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Original

The inhibitory effect of somatostatin on gastric motility in *Suncus murinus*

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Abstract

Gastric contractions show two specific patterns in many species, migrating motor contractions (MMC) and postprandial contractions (PPCs), that occur in the fasted and fed states, respectively. In this study, we examined the role of somatostatin (SST) in gastric motility both *in vivo* and *in vitro* using the Asian house shrew (*Suncus murinus*). We performed *in vivo* recordings of gastric motility and *in vitro* organ bath experiments using *S. murinus*, which was recently established as a small laboratory animal for use in tests of gastrointestinal motility. SST (1.65 μg kg^{-1} min^{-1}) was intravenously administered during phase II of MMC and PPCs. Next, the effect of SST on motilin-induced gastric contractions at phase I of MMC was measured. Cyclosomatostatin (CSST), an SST receptor antagonist, was administered at the peak of phase III of MMC. In addition, the effect of SST (10^{-11}–10^{-9} M) on motilin-induced gastric contractions was evaluated using an organ bath experiment *in vitro*. In conscious, free-moving *S. murinus*, the administration of SST decreased the occurrence of the spontaneous phase II of MMC and PPCs. Pretreatment with SST and octreotide suppressed the induction of motilin-induced gastric contractions both *in vivo* and *in vitro*. Administration of CSST before the peak of spontaneous phase III contractions had no effect on gastric contractions. Endogenous SST is not involved in the regulation of gastric MMC and PPCs, but exogenous SST suppresses spontaneous gastric contractions. Thus, SST would be good for treating abnormal gastrointestinal motility disorders.

Key words: somatostatin, motilin, *Suncus murinus*, MMC (migrating motor contractions)

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Introduction

In humans and dogs, characteristic gastric motility patterns occur in the fasted and fed states. The migrating motor contraction (MMC) patterns observed in the fasted state comprise three phases: a motor quiescent period (phase I), an irregular and low-amplitude contraction period (phase II), and a strong contraction period (phase III) (1). MMC is thought to be physiologically important for intraluminal cleaning to allow the gastrointestinal tract (GI) to receive the next meal and preventing bacterial overgrowth (2). On the other hand, postprandial contractions (PPCs) that are initiated immediately after eating are composed of a rhythmic contraction and a subsequent postprandial giant contraction (PPGC). In the stomach, it has been demonstrated that the latter half of the PPCs and the PPGC had the same properties as those of phase II and phase III contractions of MMC, respectively (3). Due to the lack of a suitable animal model to mimic human responses, the detailed mechanisms of gastric motility, especially inhibitory mechanisms, remain to be elucidated.

The small experimental animal *Suncus murinus*, also known as the Asian house shrew and referred to as 'suncus' in a laboratory context, is useful for research on GI motility (4), and detailed physiological and pharmacological experiments using this animal have expanded our understanding of the regulatory mechanisms controlling GI contractions (5). The time intervals of MMC cycles in freely moving, fasted *S. murinus* are similar to those observed in humans and dogs (90–120 min) (4). Moreover, motilin, an important GI hormone that induces gastric phase III of MMC, and its receptor (G protein-coupled receptor 38, GPR38) have already been identified in *S. murinus*, but not in rodents (6). Also, ghrelin, an orexigenic peptide in the same peptide family as motilin, and its receptor (growth hormone secretagogue receptor, GHSR), have been found to regulate gastric MMC and PPCs (7). Administration of exogenous ghrelin during phase I induces a phase II-like low-amplitude and irregular contraction in the stomach, and administration of a ghrelin receptor antagonist in phase II suppresses gastric phase II contractions (8). Phase II contractions have also been shown to be attenuated after vagotomy (9). These studies suggested that gastric phase II contractions are mainly controlled by ghrelin and the vagus nerve. It is also known that the administration of exogenous motilin in phase I of MMC induces phase III-like strong contractions in the stomach, and administration of a motilin receptor antagonist abolishes gastric phase III contractions (10). In addition, it has been reported that continuous administration of a ghrelin receptor antagonist delays spontaneous gastric phase III contractions and suppresses the effect of exogenous motilin (11). These findings indicate that coordination between motilin and ghrelin is necessary for inducing the spontaneous gastric phase III contraction during MMC.

Among the several gut hormones known to be involved in regulating GI contractions, somatostatin (SST) is known to have particularly notable inhibitory effects on gastric motility. SST is a cyclic peptide consisting of 14 or 28 amino acid residues (12). SST is widely distributed throughout the body and is abundant in the central nervous system, such as in the hypothalamus and cerebral cortex, as well as in peripheral tissues, such as the stomach, small intestine, and pancreas (13). The major known functions of SST are to inhibit the secretion of endocrine hormones, such as growth hormone, inhibit gastric acid secretion, and suppress nutrient absorption from the GI tract (14). In humans, SST is known to suppress gastric antral contraction (15), and octreotide, an SST analog with high affinity for SST receptors (SSTRs) 2, 3, and 5, also inhibits gastric antral contraction (16). Also, SST has been reported to inhibit ghrelin and motilin secretion, and plasma SST levels in the fasted state tend to be high near the peak of phase III of MMC and low in phase I (17–21). Collectively, these data suggest that SST plays an important role in regulating gastric motility. However, the inhibitory effects of SST on MMC and PPCs are still poorly understood.

