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Pharmacological action of DA-9701 on the motility of feline stomach circular smooth muscle

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DA-9701, a new prokinetic agent for the treatment of functional dyspepsia, is formulated with *Pharbitis* semen and *Corydalis* tuber. This study wasconducted to determine the pharmacological action of DA-9701 and to identify the receptorsinvolved in DA-9701-induced contractile responsesin the feline gastric corporal, fundic and antral circular smooth muscle. Concentration-response curve to DA-9701 was established. The tissue trips were exposed to methylsergide, ketanserin, ondansetron, GR 113808, atropine and dopamine before administration ofDA-9701. The contractile force was determined before and after administration of drugs by a polygraph.DA-9701 enhanced the spontaneous contractile amplitude of antrum, corpus and fundus. However, it did not change the spontaneous contractile frequency of antrum and corpus, but concentration-dependently reduced that of fundus. In the fundus, DA-9701-induced tonic contractions were inhibited by dopamine, methylsergide, ketanserine, ondansetron or GR 113808 respectively, but not by atropine, indicating that the contractile responses are mediated by multiple receptors: 5-HT_2, 5-HT_3, 5-HT_4, and dopamine receptors. In the corpus, DA-9701-induced contractions were blocked by atropine, dopamine or GR 113808, but not by methysergide, ketanserin or ondansetron, indicating that they are involved in receptors on both, smooth muscles and neurons: 5-HT_4 and dopamine receptors. However, contractile responses to DA-9701 are mainly mediated by dopamine receptors in the antrum.These results suggest that DA-9701 has important roles in gastric accommodation by enhancing tonic activity of fundus, and in gastric emptying and gastrointestinal transit by phasic contractions of corpus and antrummediated by multiple receptors.

1. Introduction

Anatomically, the stomach is divided into four distinct regions: the cardia, the fundus, the corpus (body) and the antrum (Ehrlein and Schemann 2006; Lacy and Weiser 2005). It is composed of two major functional areas: the "proximal stomach" and the "distal stomach". The proximal stomach consists of the fundus and the upper body. The primary function of the proximal stomach is to accommodate ingested food. The distal stomach consists of the lower body and antrum. This area plays a critical role in the processes of trituration and emptying (Lacy and Weiser 2005). Due to different properties of the smooth muscle cells, the gastric reservoir is characterized by tonic activity and the gastric pump by phasic activity (Ehrlein and Schemann 2006).

Disordered motility occurs when the processes of gastric emptying, reservoir function, or interdigestive motility are not properly controlled (Tack 2007). An impaired gastric accommodation may lead to a defective reservoir function, inability to ingest normal-sized meals and weight loss (Tack 2007; Tack et al. 1998). Delayed gastric emptying leads to gastroparesis syndrome with prolonged stasis and fermentation of food (Abell et al. 2006). Abnormally rapid gastric emptying causes duodenal caloric overload and dumping syndrome (Vecht et al. 1997).

Functional dyspepsia (FD) and gastroparesis are the main clinical syndromes that have been associated to gastric motor dysfunction (Tack 2007). Currently, prokinetics and fundic relaxants appear to be the drugs of choice in the treatment of FD. Gastrointestinal prokinetics are a heterogeneous class of drugs that stimulate smooth muscle contractions to enhance gastric emptying and intestinal transit. Recently studied prokinetics include antidopaminergic agents (itopride), serotonergic agents (tegaserod and others), motilin receptor agonists (mitemcinal) and ghrelin receptor agonists (TZP101) (Tack 2008).

Tegaserod, a partial 5-HT_4 receptor agonist has been shown to induce the acceleration of gastric emptying in FD patients as well as healthy volunteers (Degen et al. 2001; Vakil et al. 2008). In addition, cisapride is a non-selective 5-HT_4 receptor agonist with a partial weak 5-HT_3 antagonist effect that strongly exhibits prokinetic actions (Lee et al. 2011). However, due to the incidence of cardiovascular ischemia and serious cardiac arrhythmias, tegaserod and cisapride were withdrawn from the market (Wysowski et al. 2001). Furthermore, domperidone, a dopaminergic antagonist, may be useful in the management of FD symptoms (Halter et al. 1997), but is not approved in the US for FD (Reddymasu et al. 2007) and associated with side effects such as cardiac arrhythmia (Drolet et al. 2000) and galactorrhea (Cann et al. 1983). Therefore, it is necessary to develop safer and

