca2+ channel, enhanced the strength of the spontaneous contractions to 686 ± 151.98% of the control in a nifedipine-sensitive manner (*P*<0.05; *n* =8).

**ACh-induced contraction of the human gastric corpus smooth muscle**

ACh produced traditional tri-phasic contractions in the human gastric smooth muscle. ACh produced initial (3 ± 1.1 g) and sustained contractions (0.7 ± 0.19 g) superimposed with phasic contractions (1.7 ± 0.71 g; 1.9 ± 0.20 cycles/min) in human gastric smooth muscle preparations (*n* =20, Fig. 2A). Application of Bay K 8644 (0.4 μM) enhanced the phasic contractions in a nifedipine-sensitive manner. Nifedipine at 1, 2 and 5 μM
K<sub>ATP</sub> channel in human stomach

Effects of glibenclamide on the spontaneous phasic contractions of the human gastric smooth muscle

Glibenclamide (20 μM), known to block K<sub>ATP</sub> channel, increased ACh-induced tonic contractions to 147 ± 19.8% of the control (P<0.05; n=6). The results imply that K<sub>ATP</sub> channels might be involved in the regulation of the response to ACh in human gastric smooth muscle.

**Effects of pinacidil on the spontaneous phasic contractions of the human gastric corpus smooth muscle**

To verify the involvement of K<sub>ATP</sub> channels in human gastric smooth muscle, the effects of pinacidil, a known activator of K<sub>ATP</sub> channels, on the spontaneous contraction of human gastric smooth muscle were evaluated. As shown in Fig. 3A, pinacidil (5, 10 μM) inhibited the spontaneous contraction of human gastric smooth muscle. The spontaneous contractions were significantly inhibited to 37 ± 10.2% (to 0.14 ± 0.06 g) and 30 ± 5.7% (to 0.07 ± 0.02 g) of the control (P<0.05; n=13 and 36, respectively; Fig. 3Ab). In addition to the inhibition of spontaneous contractions, pinacidil also produced a tonic relaxation by −0.12 ± 0.04 g and −0.21 ±
0.04 g, respectively (5 and 10 μM; n=13 and 45, respectively). The inhibitory effect of pinacidil was significantly recovered by a treatment with 20 μM glibenclamide, to 59 ± 6.91% of the control (P<0.05; Fig. 3Aa and Ab).

The relaxing effect of pinacidil was studied further to exclude the involvement of other mechanisms. The inhibition of the spontaneous human gastric smooth muscle contractions by pinacidil was not affected by pretreatment with L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) production (38 ± 10.9% of the control; P<0.05; n=7; Fig. 3B) (16, 17). Neither were they affected by pretreatment with TEA (10 mM), a blocker of the Ca²⁺-activated K⁺ channels or by pretreatment with 4-AP (5 mM), a blocker of the voltage-dependent K⁺ channels (16, 17) indicating that these K⁺ channels are not involved in the effect of pinacidil (Fig. 4).
Effects of other \( K_{ATP} \) channel activators on the spontaneous contraction of human gastric corpus smooth muscle preparations

The effects of diazoxide, cromakalim, and nicorandil, known activators of the \( K_{ATP} \) channel were also studied (Fig. 5). Diazoxide (100 and 500 \( \mu \)M) inhibited the spontaneous contraction of human gastric smooth muscle to 37 ± 17.7% and 19 ± 12.4% of the control in a glibenclamide-sensitive (up to 75 ± 31.0% of the control) manner (\( n=5 \) and 6, respectively; Fig. 5A, 5B and 5D). Cromakalim (5 and 20 \( \mu \)M) inhibited the spontaneous contractions to 21 ± 15.6% and 0%, respectively, and treatment with glibenclamide recovered the contractions to 97 ± 16.9% of the control (\( n=4 \); Fig. 5C and 5D). Nicorandil (5 and 10 \( \mu \)M) also inhibited the spontaneous contractions to 69 ± 13.5% and 32 ± 23.8% of the control in a glibenclamide-sensitive manner (\( n=9 \) and 8, respectively; Fig. 5D). Diazoxide (500 \( \mu \)M), cromakalim (10 \( \mu \)M), and nicorandil (10 \( \mu \)M) produced a tonic relaxation of −0.6, −0.26, and −0.21 g in a glibenclamide-sensitive manner (\( n=4, 7, \) and 8, respectively).