In this study, we administered SST or cyclosomatostatin (CSST), an SST receptor antagonist, to free mov-

ing *S. murinus* to determine the effects of SST on several phases of gastric contraction *in vivo*. In addition, we also studied the underlying mechanisms of SST's effects on motilin-induced gastric contractions using both octreotide *in vivo* and *in vitro* organ bath experiments.

Materials and Methods

Drugs used

Acetylcholine chloride (Sigma, USA) was dissolved in distilled water. Synthesized *S. murinus* motilin (GenScript, Tokyo, Japan), human acyl ghrelin (Asubio Pharma Co., Ltd., Hyogo, Japan), somatostatin-14, (Bachem, Torrance, CA, USA), and octreotide (an SST analog; Abcam, Cambridge, UK) were dissolved in 0.1% bovine serum albumin/phosphate-buffered saline (BSA/PBS). CSST (Abcam, Cambridge, UK) was dissolved in DMSO (0.1% of final concentration). All reagents were freshly prepared immediately before each experiment, according to the manufacturer's instructions.

Animals

Experiments were performed using Asian house shrews of either sex (10–20 weeks of age, weighing 40–110 g) belonging to the outbred 'KAT' strain established from a wild population in Kathmandu, Nepal. Animals were housed individually in plastic cages equipped with an empty can for a nest under controlled environmental conditions (23 ± 2 °C, with the lights on from 8:00 to 20:00), with free access to water and commercial trout pellets (number 5P; Nippon Formula Feed Manufacturing, Yokohama, Japan). The energy content of the pellets was 344 kcal/100 g. The pellets contained 54.1% protein, 30.1% carbohydrates, and 15.8% fat. All procedures were approved by and performed in accordance with the guidelines of the Committee on Animal Research of Saitama University (approval number: H31-A-1-15). All possible efforts were made to minimize animal suffering and reduce the number of animals used in the experiments.

Animal surgery and recording gastric motility in conscious animals

After fasting for 3–5 h, each animal was anesthetized with an intraperitoneal (IP) injection of a combination of midazolam (4 mg kg^{-1}; Sandoz K.K., Yamagata, Japan), Domitor (0.3 mg kg^{-1}; Nippon Zenyaku Kogyo Co., Fukushima, Japan), and Vetorphale (5 mg kg^{-1}; Meiji Seika Pharma Co., Tokyo, Japan). The methodology used for animal surgery, transducer implantation, and recording GI motility followed that described in a previously published study (8). In brief, a strain gauge force transducer developed in our laboratory was passed through a midline laparotomy and sutured to the dorsal portion of the lower gastric body to record measurements of circular muscle contractions. Waterproofing and response properties were checked in all transducers before implantation. A silicon-coated wire (RSF 66/0.03; Sanyo Electric Wire, Osaka, Japan) from the transducer was exteriorized through the abdominal wall and passed under the skin toward the back of the middle of the neck.

For the intravenous (IV) infusion of either drugs or a vehicle, a silicone tube (1.0 mm outer diameter × 0.5 mm inner diameter; Kaneka Medics, Osaka, Japan) was inserted into the right jugular vein and exteriorized to the back of the neck on the right side. All animals were allowed a post-surgery recovery period of at least 2 days prior to the start of the experiment. In the feeding experiment, the shrews were kept in a fasted state for approximately 7 h and then administered commercial trout pellets in phase I of MMC.

GI motility was measured in conscious, freely moving animals. To record GI motility, wires from the transducer were connected to an amplifier, and then the amplified signals were converted by an analog-digital converter (ADC-20/24, Pico Technology Ltd., St. Neots, UK). The digital signals were then recorded by Pico-

Log software (Pico Technology Ltd.) with a sampling interval of 100 ms. The recorded signals were processed, with a frequency cutoff of 10 Hz, using Chart 5 Reader software (ADInstruments, Ltd., Dunedin, NZ).

Timing of drug administration in vivo

SST (1.65 μg kg^{-1} min^{-1}) was continuously infused to the test animals by IV for 2 h starting 10–20 min after the initiation of phase II of MMC or PPCs. The same volume of 0.1% BSA/PBS was administered as a vehicle.

To observe the effects of SST or octreotide on gastric motilin-induced contractions, SST (1.65 μg kg^{-1} min^{-1}) or octreotide (10 ng kg^{-1} min^{-1}) infusion by IV was initiated 10–20 min after the completion of the spontaneous phase III contractions and maintained for 30 min. A bolus dose of motilin (300 ng kg^{-1}) was injected intravenously 20 min after the start of the IV infusion of SST or octreotide.

CSST (1 μg kg^{-1}) was administered intravenously by bolus injection at the peak of the spontaneous phase III of MMC.

In vitro organ bath experiment

After being deeply anesthetized with diethyl ether, the stomachs of test animals were removed and immediately placed into freshly prepared Krebs solution (composition in mM: NaCl, 118; KCl, 4.75; $CaCl_2$, 2.5; $MgSO_4$, 1.2; NaH_2PO_4, 1.8; $NaHCO_3$, 25; and glucose, 11.5; pH: 7.2). The mesentery attachments and fatty tissues were removed, and the inside of each stomach was washed with Krebs solution through a small incision in the gastric fundus. The stomachs were then mounted in 10 ml water-jacketed organ baths and initially loaded with the weight totaling approximately 1.0 g. The temperature of the Krebs solution was maintained at 37 ± 0.5°C, and the solution was aerated continuously with a mixture of 95% O_2 and 5% CO_2.