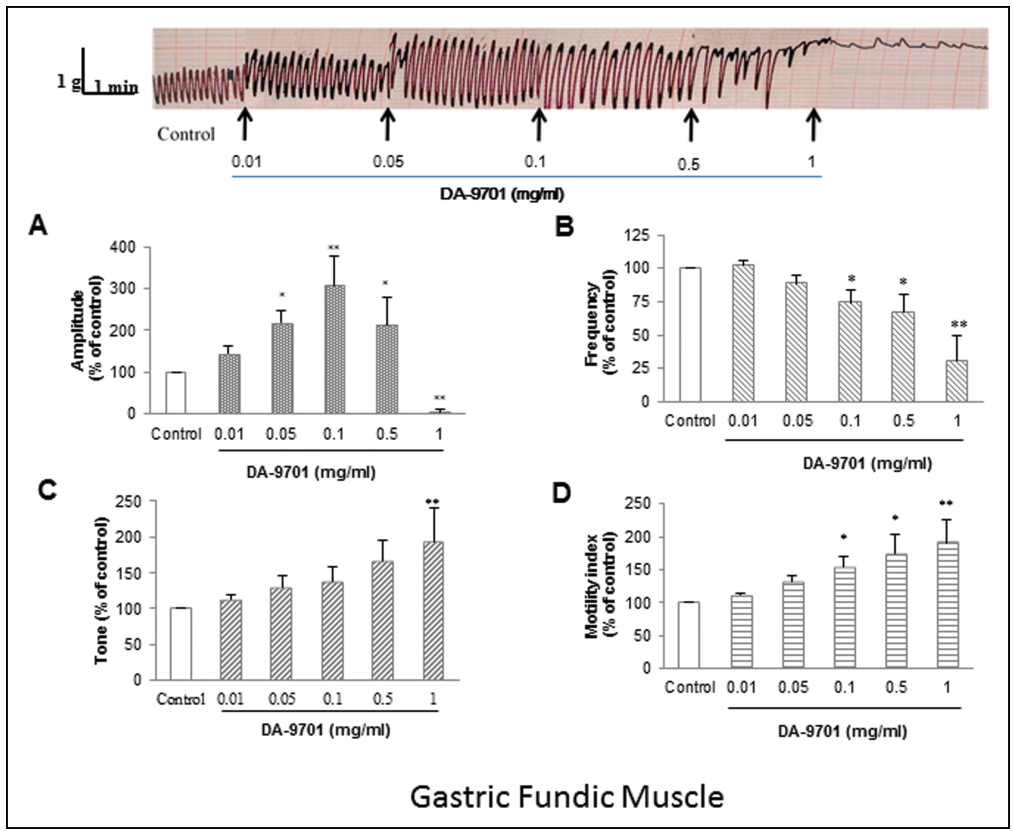

Fig. 1: Effect of DA-9701 on the spontaneous activity of the feline fundic circular smooth muscle. A: amplitude, B: frequency, C: tone, D: motility index. DA-9701 was cumulatively added (0.01 mg/ml∼ 1 mg/ml) with 15 min contact time for each concentration. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$, (**) $P < 0.01$ vs. control.

more effective gastroprokinetic agents to improve the treatment of FD.

The dried ripe seed of *Pharbitis nil* has been used for thousands of years for analgesic effects on the abdomen, purgative effect, gastroprokinetic effects and antifungal activity (Ito 1964; Koo et al. 1998). *Corydalis* tuber is also a traditional herbal medicine that has been widely used in the treatment of gastric and duodenal ulcers cardiac arrhythmia disease, rheumatism, dysmenorrheal and memory dysfunction (Hung et al. 2008). DA-9701 is the standardized extract of the seed of *Pharbitis nil* (Pharbitidis semen, Convolvulaceae) and the root of *Corydalis yahusuo* (Corydalis tuber, Papaveraceae). A previous study showed that DA-9701 has strong gastropro kinetic effects and a safety profile superior to conventional prokinetics, including cisapride and mosapride (Lee et al. 2008). DA-9701 not only accelerated gastric emptying and the GI transit of meals in normal and abnormally delayed conditions, but also enhanced gastric accommodation in conscious dogs (Lee et al. 2008). In that study, the authors examined the fundic accommodation and monitored the basal and postprandial gastric volumes at a constant operation pressure in Beagle dogs. Moreover, DA-9701 provoked pacemaker currents in the interstitial cells of Cajal (ICC) by means of intracellular mobilization of Ca^{2+} through phospholipase C, which might be one of the cellular and molecular targets for the gastroprokinetic effects of DA-9701 (Choi et al. 2009). However, specific receptorsinvolved in the prokinetic action of this drug on the stomach are not fully elucidated yet.Therefore, this research was carried out to accurately characterize the pharmacological activity of DA-9701 and to clarify thereceptors mediating the DA-9701-induced contraction of feline gastric corporal fundic, and antral circular smooth muscle using atropine, selective 5-HT receptor antagonists and dopamine.

2. Investigations and results

2.1. Effects of DA-9701 on the spontaneous activity of fundic circular smooth muscle

Circular muscle strips were isolated from feline gastric corpus, fundus and antrum and displayed spontaneous activity. DA-9701 evoked both, tonic and phasic contractile activities in the gastric fundic, corporal and antral strips. A typical tracing from the fundus revealed spontaneous activity and concentration-dependent effects of DA-9701 (Fig. 1). A spontaneous phasic motility was observed in the fundus after an incubation of the smooth muscle strips for approximately 90 min. The application of DA-9701 (0.01 mg/ml to 1 mg/ml) in an organ bath induced a dose-dependent significant increase in the basal tone and motility index. The maximal amplitude was observed at 0.1 mg/ml of DA-9701 and then dramatically declined. Compared with control, the basal tone significantly increased by 200% and the motility index (MI) increased to 190% at a concentration of 1 mg/ml DA-9701. Conversely, DA-9701 induced concentration-dependent decreases in frequency of the feline gastric fundus. These results suggest that the spontaneous phasic contraction changed to tonic responseat a high concentration of DA-9701.

2.2. Effects of DA-9701 on the spontaneous activity of corporal circular smooth muscle

DA-9701 (0.01 mg/ml ∼ 1 mg/ml) evoked concentration-dependent contractions of feline corporal smooth muscle strips (Fig. 2). At 1 mg/ml, DA-9701 caused a significant increase in the amplitude to 250%, compared with control. There was also a statistical increase in the tone and the MI at concentrations starting at 0.05 mg/ml of DA-9701. The basal tone and