Regulatory effects of \( K_{ATP} \) channel activators on ACh-induced phasic contraction of the human gastric corpus smooth muscle

The ACh-induced phasic contractions of the stomach were inhibited by the application of pinacidil, diazoxide, and cromakalim in a glibenclamide-sensitive manner. Pinacidil (10 \( \mu \)M), diazoxide (500 \( \mu \)M), and cromakalim (20 \( \mu \)M) inhibited the ACh-induced phasic contractions to 6.4 ± 3.9%, 14 ± 9.7%, 25 ± 18.2% of the control (\( n=13, 5 \) and 4, respectively; Fig. 6A–6E). Nicorandil (50 \( \mu \)M) inhibited the contractions to 37 ±
23.4% of the control (n=4, Fig. 6E). The inhibition by pinacidil was recovered to 70 ± 8.6% of the control by treatment with glibenclamide (n=8; Fig. 6A and 6D).

**Effects of K<sub>ATP</sub> channel activators on the spontaneous contraction of the human gastric antrum smooth muscle preparations**

The regulation of human gastric antral contractility by K<sub>ATP</sub> channels was studied. Human gastric smooth muscle preparations from the antrum showed spontaneous contractions of 0.4 ± 0.13 g (2.0 ± 0.88 cycles/min)
K<sub>ATP</sub> channel in human stomach

Fig. 5. Effects of K<sub>ATP</sub> channel openers on isometric contraction of human gastric smooth muscle preparations. A. Daizoxide produced relaxation in a concentration-dependent manner in human gastric smooth muscle. B. Daizoxide (200 μM) inhibited spontaneous contraction which was reversed by glibenclamide (20 μM). C. Cromakalim (20 μM) also inhibited spontaneous contraction in a glibenclamide-sensitive manner. D. Inhibitory effects of diazoxide, cromakalim, and nicorandil on spontaneous contraction of human gastric smooth muscle preparations are summarized as a bar graph. Diazoxide (500 μM), Cromakalim (5 μM), and nicorandil (10 μM) inhibited the spontaneous contraction of human gastric smooth muscle preparations to 19%, 21%, and 32% of the control in a glibenclamide-sensitive manner (n=5, 4, and 4, respectively).

(Fig. 7A). High K⁺ conditions produced contractions of 0.8 ± 0.19 g (n=20; data not shown). As shown in Fig. 7A and 7D, pinacidil (20 μM) inhibited the spontaneous and ACh-induced contractions of human gastric smooth muscle preparations from the antrum. Pinacidil (10 μM) inhibited spontaneous contractions to 9 ± 5.7% of the control (n=5; P<0.05; Fig. 7A). Pinacidil (10 and 20 μM) inhibited the ACh-induced contractions to 29 ± 13.8% and 6 ± 5.7% of the control in a glibenclamide-sensitive manner (n=18 and 4, respectively; P<0.05; Fig. 7D). Glib-
Fig. 6. Effects of $K_{ATP}$ channel openers on ACh-induced contraction of human gastric smooth muscle preparations.

A. ACh-induced contraction was inhibited by pinacidil (10 μM) and it was reversed by glibenclamide (20 μM). Similar inhibitory effects of $K_{ATP}$ channel openers on ACh-induced contraction were also observed for diazoxide and cromakalim (B and C). Inhibitory effects of diazoxide and cromakalim on ACh-induced contraction were reversed by glibenclamide. D. Inhibitory effect of pinacidil on ACh-induced phasic contraction of human gastric smooth muscle is summarized as a bar graph. Pinacidil inhibited ACh-induced phasic contraction of human gastric smooth muscle preparations to 6.4% of the control in a glibenclamide-sensitive manner ($n=13$). E. Diazoxide (500 μM), cromakalim (20 μM) and nicorandil (50 μM) also inhibited ACh-induced phasic contractions of human gastric smooth muscle to 14%, 25%, and 37% of the control ($n=5, 4$ and 4, respectively).
enclamide recovered the pinacidil-induced inhibition of ACh-induced contractions to 113 ± 18.4% of the control (n=15, P<0.05; Fig. 7D). Cromakalim (0.5 and 5 μM) also inhibited the spontaneous contractions to 34 ± 10.1% and 21 ± 15.6% of the control (n=6 and 4, respectively; P<0.05; Fig. 7B). As we did in the corpus smooth muscle (Fig. 2C), functional involvement of KATP channel on ACh-induced phasic contractions of human gastric smooth muscle of antrum was also evaluated (Fig. 7C). Glibenclamide (20 μM) increased ACh-induced phasic contractions to 128 ± 4.6% of the control, as it did so in the corpus smooth muscle (n=7; p<0.05). The results imply that KATP channels might be involved in the regulation of the response to ACh in human gastric smooth muscle.