The contractile activity of the stomach in response to motilin treatment was monitored using an isometric force transducer (UM-203; Iwashiya Kishimoto Medical Instruments, Kyoto, Japan) and specialized software (PicoLog for Windows; Pico Technology Ltd., St. Neots, UK). To normalize the contractions measured in this experiment, we added acetylcholine (ACh; 10^{-5} M) to the organ bath twice before the cumulative administration of motilin in the absence or presence of SST (10^{-11}–10^{-9} M). At the end of the experiment, ACh (10^{-5} M) was added to the organ bath once again, and the percent occurrence of different contraction responses was calculated by averaging the tonic responses induced by these three administrations. It should be noted that the ACh administration evoked almost the same tonic gastric contractions in each case. The effects of motilin in the absence or presence of SST were then expressed as percentages relative to the frequencies of the control contractions. Concentration-response bar graphs were made using data obtained after the cumulative addition of motilin with or without SST at appropriate intervals to the organ bath. To examine the role of cholinergic effects on the SST pathway, ACh (10^{-5} M) was administered after pretreatment with SST.

Statistical analyses

The definition of each contraction type and the statistical analyses performed followed those used in a previous study (3). PPCs were defined as the continuous irregular and low-amplitude contractions that began after feeding and lasted to the initiation of phase I of MMC, and PPGCs were defined as the strong contractions that occurred at the end of the PPCs, prior to phase I of MMC. Gastric motility was quantified by calculating the motility index (MI; %) as the percentage of the area under the curve (AUC), which was equivalent to the integrated area between the contractile wave and the baseline of the adjacent phase I contraction. For the data shown in Figs. 1 and 2, the MI was calculated as the AUC for the 30 min from the start to continuous SST or

vehicle infusion divided by the AUC from the start to the end of the endogenous phase III contraction that occurred prior to SST or vehicle infusion. For the data in Fig. 3, the MI was calculated as the AUC for the 10 min from the start to motilin administration divided by the AUC from the start to the end of the endogenous phase III contraction that occurred prior to vehicle, SST, or octreotide infusion. For the data in Fig. 4, the percentage of contraction responses induced by ACh was calculated by averaging the tonic response induced by each of the three individual administrations. For the data in Fig. 6, the MI was calculated as the AUC from the start to the CSST administration at the end of phase III divided by the AUC from the start to the vehicle administration during the endogenous phase III contraction. Four to seven animals were used in each experiment. All data are presented as mean ± standard error of the mean (S.E.M.) values. Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Student's *t* tests were used to analyze all data except for those presented in Fig. 4, for which a two-way analysis of variance (ANOVA) was used followed by post-hoc comparisons with Bonferroni's test.

Results

Effects of SST on phase II contractions and PPCs

We administered SST in phase II of MMC for 2 h. Although vehicle infusion did not change gastric motility (Fig. 1A), SST (1.65 μg kg^{-1} min^{-1}) infusion significantly attenuated the occurrence of spontaneous gastric contractions in phase II of MMC (Fig. 1B). The MI during the 30 min after SST administration was significantly decreased with SST (−0.3 ± 0.6%) compared to that after vehicle administration (35.4 ± 9.6%) (Fig. 1C). In addition, SST and vehicle were infused intravenously for 2 h during PPCs. Infusion with the vehicle resulted in no change (Fig. 2A), but SST infusion suppressed the spontaneous contractions occurring during the PPCs (Fig. 2B). Statistical analysis results showed that the MI following SST administration (1.9 ± 6.1%) was lower than that following vehicle administration (22.5 ± 5.6%) (Fig. 2C).

Effects of SST and octreotide on motilin-induced gastric contractions

Although administration of motilin (300 ng kg^{-1}) during vehicle infusion at phase I of MMC induced strong phase III-like contractions in the stomach (Fig. 3A), the same dose of motilin during infusion of SST (1.65 μg kg^{-1} min^{-1}) did not induce strong contractions (Fig. 3B). SST significantly decreased motilin-induced contractions in the motility index (89% decrease vs. vehicle, Fig. 3C). Similarly, motilin induced strong contractions during vehicle infusion at phase I of MMC; however, infusion of octreotide (10 ng kg^{-1} min^{-1}) inhibited motilin-induced contractions (Fig. 3D and E). The motility index was significantly decreased by octreotide (98% vs. vehicle, Fig. 3F).

Effects of SST on motilin-induced gastric contraction in vitro

Addition of 10^{-9} M motilin started to evoke strong contractions *in vitro* without SST (Fig. 4A). In contrast, motilin induced strong contractions from 10^{-8} M in the presence of 10^{-11} or 10^{-10} M SST (Fig. 4B and C). In addition, strong contractions were caused by 10^{-7} M motilin with a pretreatment of 10^{-9} M SST (Fig. 4D). Analysis of contraction responses showed that pretreatment with SST inhibited 10^{-9} M motilin-induced contractions in a dose-dependent manner, and 10^{-9} M SST decreased 10^{-8} M motilin-induced contractions by 21% (Fig. 4E). However, contractions induced by a higher concentration of motilin (10^{-7} M) were not inhibited by pretreatment with SST. Administration of 10^{-5} M ACh immediately induced strong contractions (Fig. 5A), and pretreatment with SST (10^{-9} M) had no effect on ACh-induced contractions (Fig. 5B and C).