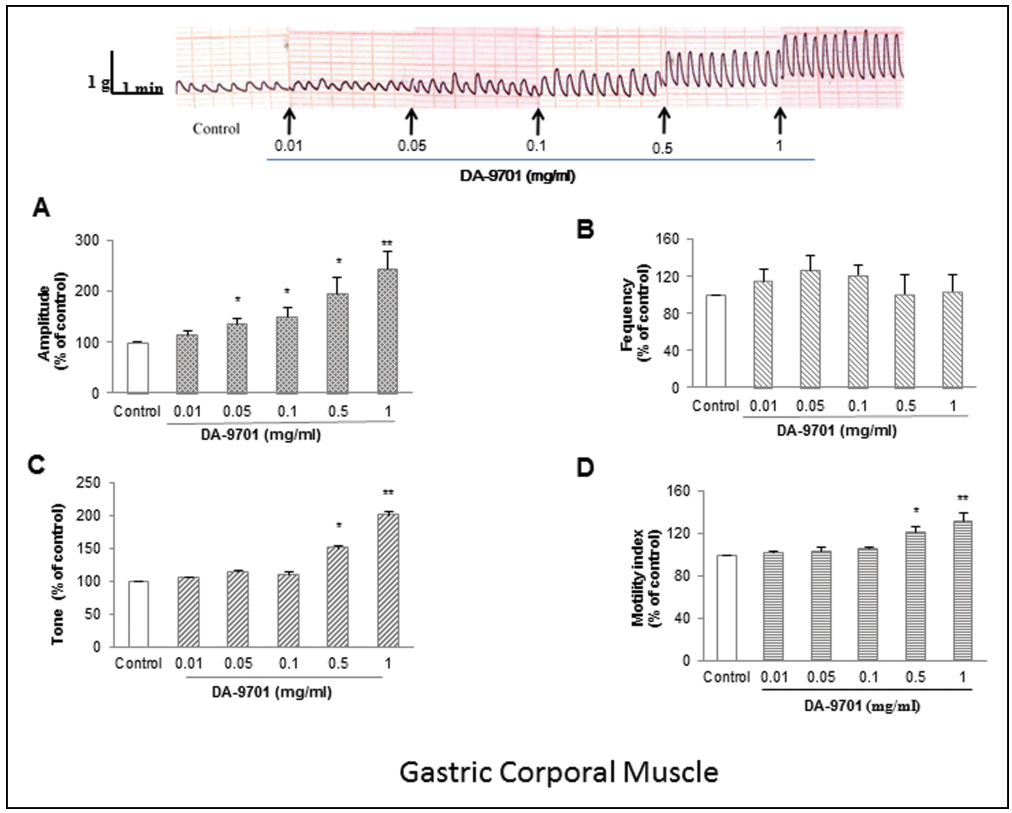

Fig. 2: Effect of DA-9701 on the spontaneous activity of the feline corporal circular smooth muscle. A: amplitude, B: frequency, C: tone, D: motility index. DA-9701 was cumulatively added (0.01 mg/ml ~ 1 mg/ml) with 15 min contact time for each concentration. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$, (**) $P < 0.01$ vs. control.

the percentage of MI increased by185% and 130% at 1 mg/ml DA-9701, respectively. However, DA-9701 did not cause a dose-dependent increase in the frequency. Taken together, these data suggest that an increase in the contractile responses of gastric corpus by DA-9701 may contribute to an increase in the intra-gastric pressure.

2.3. Effects of DA-9701 on the spontaneous activity of antral circular smooth muscle

The effects of DA-9701 on isolated antral smooth muscle motility were investigated as the antral smooth muscle layer is thickest in the stomach and may play an important role in gastric empty-

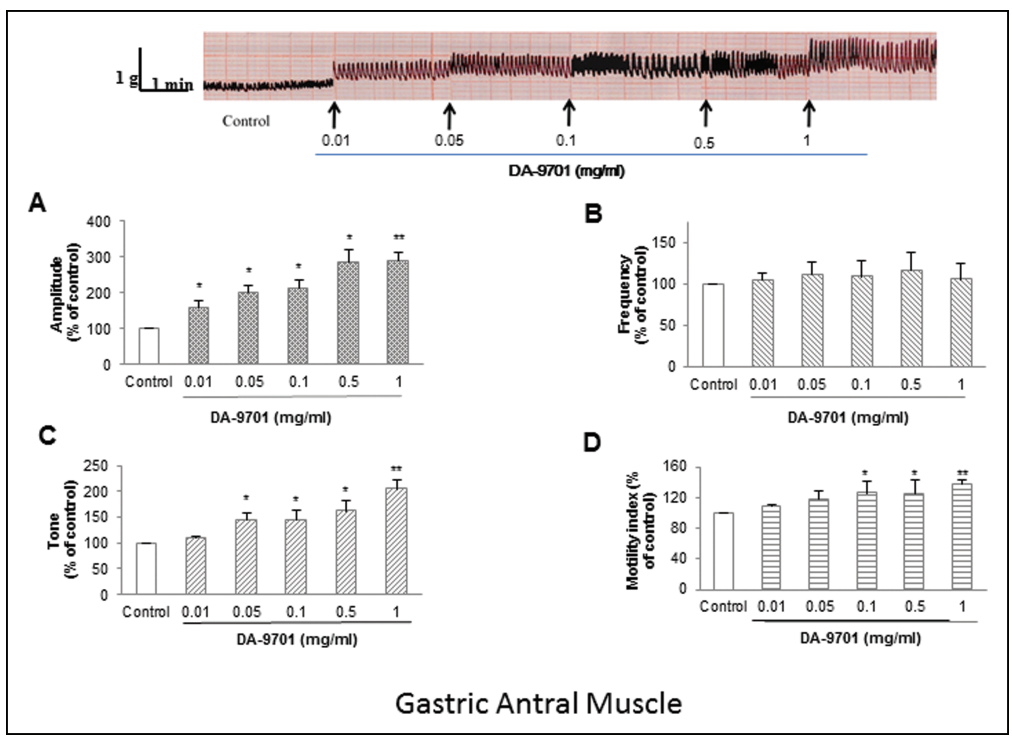

Fig. 3: Effect of DA-9701 on the spontaneous activity of the feline antral circular smooth muscle. A: amplitude, B: frequency, C: tone, D: motility index. DA-9701 was cumulatively added (0.01 mg/ml ~ 1 mg/ml) with 15 min contact time for each concentration. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$, (**) $P < 0.01$ vs. control.

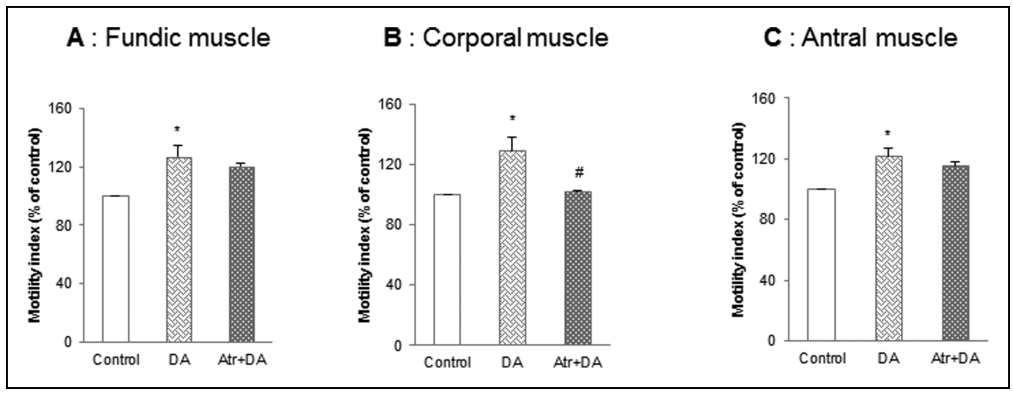

Fig. 4: Effect of atropine on the DA-9701-induced contractions of the feline gastric circular smooth muscle.A: fundus, B: corpus, C: antrum. Atropine (Atr) 10^{-6} M was pre-incubated 15 min before DA-9701 administration. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$ vs. control, (#) $P < 0.05$ vs. motility after DA-9701 treatment.

ing. The application of 1 mg/ml DA-9701 induceda gastric antral circular smooth muscle contraction and produced an increase in the amplitude to 290%, in the tone to 175% and in the percentage of MI to 140%, compared with that of the control. However, DA-9701 did not significantly change the frequency of the gastric antral circular smooth muscle contraction (Fig. 3). These results demonstrate that DA-9701 can increase in antral circular smooth muscle motility and might contribute to an increase in the intra-gastric pressure.