**Effects of CGRP on the human gastric antrum smooth muscle preparations**

As shown in Fig. 8, CGRP inhibited the spontaneous and ACh-induced contractions of the human gastric antrum preparations. As shown in Fig. 8Aa and b, CGRP (100, 200 nM) inhibited the spontaneous contractions to 37 ± 11.8% and 29 ± 8.97% of the control (n=5 and 4, respectively; P<0.05). The contractions were recovered to 56 ± 32.1% of the control by treatment with 20 μM glibenclamide (n=4). The ACh-induced contractions were also inhibited by CGRP (100 nM) to 40 ± 17.0% of the control in a glibenclamide-sensitive manner (n=6; P<0.05; Fig. 8B). In the gastric corpus smooth muscle preparations, the effect of CGRP was relatively weak compared to that of the antrum (data not shown).

**Molecular basis of human KATP channels in human gastric smooth muscle**

The expression of the molecular KATP channel components of the human gastric corpus and antrum smooth muscle preparations were analyzed using Western blots. Kir 6.2 and SUR2B were expressed in both corpus and antrum muscle preparations (Fig. 9). However, the expressions of Kir 6.1, SUR1, and SUR2A were undetected in the samples.
In this study, we firstly reported that K$_{ATP}$ channels of human gastric smooth muscle produce relaxation via the activation of the Kir6.2 and SUR2B subunits. KCOs, such as pinacidil and cromakalim, produced relaxation that was recovered by glibenclamide treatment. CGRP induced relaxation of human gastric smooth muscle, which was recovered by the application of glibenclamide. This implies that K$_{ATP}$ channels might play an important role in the regulation of human gastric motility.

Gastric smooth muscle produces peristalsis to grind and mix boluses with gastric juice to ingest nutrients. Even between meals, the stomach antrum initiates strong contractions called migrating myoelectric motor complexes, which is activated by motilin (8). These entire processes depend on the energy produced by the catalysis of ATP in cells. The breakdown of ATP decreases gastric motility and is tightly linked to constant metabolic changes. The cellular ratio of ATP/ADP during muscle contraction is responsible for the negative feedback control of muscle strength (1, 4). A single K$_{ATP}$ channel was isolated and its ionic current was evalu-

![Effects of CGRP on spontaneous and ACh-induced contraction of human gastric smooth muscle preparations.](image)

Aa and Ab. CGRP produced relaxation of human gastric smooth muscle of the antrum in a glibenclamide-sensitive manner. B. ACh-induced contraction was inhibited by CGRP in a glibenclamide-sensitive manner in human gastric smooth muscle preparations of the antrum.

**Discussion**

In this study, we firstly reported that K$_{ATP}$ channels of human gastric smooth muscle produce relaxation via the activation of the Kir6.2 and SUR2B subunits. KCOs, such as pinacidil and cromakalim, produced relaxation that was recovered by glibenclamide treatment. CGRP induced relaxation of human gastric smooth muscle, which was recovered by the application of glibenclamide. This implies that K$_{ATP}$ channels might play an important role in the regulation of human gastric motility.

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The channel was shown to have 37 pS conductance and to be composed of Kir6.1/SUR2B subunits (6). Intracellular ATP was shown to block the single channel current in guinea pig stomachs. Since K\textsubscript{ATP} channels were isolated in the GI smooth muscle of animals, the next obvious step was to elucidate its molecular identity and physiological function in the human stomach. Moreover, unlike pancreatic β-cells, human GI muscle diseases and disorders involving K\textsubscript{ATP} channels have not been studied yet.