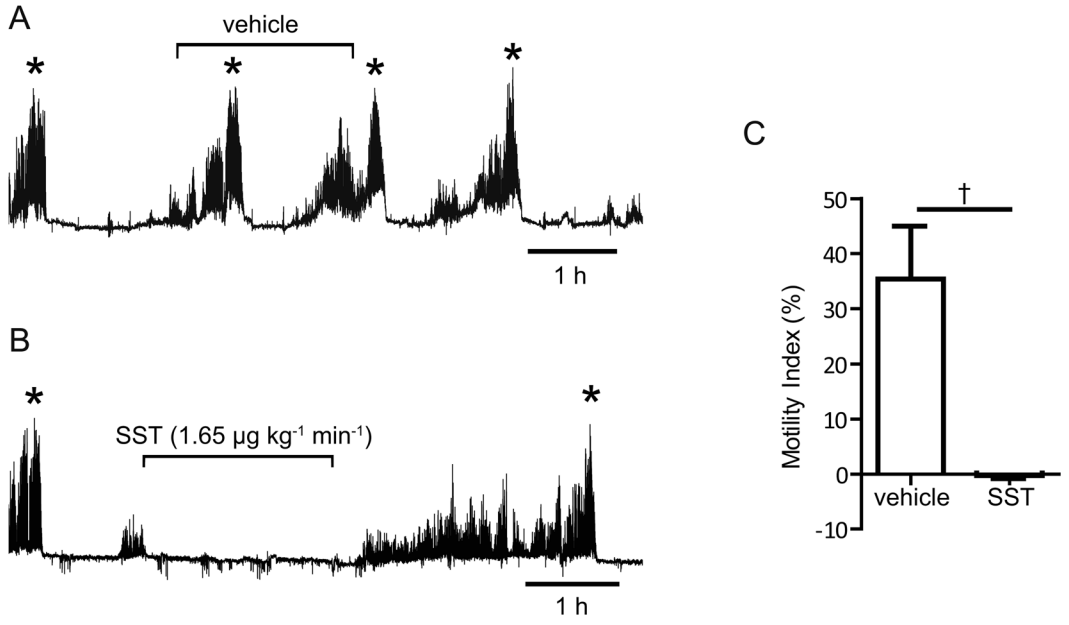

Fig. 1. Effects of SST on phase II of MMC.
Vehicle or SST (1.65 μg kg^{-1} min^{-1}) was intravenously infused for 2 h during phase II of MMC in the same animal. The continuous infusion of vehicle did not change the occurrence of spontaneous gastric contractions (A). On the other hand, SST immediately inhibited gastric contractions in phase II (B), and the MI was significantly decreased (C). Each bar represents the mean ± S.E.M. (n=4) of a treatment group. Statistical analysis was performed using paired t test. †: P<0.05 vs. vehicle; *: phase III contractions.

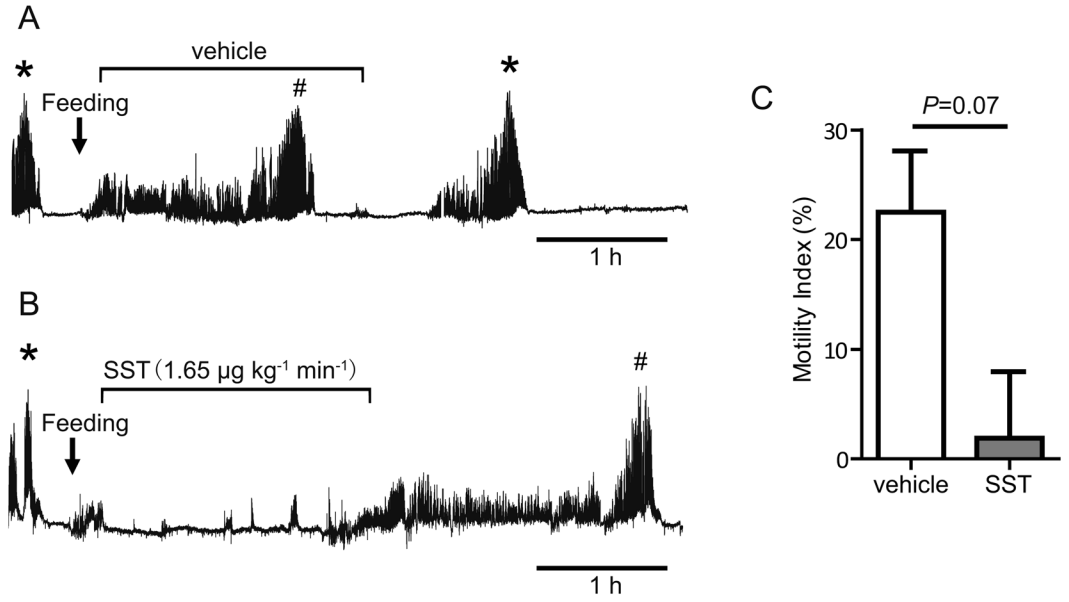

Fig. 2. Effects of SST on PPCs.
Vehicle or SST (1.65 μg kg^{-1} min^{-1}) was intravenously infused for 2 h during PPCs. Although the continuous infusion of vehicle did not change gastric contractions (A), SST immediately inhibited gastric contractions during PPCs (B). The MI was decreased by SST administration (C). Each bar represents the mean ± S.E.M (n=4) of a treatment group. Statistical analysis was performed using paired t test. *: phase III contractions; #: PPGC.