2.4. Effects of atropine on the DA-9701-induced tonic and phasic contractions

Previous results showed that 1 mg/ml DA-9701 significantly induced tonic contractions in gastric fundus and phasic responses in gastric corpus and antrum. Therefore, this concentration was used for the following experiments. Atropine is a central and peripheral muscarinic cholinergic receptor antagonist. The muscle strips were pre-incubated with 10^{-6} M atropine to test whether the cholinergic neural effect on the DA-9701induced phasic contractions of the gastric corpus and antrum and tonic responses of the fundic stomach. The administration of atropine to the incubation chamber significantly inhibited the contractile responses induced by DA-9701 in isolated stomach corpus strips (Fig. 4. Compared to the control,the responses of gastric corpus strips amounted to 129%without prior treatment with atropine, but the responses were statistically reduced to 102% when pretreated with atropine. On the other hand, the administration of atropine did not significantly change the responses of gastric fundus and antrum. These results suggest that the DA-9701–induced contractions are mediated by a cholinergic pathway in the gastric corpus, but the cholinergic innervation was not implicated in the DA-9701-induced contractile responses in feline fundic and antral circular smooth muscles.

2.5. Effects of dopamine on the DA-9701-induced tonic and phasic contractions

We knew dopamine to exert an inhibitory effect '*in vivo*' on gastrointestinal activity in animals and man. '*In vivo*' experiments using the intact guinea pig stomach have also shown dopamine to reduce gastric motility. To test whether dopamine influences DA-9701-induced contractions, muscle strips were pre-treated with 10^{-6} M dopamine. The DA-9701-induced contractile responses were significantly reduced in all parts of the stomach at this dopamine concentration (Fig. 5).

2.6. Effects of 5-HT receptor antagonists onDA-9701-induced tonic contractions of the fundic circular smooth muscle

Specific 5-HT receptor antagonists were used to pharmacologically define the serotonin receptors involved in the DA-9701-induced contractile responses of gastric corpus, fundus and antrum. In order to test whether the 5-HT_1 or the 5-HT_2 receptor is involved in the DA-9701-induced tonic contractions, muscle strips were pretreated with methysergide (nonselective 5-HT_1 or 5-HT_2 receptor antagonist, 10^{-6} M) or ketanserin (selective 5-HT_2 receptor antagonist, 10^{-6} M) for 15 min before addition of motillitione. Both, methysergide and ketanserin significantly inhibited the DA-9701-induced tonic contractions of

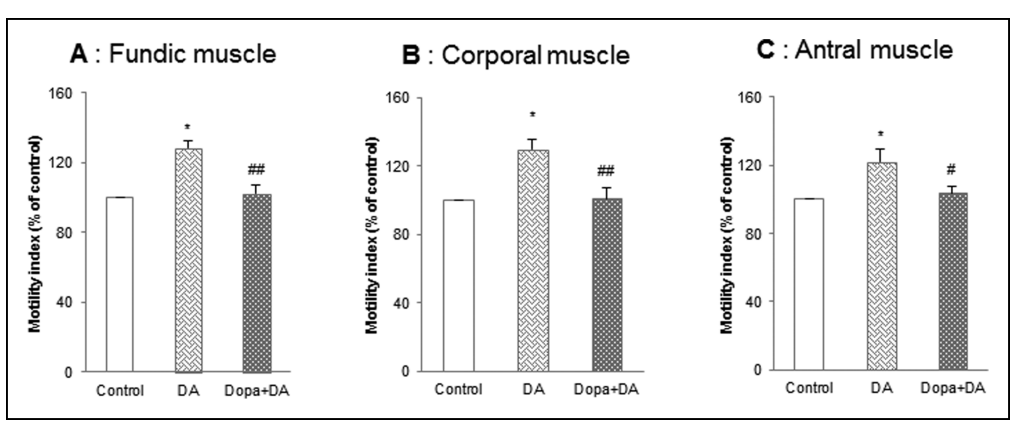

Fig. 5: Effect of dopamine on the DA-9701-induced contractions of the feline gastric circular smooth muscle.A: fundus, B: corpus, C: antrum. Dopamine (Dopa) 10^{-6} M was pre-incubated 15 min before DA-9701 administration. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$ vs. control. (#) $P < 0.05$, (##) $P < 0.01$ vs. motility after DA-9701 treatment.

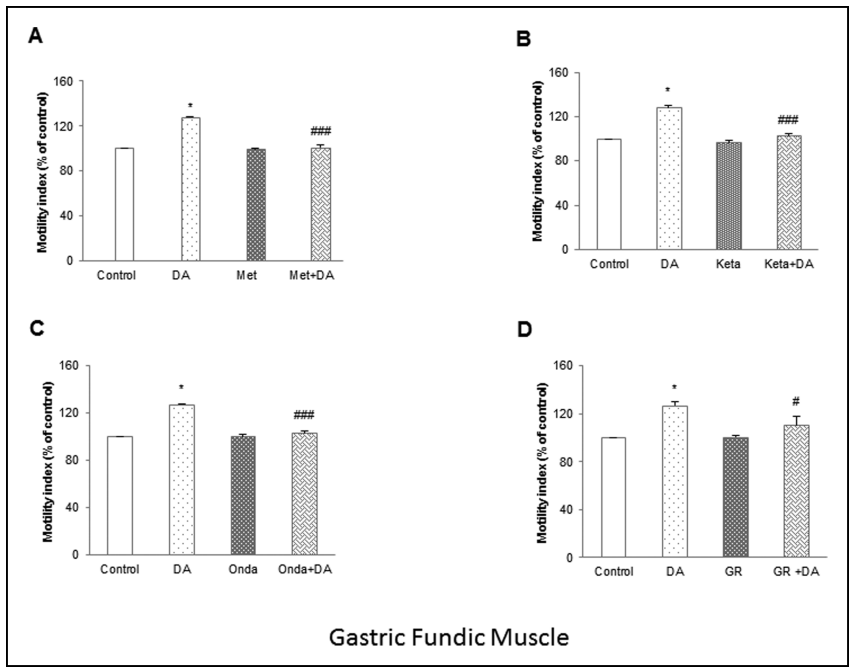

Fig. 6: Effects of 5-HT receptor antagonists on the DA-9701-induced tonic contractions of the the fundic circular smooth muscle. Methysergide (Met) 10^{-6} M (A), ketanserin (Keta) 10^{-6} M (B), ondansetron (Onda) 10^{-6} M (C) or GR 113808 (GR) 10^{-6} M (D) was applied for 15 min before addition of DA-9701. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$ vs. control. (#) $P < 0.05$, (###) $P < 0.001$ vs. motility after DA-9701 treatment.