The effects of a number of KCOs, such as pinacidil, cromakalim, diazoxide, and nicorandil, on diverse organs have been studied (4, 6, 22). Their effects were antagonized by glibenclamide, a known K\textsubscript{ATP} channel blocker, in several tissues and cells (4, 6, 22). In the GI tract, KCOs produced activation of K\textsubscript{ATP} channel current and relaxation of guinea pig and rat (6, 23). At the molecular level, KCOs stimulate the SUR subunits in K\textsubscript{ATP} channels, which opens the K\textsuperscript{+} channels. In turn, they oppose membrane excitability by the efflux of K\textsuperscript{+} ions and produce hyperpolarization and relaxation. These phenomena are associated with the inhibition of the voltage-dependent Ca\textsuperscript{2+} channel and muscle relaxation (8). As shown in Figs. 3, 4, 5, 7A, and 7B, pinacidil, cromakalim, and diazoxide produced muscle relaxation and their inhibitory effects were recovered by the application of glibenclamide. Both the spontaneous and ACh-induced phasic contractions of human gastric smooth muscle preparations from both the corpus and antrum (A and B).
preparations were inhibited by the activation of $K_{ATP}$ channels in a glibenclamide-sensitive manner. As $K_{ATP}$ channels are functionally expressed, but not basally activated in human gastric corpus smooth muscle (7), the results strongly suggest that some portion of the ACh-induced phasic contractions was negatively regulated by the activation of $K_{ATP}$ channels in the human gastric smooth muscle preparations. As glibenclamide enhanced the phasic contractions in the presence of ACh, ACh seems to activate $K_{ATP}$ channels which negatively regulate the ACh-induced phasic contractions in human gastric smooth muscle. This phenomenon was also recorded in preparations of the antrum of the human stomach and its effect was stronger than that from the corpus of the human stomach (Fig. 7C). In neurons and cardiac muscle, KCOs are known to protect against damage (1, 4, 24, 25). In the small intestine, diazoxide was shown to attenuate the indomethacin-induced intestinal damage in rats (26). $K_{ATP}$ channels are critical for the homeostasis of glucose via the activation of the release of insulin and glucagon (2, 4). Glucose uptake and/or release by skeletal muscle and the liver are also associated with the function of $K_{ATP}$ channels (4). For these reasons, a malfunction of $K_{ATP}$ channels by denaturation, such as mutations, might give rise to serious problems in the human stomach. In addition, since the human gastric corpus and antrum are important for gastric emptying, major contractions caused by the vagus nerve and its neurotransmitters, such as ACh, might be regulated negatively via activation of $K_{ATP}$ channel function.

As shown in Fig. 8A, CGRP produced relaxation in the human gastric antrum smooth muscle preparations. Its relaxant effect was antagonized by glibenclamide, indicating that CGRP-induced human gastric relaxation was at least partially responsible for the activation of $K_{ATP}$ channels because $K_{ATP}$ channels are not basally activated in human gastric corpus smooth muscle (7). CGRP is a 37-amino acid neuropeptide released from capsaicin-sensitive neurons in the peripheral and central nerves (27–29). Specifically, CGRP is located in the visceral sensory nerve fibers arising in the gut (13, 14). In the GI tract, CGRP is present in the gastric mucosa, myenteric nerve plexus, and muscles (15). CGRP receptors have also been found throughout the antrum and play important physiological roles in the GI, cardiovascular, and other systems (15, 16, 28). CGRP has been reported to produce muscle relaxation and is known to be one of the most potent vasodilators (16, 17). The release of CGRP during abdominal surgery is believed to inhibit GI transit (30, 31). Increased postoperative gastric motility and gastric emptying by specific antibody neutralization of the CGRP receptor has also been reported (32, 33).