Effects of CSST on endogenous phase III gastric contractions

Phase III is a series of periods that elicit rapid and strong contractions and terminate quickly. To examine whether SST involves the termination of endogenous gastric phase III contractions, CSST (1 μg kg^{-1}) was administered intravenously by bolus injection at the peak of the spontaneous phase III contractions. CSST treatment had no effect on the gastric phase III contractions observed (Fig. 6A–C).

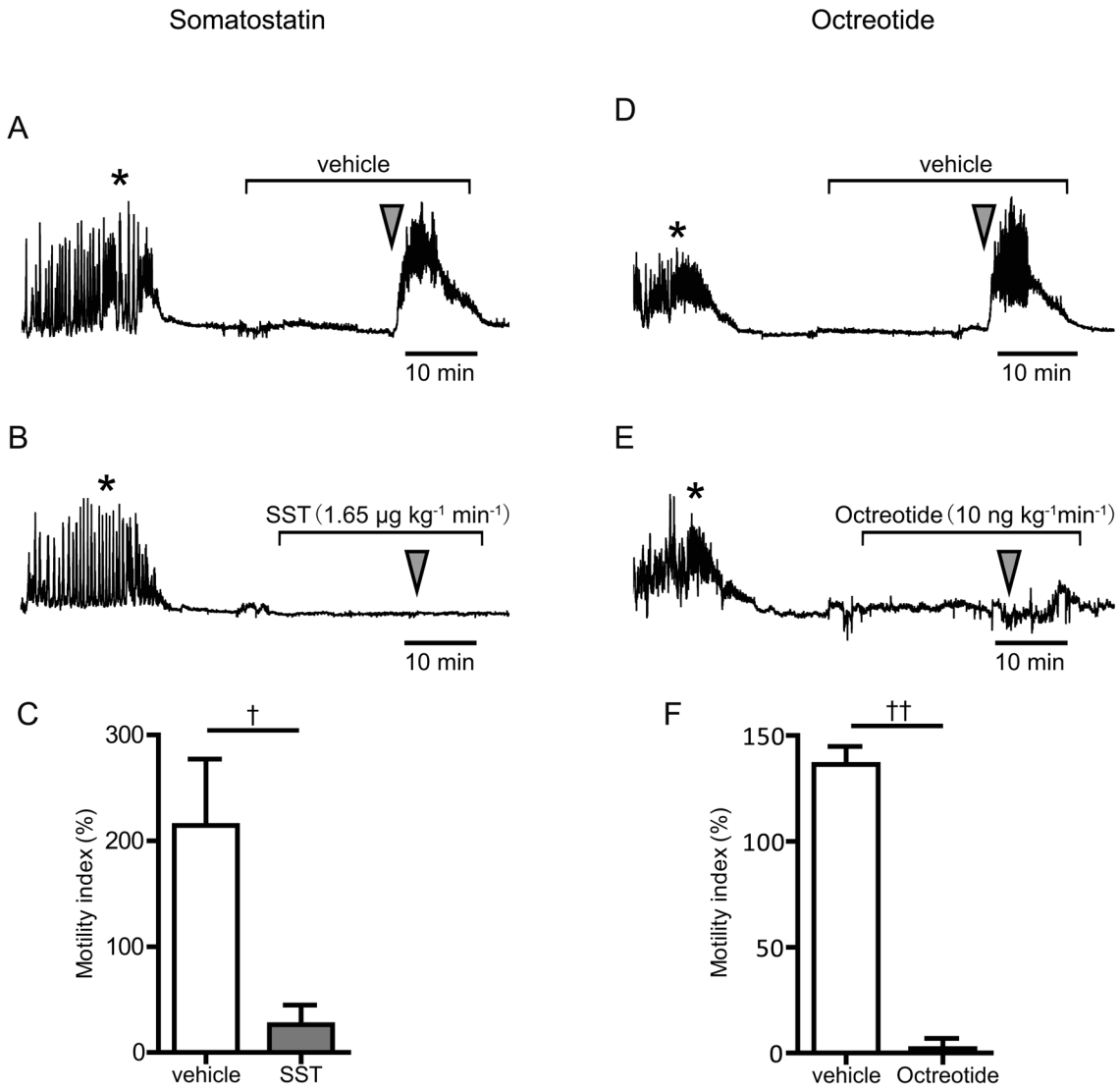

Fig. 3. Effects of SST and octreotide on motilin-induced gastric contractions.
Vehicle or SST (1.65 μg kg^{-1} min^{-1}) was continuously infused for 30 min, beginning at the end of spontaneous phase III contractions. Motilin (300 ng kg^{-1}) was administered 20 min after the start of infusion (grey triangles). Although motilin induced phase III-like contractions during infusion with vehicle (A), infusion with SST significantly attenuated motilin-induced contractions (B). The MI during the 10 min after motilin administration was significantly decreased (C). We conducted the same experiment using octreotide (10 ng kg^{-1} min^{-1}) instead of SST. Motilin administration stimulated gastric phase III-like contractions during infusions with vehicle (D). However, motilin-induced contractions did not occur during octreotide infusion (E). The MI was again significantly decreased after motilin administration (F). Each bar represents the mean ± S.E.M. (n=4) of a treatment group. Statistical analysis was performed using paired t test. †: P<0.05 vs. vehicle; ††: P<0.01 vs. vehicle; *: phase III contractions