the fundic strips, but did not change the spontaneous activities (Figs. 6A and B) suggesting that the 5-HT_2 and/or 5-HT_1 receptors might mediate the DA-9701-induced tonic contractions of feline gastric fundus. The fundic circular smooth muscle strips were incubated with ondansetron (selective 5-HT_3 receptor antagonist, 10^{-6} M) or GR 113808 (selective 5-HT_4 receptor antagonist, 10^{-6} M) for 15 min before addition of DA-9701 to define whether 5-HT_3 or 5-HT_4 receptor is involved in the DA-9701-induced tonic contractions. Either ondansetron or GR 113808 significantly blocked DA-9701-induced contractions, but did not alter the spontaneous responses (Fig. 6C and D). These results indicate that 5-HT_3 and 5-HT_4 receptors might be involved in the tonic contractions induced by DA-9701 in the feline fundic circular smooth muscle.

2.7. Effects of 5-HT receptor antagonists on the DA-9701-induced contractions of the corporal circular smooth muscle

Pretreatment with methysergide (10^{-6} M), ketanserin (10^{-6} M) or ondansetron (10^{-6} M) had no effects on the DA-9701-induced contractions of the corporal strips (Fig. 7A, B, and C).

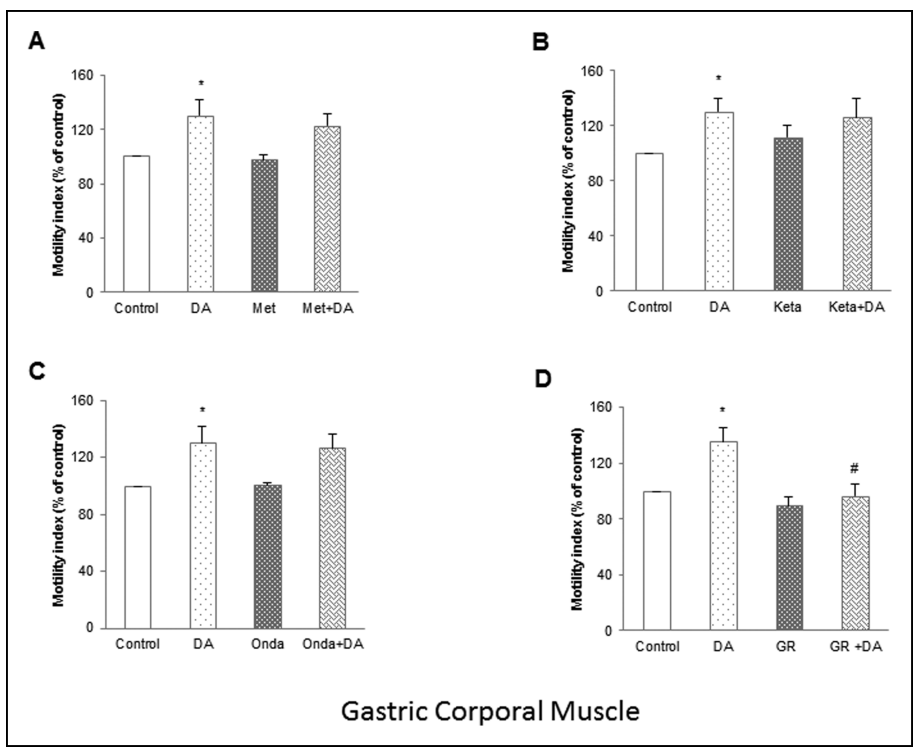

Fig. 7: Effects of 5-HT receptor antagonists on the DA-9701-induced contractions of the corporal circular smooth muscle.Methysergide (Met) 10^{-6} M (A), ketanserin (Keta) 10^{-6} M (B), ondansetron (Onda) 10^{-6} M (C) or GR 113808 (GR) 10^{-6} M (D) was applied for 15 min before addition of DA-9701. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$ vs. control. (#) $P < 0.05$ vs. motility after DA-9701 treatment.