As shown in Fig. 8B, CGRP inhibited ACh-induced contractions in a glibenclamide-sensitive manner. In the guinea-pig corpus and rat fundus, inhibitory receptors for CGRP were reported to be related to the sustained relaxation of longitudinal muscles (34). CGRP also inhibited the strong contractions produced by carbachol in isolated guinea pig gastric smooth muscle cells (35). Gastric distention-induced adaptive relaxation and lower esophageal sphincter (LES) relaxation were also reported to be significantly attenuated by CGRP antagonists (36, 37). However, in the guinea pig stomach, gastric adaptive relaxation was reported to be mediated by the release of NO from nerves, not by CGRP (38). In opossum, NO and vasoactive intestinal peptide, not CGRP, produced LES relaxation (37). Therefore, regional- and species-specific relaxant effects of CGRP on gastric smooth muscle might exist.

Gastroparesis (gastric paralysis), where gastric emptying is delayed, is common in diabetic patients (38). Since gastric emptying is controlled by the enteric nerve and motility of the gastric corpus and antrum, a malfunction of the gastric muscle and nerve are responsible for gastroparesis and delayed gastric emptying. Some reports have shown that the changes in CGRP in gastric emptying were indirectly associated with gastric muscular innervation (38). Since the enteric nerves modulate gastric motility, the release of CGRP from the stomach might lead to gastroparesis in diabetic patients. Furthermore, decreased levels of CGRP in diabetic patients might result in decreased blood circulation for muscle contractility, causing gastroparesis. Therefore, more
investigations on the effect of CGRP in the human stomach are needed in the future. In addition, given that the roles of K$_{ATP}$ channels and CGRP in delayed gastric emptying are not well known yet (27), the physiological meaning of CGRP via activation of K$_{ATP}$ channels in the human stomach should be studied more closely.

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**Conflict of Interest**

No conflict of interest exists for this study.

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**References**

1. Noma A. ATP-regulated K$^+$ channels in cardiac muscle. Nature. 1983; 305(5930): 147–8. [Medline] [CrossRef]
2. Cook DL, Hales CN. Intracellular ATP directly blocks K$^+$ channels in pancreatic B-cells. Nature. 1984; 311(5983): 271–3. [Medline] [CrossRef]
3. Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol Rev. 1997; 77(4): 1165–232. [Medline] [CrossRef]
4. Flagg TP, Enkvetchakul D, Koster JC, Nichols CG. Muscle KATP channels: recent insights to energy sensing and myoprotection. Physiol Rev. 2010; 90(3): 799–829. [Medline] [CrossRef]
5. Kyeong KS, Hong SH, Kim YC, Cho W, Myung SC, Lee MY, You RY, Kim CH, Kwon SY, Suzuki H, Park YJ, Jeong EH, Kim HS, Kim H, Lim SW, Xu WX, Lee SJ, Ji IW. Myometrial relaxation of mice via expression of two pore domain acid sensitive K(+) (TASK-2) channels. Korean J Physiol Pharmacol. 2016; 20(5): 547–56. [Medline] [CrossRef]
6. Sim JH, Yang DK, Kim YC, Park SJ, Kang TM, So I, Kim KW. ATP-sensitive K(+) channels composed of Kir6.1 and SUR2B subunits in guinea pig gastric myocytes. Am J Physiol Gastrointest Liver Physiol. 2002; 282(1): G137–44. [Medline] [CrossRef]
7. Lee JY, Ko EJ, Ahn KD, Kim S, Rhee PL. The role of K$^+$ conductances in regulating membrane excitability in human gastric corpus smooth muscle. Am J Physiol Gastrointest Liver Physiol. 2015; 308(7): G625–33. [Medline] [CrossRef]
8. Altaf MA, Sood MR. The nervous system and gastrointestinal function. Dev Disabil Res Rev. 2008; 14(2): 87–95. [Medline] [CrossRef]
9. Yin J, Chen JD. Gastrointestinal motility disorders and acupuncture. Auton Neurosci. 2010; 157(1-2): 31–7. [Medline] [CrossRef]
10. Sung R, Kim YC, Yun HY, Choi W, Kim HS, Kim H, Lee KJ, You RY, Park SM, Youn SJ, Kim MJ, Kim WS, Song YJ, Kim SY, Xu WX, Lee SJ. Interstitial cells of Cajal (ICC)-like-c-Kit positive cells are involved in gastritis and carcinogenesis in human stomach. Oncol Rep. 2011; 26(1): 33–42. [Medline]
11. Yun HY, Sung R, Kim YC, Choi W, Kim HS, Kim H, Lee GJ, You RY, Park SM, Yun SJ, Kim MJ, Kim WS, Song YJ, Xu WX, Lee SJ. Regional distribution of interstitial cells of Cajal (ICC) in human stomach. Korean J Physiol Pharmacol. 2010; 14(5): 317–24. [Medline] [CrossRef]
12. Bolton TB. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev. 1979; 59(3): 606–718. [Medline] [CrossRef]
13. Green T, Dockray GJ. Calcitonin gene-related peptide and substance P in afferents to the upper gastrointestinal tract in the rat. Neurosci Lett. 1987; 76(2): 151–6. [Medline] [CrossRef]
14. Holzer P. Neural emergency system in the stomach. Gastroenterology. 1998; 114(4): 823–39. [Medline] [CrossRef]
15. Cottrell GS, Alemi F, Kirkland JG, Grady EF, Corvera CU, Bhargava A. Localization of calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 1 (RAMP1) in human gastrointestinal...
tinal tract. Peptides. 2012; 35(2): 202–11. [Medline] [CrossRef]