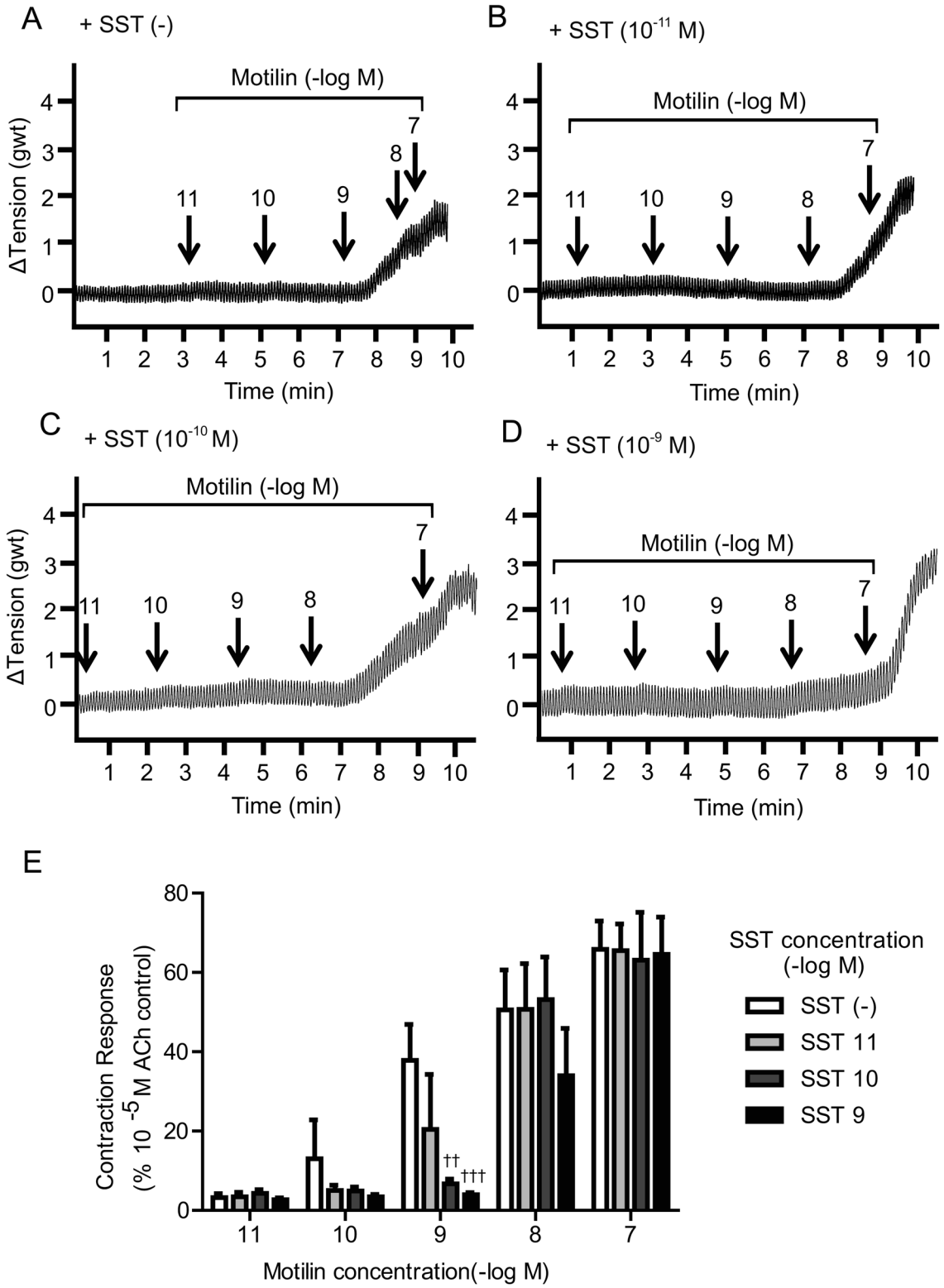

Fig. 4. Effects of SST on motilin-induced gastric contractions *in vitro*.
Motilin induced gastric contractions in a dose-dependent manner (10^{-9}–10^{-7} M) (A). Pre-treatment with SST reduced the occurrence of motilin-induced gastric contractions (B, C, and D). SST (10^{-10} and 10^{-9} M) significantly inhibited motilin-induced gastric contractions at doses of 10^{-9} M motilin (E). Each bar represents the mean ± S.E.M. (n=4) of a treatment group. Statistical analysis was performed as a two-way ANOVA with post-hoc comparisons using Bonferroni's test. ††: P<0.01 vs. motilin; †††: P<0.001 vs. motilin for 10^{-9} M SST (−).