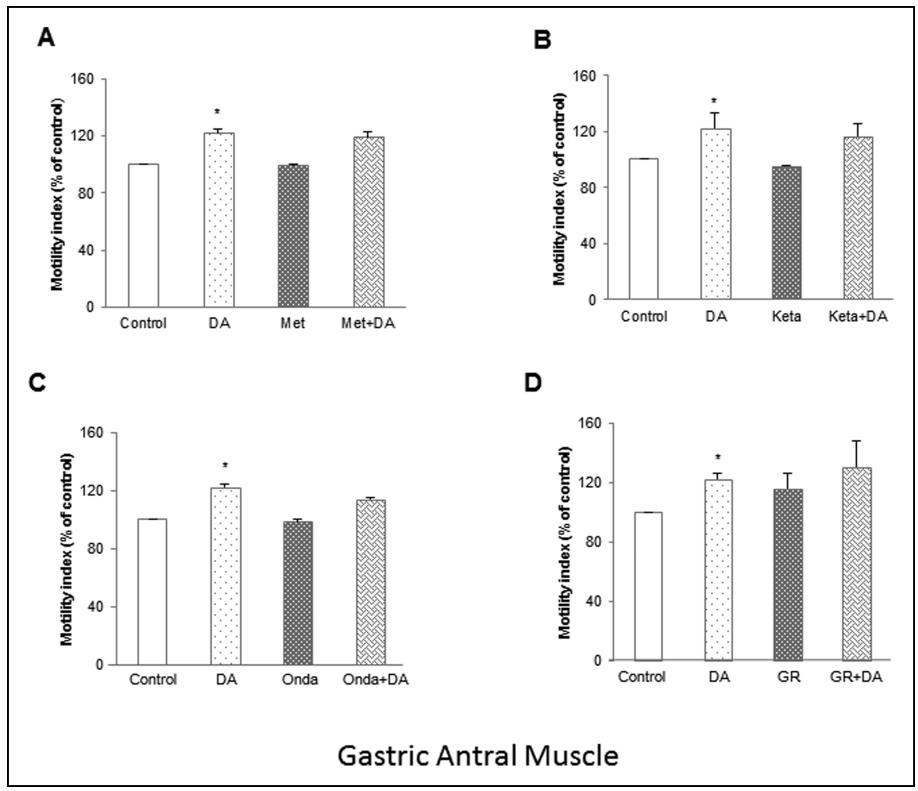

Fig. 8: Effects of 5-HT receptor antagonists on the DA-9701-induced contractions of the antral circular smooth muscle.Methysergide (Met) 10^{-6} M (A), ketanserin (Keta) 10^{-6} M (B), ondansetron (Onda) 10^{-6} M (C) or GR 113808 (GR) 10^{-6} M (D) was applied for 15 min before addition of DA-9701. Each point represents the mean ± SE (n = 7). (*) $P < 0.05$ vs. control.

However, GR 113808 (10^{-6} M) significantly reduced the DA-9701-induced contractions of the corporal strips (Fig. 7D). In the spontaneous activities, all of these antagonists had no effect also. Those results show the 5-HT_4 receptor might mediate the DA-9701-induced contractions of the feline gastric corpus.

2.8. Effects of 5-HT receptor antagonists on the DA-9701-induced contractions of the antral circular smooth muscle

DA-9701-induced contractions and the spontaneous activities of the gastric antrum were not antagonized by methysergide, ketanserin, ondansetron or GR 113808, suggesting that all 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 are not involved in the contractions induced by DA-9701 and the spontaneous activities (Fig. 8).

3. Discussion

The main clinical syndromes of a gastric motor dysfunction are FD and gastroparesis. The genesis of gastroparesis includes impaired fundal tone, antral hypomotility, antroduodenal dyscoordination, gastric pacemaker dysrhythmias and excessive inhibitory feedback from the small bowel to the stomach (Lacy and Weiser 2005). It is also clear that dyspeptic symptoms have a heterogeneous origin: delayed gastric emptying, hypersensitivity to gastric distention and impaired accommodation to a meal (Sarnelli et al. 2003; Stanghellini et al. 1996; Tack et al. 2001, 1998).

The results in this study demonstrate that DA-9701 evokesthe tonic contractile activity in the gastric fundus and phasic contractile responses in the corporal and antral stomach, which is considered to be a key factor to increase the intra-gastric pressure responsible for the acceleration of gastric emptying (Boivin et al. 1997; Collins et al. 1988, 1991; Coulie et al. 1998). These findings suggest that DA-9701 may have potential as a gastroprokinetic agent in the treatment of FD and gastroparesis. It is consistent with the research of Hung et al. (2008), which indicated that pseudocoptisine, a quaternary alkaloid with benzylisoquinoline skeleton, isolated from *Corydalis* tuber, inhibited acetylcholinesterase activity in a dose-dependent manner. However, this is controversialto the results of Kobayyashi et al. (2001) where the contraction of isolated mice ileum induced by acetylcholine was depressed by a hot water extract solution of Corydalis tuber. These different pharmacological effects may be due to the different animals and different parts of the body.

At present, there is strong agreement that ICC are the pacemarker cells that generate slow waves (Thomson et al. 1998; Van Helden et al. 2010), which govern the frequency of the spontaneous smooth muscle contractions (Dickens et al. 1999; Thomson et al. 1998), resulting in normal peristalsis (Malysz et al. 1996; Sanders and Ward 2007). It has been shown that a Ca^{2+}-inhibited non-selective action conductance (Jin et al. 2009) and a Ca^{2+} activated Cl^- conductance (Zhu et al. 2009) play a role in pacemaker activities. Recently, volume-activated Cl^- currents are found to be present in gastric epithelial cells (Jin et al. 2003) and smooth muscle cells (Xu et al. 1997), and may contribute to a depolarization of ICC and an increase in cell excitability (Park et al. 2005).

In addition, a previous study showed that DA-9701 depolarized the membrane potential and induced tonic inward currents in the ICC.It may be mediated by the activation of non-selective cationic channels *via* external Ca^{2+} and intracellular Ca^{2+} release from internal storage by the action of inositol triphosphate *via* PLC activation in a G protein-independent and protein kinase C-independent manner (Choi et al. 2009). In this study, the differential effects of DA-9701 on the frequency of spontaneous motility in fundus, corpus and antrum may be due to diverse regulation of these conductance expressed in different types of ICC such as ICC-IM and ICC-MY.

Currently, new agents for the treatment of gastrointestinal motor disorders include several major classes such as serotonergic agents, antidopaminergic agents, motilin receptor agonists and ghrelin receptor agonists (Tack 2008). In the present study, we investigated the effect of atropine on the DA-9701-induced contractions of gastric fundus, corpus and antrum as dopamine (Nagahata et al. 1995), motilin (Okano et al. 1996), and ghrelin (Depoortere et al. 2005) receptors have been shown to regulate gastric emptying partially through the direct and/or indirect cholinergic pathway. The results of this study show that atropine significantly blocked the DA-9701-induced contractile responses in the corporal smooth muscle only, suggesting the involvement of a cholinergic pathway.

Neural mediators are known to play important roles in the regulation of gastrointestinal motility. Many studies have indicated that muscarinic receptors seem to play an important role in regulating gastric slow waves. It is well known that slow waves in the stomach originate from ICC in the myenteric region (ICC-MY) and conduct passively into smooth muscle cells (Huizinga 2001; Ördög et al. 1999). Intramuscular ICC (ICC-IM) may have a common role in neurotransmission (Daniel 2001; Vecht et al. 1997). A recent study suggested that ICC-IM mediate cholinergic inputs to the circular muscle of the antrum (Hirst et al. 2002). Therefore it is speculated that the cholinergic modulation of DA-9701-induced contractile response is a peripheral mechanism involving the interaction of ICC-IM and ICC-MYin the feline gastric corpus.