16. Luo XJ, Liu B, Dai Z, Yang ZC, Peng J. Stimulation of calcitonin gene-related peptide release through targeting capsaicin receptor: a potential strategy for gastric mucosal protection. Dig Dis Sci. 2013; 58(2): 320–5. [Medline]

17. Bartho L, Benko R, Holzer-Petsche U, Holzer P, Undi S, Wolf M. Role of extrinsic afferent neurons in gastrointestinal motility. Eur Rev Med Pharmacol Sci. 2008; 12(Suppl 1): 21–31. [Medline]

18. Kim DH, Kim YC, Choi W, Yun HY, Sung R, Kim HS, Kim H, Yoo RY, Park SM, Yun SJ, Song YJ, Xu WX, Lee SJ. High K(+) -induced relaxation by nitric oxide in human gastric fundus. Korean J Physiol Pharmacol. 2012; 16(5): 297–303. [Medline] [CrossRef]

19. Kim YC, Choi W, Yun HY, Sung R, Yoo RY, Park SM, Yun SJ, Kim MJ, Song YJ, Xu WX, Lee SJ. Nitric oxide-mediated relaxation by high K+ in human gastric longitudinal smooth muscle. Korean J Physiol Pharmacol. 2011; 15(6): 405–13. [Medline] [CrossRef]

20. Kim YC, Choi W, Sung R, Kim H, You RY, Park SM, Youn SJ, Kim MJ, Song YJ, Xu WX, Lee SJ, Yun HY. Relaxation patterns of human gastric corporal smooth muscle by cyclic nucleotides producing agents. Korean J Physiol Pharmacol. 2009; 13(6): 503–10. [Medline] [CrossRef]

21. Lee S.E., Kim D.H., Kim Y.C., Han J.H., Choi W, Kim C.H., Jeong HW, Park SM, Yun SJ, Choi SY, Sung R, Kim YH, Yoo RY, Sun PH, Kim H, Song YJ, Xu WX, Yun HY, Lee SJ. H. H(2) receptor-mediated relaxation of circular smooth muscle in human gastric corpus: the role of Nitric Oxide (NO). Korean J Physiol Pharmacol. 2014; 18(5): 425–30. [Medline] [CrossRef]

22. Hong SH, Kyeong KS, Kim CH, Kim YC, Choi W, Kim HS, Park YJ, Ji IW, Jeong EH, Kim HS, Xu WX, Lee SJ. Regulation of myometrial contraction by ATP-sensitive potassium (KATP) channel via activation of SUR2B and Kir 6.2 in mouse. J Vet Med Sci. 2016; 78(7): 1153–9. [Medline] [CrossRef]

23. Shimbo T, Adachi T, Fujisawa S, Hongoh M, Ohba T, Ono K. In vitro effect of nicorandil on the carbachol-induced contraction of the lower esophageal sphincter of the rat. J Pharmacol Sci. 2009; 131(4): 267–74. [Medline] [CrossRef]

24. Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G, H overlook M, Liersch R, Schulze-Bahr E, Wilde A, Kääb S, Koster J, Rudy Y, Le Marec H, Schott JJ. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. J Cardiovasc Electrophysiol. 2009; 20(1): 93–8. [Medline] [CrossRef]

25. Ohno T, Hattori Y, Komine R, Ae T, Mizuguchi S, Ara i K, Saeki T, Suzuki T, Hosono K, Hayashi I, Oh-Hashi Y, Kurihara Y, Kurihara H, Amagase K, Okabe S, Saigenji K, Majima M. Roles of calcitonin gene-related peptide in maintenance of gastric mucosal integrity and in enhancement of ulcer healing and angiogenesis. Gastroenterology. 2008; 134(1): 215–25. [Medline] [CrossRef]

26. Menozzi A, Pozzoli C, Poli E, Passeri B, Gianelli P, Bertini S. Diazoxide attenuates indomethacin-induced small intestinal damage in the rat. J Pharmacol Sci. 2011; 650(1): 378–83. [Medline] [CrossRef]

27. Clark SY, Gangula PR. Role of calcitonin gene-related peptide in gastric motility function: animal and human studies. J Gastrointest Dig Syst. 2015; 5: 2.

28. Ghatta S, Nimmagadda D. Calcitonin gene-related peptide: understanding its role. Indian J Pharmacol. 2004; 36(5): 277–83.

29. Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature. 1983; 304(5922): 129–35. [Medline] [CrossRef]

30. Zittel TT, Reddy SN, Plourde V, Raybould HE. Role of spinal afferents and calcitonin gene-related peptide in the postoperative gastric ileus in anesthetized rats. Ann Surg. 1994; 219(1): 79–87. [Medline] [CrossRef]

31. Zittel TT, Lloyd KC, Rothenhöfer I, Wong H, Walsh JH, Raybould HE. Calcitonin gene-related peptide and spinal afferents partly mediate postoperative colonic ileus in the rat. Surgery. 1998; 123(5): 518–27.
K$_{ATP}$ channel in human stomach

32. Glowka TR, Steinebach A, Stein K, Schwandt T, Lysson M, Holzmann B, Tsujikawa K, de Jonge WJ, Kalff JC, Wehner S. The novel CGRP receptor antagonist BIBN4096BS alleviates a postoperative intestinal inflammation and prevents postoperative ileus. Neurogastroenterol Motil. 2015; 27(7): 1038–49. [Medline] [CrossRef]

33. Plourde V, Wong HC, Walsh JH, Raybould HE, Taché Y. CGRP antagonists and capsaicin on celiac ganglia partly prevent postoperative gastric ileus. Peptides. 1993; 14(6): 1225–9. [Medline] [CrossRef]

34. Katsoulis S, Conlon JM. Calcitonin gene-related peptides relax guinea pig and rat gastric smooth muscle. Eur J Pharmacol. 1989; 162(1): 129–34. [Medline] [CrossRef]

35. Chijiiwa Y, Kabemura T, Misawa T, Kawakami O, Nawata H. Direct inhibitory effect of calcitonin gene-related peptide and atrial natriuretic peptide on gastric smooth muscle cells via different mechanisms. Life Sci. 1992; 50(21): 1615–23. [Medline] [CrossRef]

36. Taniguchi M, Mashita Y, Matsuzaka Y, Kato S, Takeuchi K. Role of capsaicin-sensitive afferent neurons in receptive relaxation induced by gastric distension in rats. Inflammopharmacology. 2007; 15(6): 273–7. [Medline] [CrossRef]

37. Uc A, Oh ST, Murray JA, Clark E, Conklin JL. Biphasic relaxation of the opossum lower esophageal sphincter: roles of NO., VIP, and CGRP. Am J Physiol. 1999; 277(3): G548–54. [Medline]

38. Uc A, Murray JA, Conklin JL. Effects of calcitonin gene-related peptide on opossum esophageal smooth muscle. Gastroenterology. 1997; 113(2): 514–20. [Medline] [CrossRef]