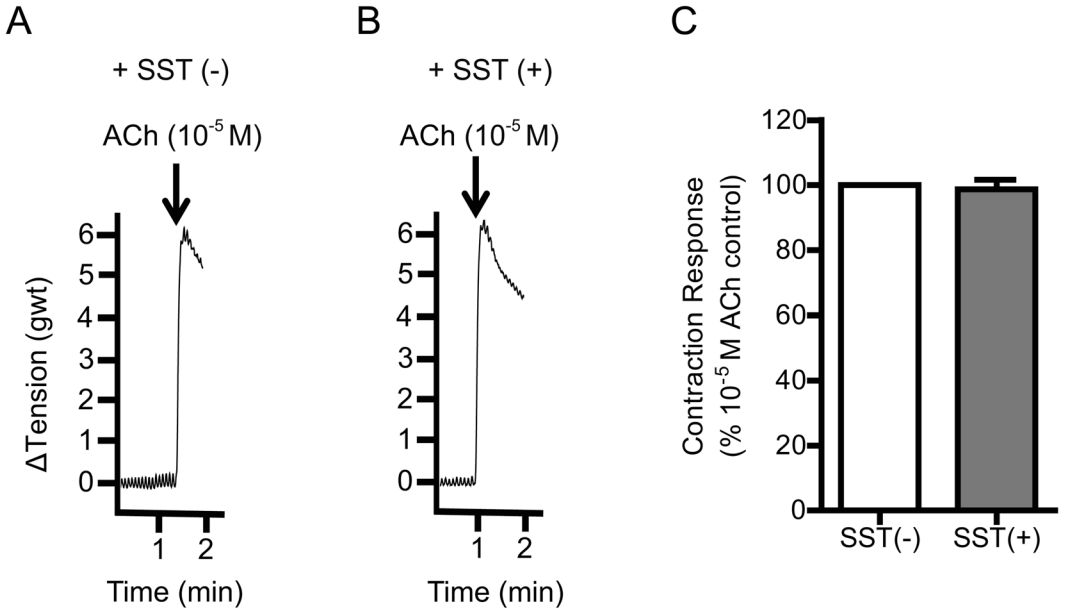

Fig. 5. Effects of SST on ACh-induced gastric contractions *in vitro*.
ACh (10^{-5} M) evoked strong gastric contractions (A). Pre-treatment with SST (10^{-9} M) had no effect on ACh-induced gastric contractions (B, C). Each histogram represents the mean ± S.E.M. (n=4) of a treatment group. Statistical analysis was performed as using paired t test.

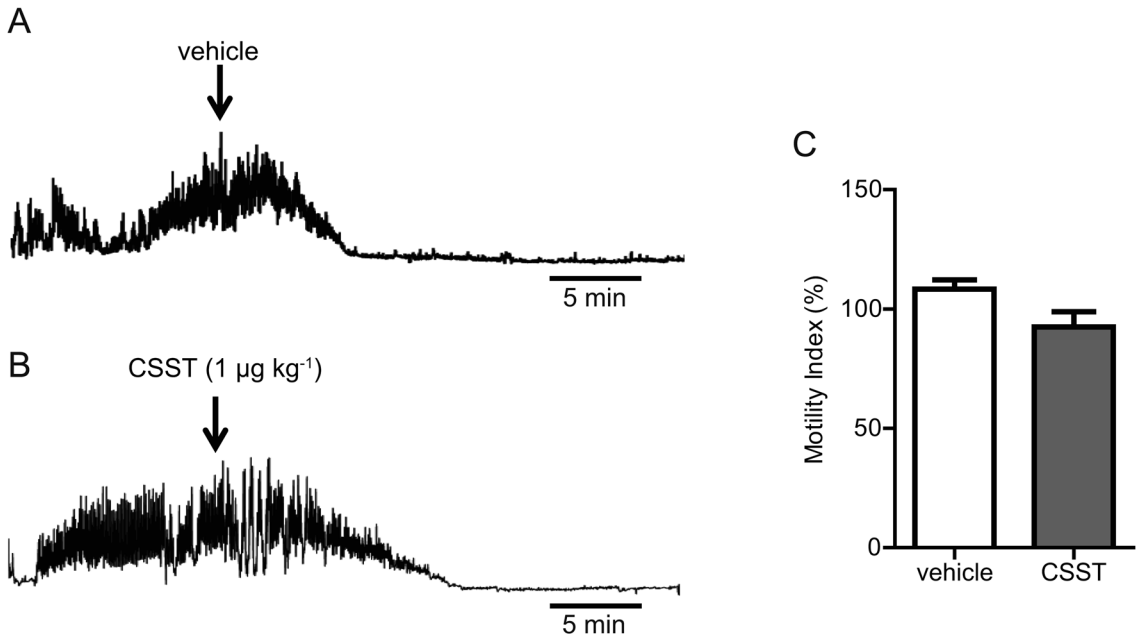

Fig. 6. Effects of CSST on phase III of MMC.
An intravenous bolus administration of vehicle had no effect on phase III contractions (A). CSST (1 μg kg^{-1}) administration at the peak of phase III of MMC did not change the nature of these contractions (B). The MI after administration during phase III did not differ significantly between groups (C). Each bar represents the mean ± S.E.M. (n=4) of a treatment group. Statistical analysis was performed using paired t test.