On the other hand, the contractions in the fundic and antral stomach induced by DA-9701 were not affected by atropine. The different neuronal mechanisms between fundus, antrum and corpus might be due to the regional and functional differences. Based on these results, it is likely that the contractile responses of feline gastric fundus and antrum to DA-9701 were mediated *via* other nervous pathways or *via* receptors located directly on the circular fundic and antral stomach smooth muscle of feline. Dopamine is known as a catecholamine neurotransmitter regulating different functions in both, the central and peripheral nervous systems, hormone/transmitter synthesis and release, blood pressure and intracellular ion transport (Palermo-Neto 1997). In the guinea-pig gastric corpus, dopamine was spontaneously released at a rate similar to that found in central dopaminergic neurons. Dopamine release is augmented by transmural electrical stimulation through a mechanism sensitive to tetrodotoxin (a neuronal Na^+-channel blocker) and dependent on the external Ca^{2+} concentration, which suggests neuronal release (Shichijo et al. 1997; Tonini et al. 2004). Enteric dopamine has also been known to mediate the inhibition of gut motility and to decrease the antroduodenal coordination in the gut muscle, thereby inhibiting acetylcholine release from the cholinergic nerve by activation of the neuronal dopamine D_2 receptor (Iwanaga et al. 1990; Lee et al. 2011).

In this study, the DA-9701-induced contractile responses were significantly reduced by 10^{-6} M dopamine in all parts of the stomach. These results suggest that DA-9701, which had effects on the cholinergic neurons, might prevent the inhibitory effect of endogenous dopamine, resulting in a potentiation of the release of acetylcholine. This is consistent with the results of previous research, which indicated that THB from *Corydalis* tuber has micromolar affinity to a dopamine D_2 receptor (Lee et al. 2011). In general, the proximal stomach is well known as a reservoir for food as it relaxes upon food intake. After relaxation, slow tonic contraction of the proximal stomach will gradually drive its contents to the antrum. The regulation of the fundic tone is mediated through serotonin. The results in this study showed that the DA-9701-evoked tonic contractions were significantly inhibited by methysergide, ketanserin, ondansetron and GR 113808in the fundus, suggesting the involvement of 5-HT_2, 5-HT_3, 5-HT_4 and/or 5-HT_1 contractile receptors. It supports the hypothesis that the effects of DA-9701 on the feline gastric fundus might be similar to motilin and serotonin, strong gastroprokinetics, which also enhance the fundic tone in humans, rats, murine, porcine, guinea pigs and canine *via* a non-cholinergic pathway (Cuomo et al. 2006; Janssen et al. 2002a, b; Komada and Yano 2007; Xue et al. 2006).

Relaxation of the fundus by 5-HT_1 receptors has been reported in the guinea pig (Coulie et al. 1999; Kojima et al. 1992; Takemura et al. 1999). The fundic integrated relaxatory response to a meal is known as fundic accommodation (Xue et al. 2006). An activation of the 5-HT_{1A} receptor results in a release of nitric oxide (NO) for the relaxation of the gastric fundus and a decrease of the gastric fundus tone (Coulie et al. 1999; Desai et al. 1991). The stimulation of 5-HT_{1B}/$_{1D}$ receptors induces the gastric accommodation in humans and dogs, and delays the gastric emptying in humans (De Ponti et al. 2003; Houghton et al. 1992; Tack et al. 2006, 2000). In addition, Meulemans et al. (1993) Takemura et al. (1999) Coulie et al. (1999), and Tack et al. (1999, 2000) have also shown 5-HT_1 receptors mediatethe relaxation inthe proximal stomach of cat and man. Moreover, the previous study also showed that DA 9701, corydaline and tetrahydroberberine from *Corydalis* tuber induced significant gastric relaxation and drastically increased gastric accommodation (Lee et al. 2008, 2010, 2011). Taken together, it might be hypothesized that DA-9701 might induce gastric tonic contractions via 5-HT_2, 5-HT_3, 5-HT_4 receptors and might involve fundic relaxations and gasric accommodation via 5-HT_1 receptorsin the feline gastric fundus. 5-HT_1 receptors are known to mediate fundic relaxation, whereas 5-HT_2, 5-HT_3, and 5-HT_4 receptors were found to mediate contractious in the fundus, corpus and antrum of guinea-pigs, rats and canine (Baxter et al. 1994; Janssen et al. 2002a; Takemura et al. 1999). In the stomach of rats, the fundus presents the relaxant 5-HT_{2A} receptor and the contractile 5-HT_{2B} (Komada and Yano 2007). In humans, the blockade of 5-HT_3 receptors inhibits the gastrointestinal motility (Komada and Yano 2007). Furthermore, m-chlorophenylbiguanide (a selective 5-HT_3 receptor agonist) stimulates the antral motility in conscious dogs (Nagakura et al. 1997). The stimulation of 5-HT_4 receptors elicits gastrointestinal contractile activity and increases the rate of gastric emptying in humans and dogs (Fraser et al. 1993; Komada and Yano 2007; Lux et al. 1994). Tegaserod, a partial 5-HT_4 receptor agonist and 5-HT_{2B} receptor antagonist (Beattie et al. 2004; De Maeyer et al. 2008), accelerates gastric emptying and enhances gastric accommodation in healthy volunteers (Degen et al. 2001; Tack et al. 2003). In addition, it is also able to normalize delayed gastric emptying, enhance gastric accommodation and to improve impaired antroduodenal motility in functional dyspepsia and gastroparesis (Thumshirn et al. 2007). In another study, 5-HT_4 receptor agonists potentiate electrically evoked contractions in the longitudinal muscle of the dog gastric corpus and in the circular muscle of the guinea-pig gastric fundus and corpus (Hegde and Eglen 1996; Prins et al. 2001). However, the results in this study show that only 5-HT_4 receptors mediated the DA-9701-induced contractions through cholinergic pathway, suggesting a species-specific involvement of 5-HT receptorsin the feline gastric corpus.