Discussion

In this study, we showed that the occurrence of spontaneous phase II contractions during MMC and PPCs was suppressed by the continuous administration of SST. Previous studies from our laboratory have shown that vagotomy suppressed both phase II of MMC and PPCs (9). It has been shown that several factors are involved in the regulation of vagus afferent nerves. For instance, in the rat jejunum, octreotide and a selective SSTR2 agonist have been reported to inhibit spontaneous afferent firing by activating SSTR2, which is located at the peripheral terminal of the primary afferent nerve (22). Moreover, GHSR mRNA was expressed in the nodose ganglion, and SST is known to suppress ghrelin secretion (17). Therefore, it is possible that SST suppresses phase II of MMC and PPCs by binding to the SSTR on vagus afferent nerves or by inhibiting ghrelin secretion. Other possible explanations for SST-induced inhibition of gastric contraction involve the enteric nervous system and non-adrenergic non-cholinergic nerves. A previous study revealed that ghrelin-induced gastric contractions are completely inhibited by hexamethonium, a nicotinic ACh receptor antagonist, and significantly suppressed by naloxone, a blocker of the effects of opioids, during *in vitro* organ bath experiments using *S. murinus* (23). L-NAME (N omega-nitro-L-arginine methyl ester), a nitric oxide (NO) synthase inhibitor, has been shown to enhance GI contractions (23). These results indicate that ghrelin-induced contractions are regulated through cholinergic neurons, opioidergic neurons, and nitric oxide synthase. In rats, it has been reported that the release of SST, NO, and GABA (gamma-aminobutyric acid) was increased during colon relaxation, and the release of [Met]-enkephalin reduced colonic motility (24). In addition, treatment with SST increases the release of GABA and NO and suppresses the release of [Met]-enkephalin from the colon (24). In the small intestine of guinea pigs, secretion of ACh from the myenteric plexus is suppressed by SST (25). These reports suggest that SST may suppress phase II contractions through direct inhibition of ACh secretion or non-adrenergic non-cholinergic nerves. The detailed neural pathway of the inhibitory effect of SST should be explored in the future using several *in vitro* methods (e.g., electrical field stimulation).

Since motilin induces phase III contractions (6), the effect of SST on motilin-induced contractions was examined in this study. During the continuous administration of SST, the occurrence of motilin-induced phase III-like contractions was suppressed. Octreotide also suppressed motilin-induced contractions, suggesting that SST presumably acted on SSTR2, 3, or 5 to suppress gastric contractions in *S. murinus*; this makes sense, given that octreotide is known to have a high binding affinity for SSTR2 and moderate affinity for SSTR3 and SSTR5 in humans (26, 27). We found that pretreatment with SST significantly suppressed motilin-induced gastric contractions, and ACh strongly induced gastric contraction after pretreatment with SST *in vitro*. It has previously been reported that octreotide administration suppresses gastric contraction in humans (28), and *in vitro* experiments using guinea pigs' small intestines indicated that SST suppressed the secretion of ACh from the myenteric plexus therein (25). Although SST has inhibitory effects on motilin secretion (19, 20), our results indicate that suppression of gastric contraction by SST is not involved in the inhibition of motilin secretion. In *S. murinus*, *in vitro* organ bath experiments showed that motilin-induced contractions are completely abolished by treatment with a muscarinic receptor antagonist and their occurrence is significantly reduced by a nicotine receptor antagonist (29). Taken together, our results suggest that the inhibitory effects of SST on motilin-induced gastric contractions were exerted without affecting extrinsic nerves, and SST may primarily suppress motilin-induced contractions by acting on SSTRs to inhibit the release of ACh from cholinergic neurons in the myenteric plexus.

It is well-known that when the gastric phase III of MMC terminates, the plasma motilin concentration remains high (1). In the *in vitro* organ bath experiment, we found that SST dose-dependently inhibited physi-

ological concentrations of motilin (10^{-9} M)-induced contractions (Fig. 4E). Therefore, we hypothesized that SST is involved in the termination of the spontaneous gastric phase III contractions during MMC. To clarify the role that SST plays in the termination of phase III, we suppressed the SST pathway by administering CSST, a non-selective SSTR antagonist, at the peak of the spontaneous phase III contractions. However, CSST had no effect on spontaneous gastric phase III contractions (Fig. 6). This result indicates that exogenous SST has the ability to suppress gastric contractions, but endogenous SST does not contribute to spontaneous phase III contractions under normal physiological conditions.

In conclusion, this study clarified that exogenous SST administration suppresses PPCs, as well as contractions in phases II and III of MMC, but an SSTR antagonist had no effect on phase III contractions. These results indicate that endogenous SST may not be involved in spontaneous gastric contractions. Future research should evaluate whether the inhibitory effect of SST is involved in the pathophysiology and further development of therapeutic applications in humans.

Author Contribution

HS collected the data and wrote the manuscript. NY collected the data. ST wrote the manuscript and analyzed the data. KN, HO, TS, and IS were involved in directing the experiment and writing the manuscript.

Conflict of Interest

TS and IS have received grant support from Ono Pharmaceutical Co., Ltd. The other authors declare no conflict of interests.

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