In conclusion, DA-9701 might increase the spontaneous motility in the stomach, but decrease the spontaneous contractile frequency in the fundus only, implying that it has an essential role in gastric accommodation. Tonic contractile responses to DA-9701 in the fundus seem to be mediated by multiple receptors located on smooth muscles: 5-HT_2, 5-HT_3, 5-HT_4 and dopamine receptors. However, DA-9701-induced phasic contractions in the corpus are involved in receptors on both, smooth muscles and neurons: 5-HT_4 and dopamine receptors. In the antrum, contractile responses to DA-9701 are mainly meditated

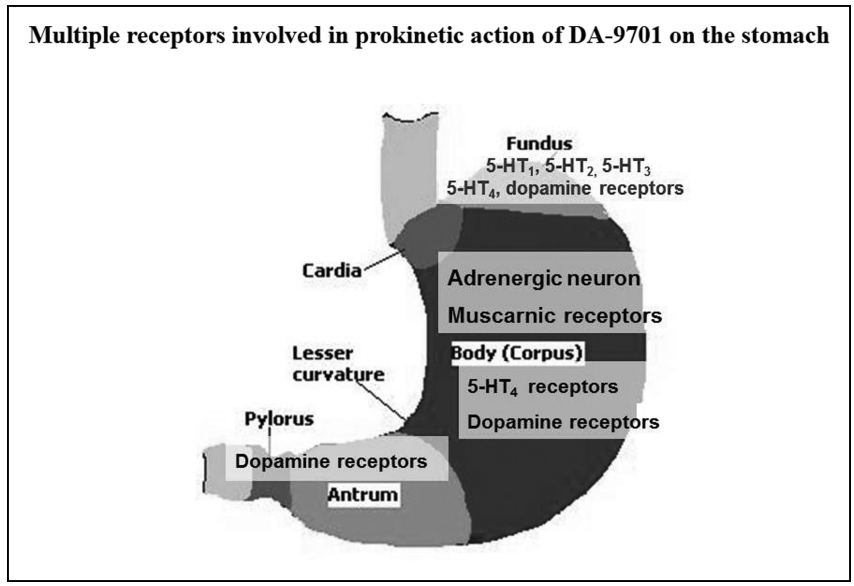

Fig. 9: Multiple receptors involved in prokinetic action of DA-9701 on the stomach.DA-9701 induced tonic contractile activity in gastric fundus. Tonic contractile responses to DA-9701 seem to be mediated by multiple receptors, 5-HT_2, 5-HT_3, 5-HT_4 and dopamine receptors. DA-9701- induced tonic contractions in fundus might contribute to an increase in the gastric accommodation. In corpus, phasic contractions induced by DA-9701 are involved by receptors on both smooth muscle and neurons: 5-HT_4 and dopamine receptors. DA-9701- induced phasic contractions in antrum are mediated by dopamine receptors. The increase in the contractile responses of gastric corpus and antrum by DA-9701 may contribute to an increase in the intra-gastric pressure, which can enhance gastric emptying and gastrointestinal transit, implying DA-9701 could be agastroprokinetic agent.

by dopamine receptors located directly on the smooth muscle. As shown in Fig. 9, these mechanisms may explain theactions of DA-9701which include not only the treatment of functional dyspepsia, but also conventional effects, such as enhanced gastric emptying and gastrointestinal transit, implying that it could be a promising gastroprokinetic agent.

4. Experimental

4.1. Solutions and drugs

Tissues were maintained in Kreb's buffer solution including 116.6 mM NaCl, 21.9 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 3.4 mM KCl, 2.5 mM $CaCl_2$, 5.4 mM glucose and 1.2 mM $MgCl_2$. Methysergide maleate salt, ketanserin (+)-tartrate salt, ondansetron hydrochloride dihydrate, GR113808 (1-[2-[(methylsulfonyl)amino] ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate), and dopamine monohydrochlride were purchased from Sigma Chemical Co. (St Louis, MO, USA). Atropine sulfate was purchased from Merck (Whitehouse Station, NJ, USA), Zoletil 50® was purchased from Virbac Korea (Songpa-Gu, Seoul, Republic of Korea).DA-9701 was suppliedfrom Dong-A Pharmaceutical Co. Ltd, (Yong-In, Gyeonggi-do, Republic of Korea).The solutions were prepared on the day of experiment. Doses of all compounds are reported in molar concentrations and refer to their final concentration in the organ bath.

4.2. Isolation of stomach

All animal experiments were performed under the NIH guideline "Principles of laboratory animal care" (NIH publication No.85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee of Chung-Ang University, Seoul, Republic of Korea. Adult cats of either sex weighing between 2.5 and 4 kg were anesthetizedwith Zoletil 50® (25 mg/kg). The chest and abdomen were opened with a mid-line incision exposing the esophagus and stomach. The esophagus and stomach were removed together and then transferred to a bath of normal Ca^{2+}-containing Krebs solution equilibrated with 95% O_2 and 5% CO_2 at 37 °C and maintained at pH 7.4 ± 0.05. The organs were pinned on a wax block at their *in vivo* dimensions and orientation. The stomach was opened along the lesser curvature and cleaned of surrounding connective tissue. The location of the squamo-columnar junction was identified and the mucosa was peeled off.

4.3. Tissue bath studies

Transversely oriented muscle strips measuring 2 mm wide and 7 mm long were taken from the fundus, corpus and antrum. The strips were then cut into 3–4 minor strips and silk ligatures were tied at both ends. The muscle strips were mounted in separate 1 ml muscle chambers. One wire was fixed to the bottom of the muscle chamber while the other was attached to a force transducer (FT03 Grass InstrumentsCo., Quincy, MA). Changes in isometric force were recorded on a polygraph (Grass model 79). They were initially stretched to 1 g to bring them to near conditions of optimal force development and were equilibrated for 90 min while continuously being perfused with oxygenated Kreb's buffer. During this time, tension in the muscle strips decreased rapidly and stabilized at less than 0.5 g. The solution was equilibrated and maintained with a gas mixture containing 95% O_2 and 5% CO_2 at pH 7.4 and 37 °C throughout the study.

4.4. Assessment of drug responses

A concentration-response curve to DA-9701 was established by increasing the concentration of the drug added to the organ bath with a contact time of 15 min. The tissue trips were exposed to the antagonists for 15 min and further administrated with DA-9701 for 20 min to evaluate the effects of 5-HT receptor antagonists. The responses to DA-9701 were determined in the absence and presence of 5-HT antagonists such as methysergide, ketanserin, ondansetron and GR 113808. The effects of DA-9701-induced contraction were also studied in the absence and presence of atropine and dopamine. Muscle trips were equilibrated for 1 h after washing with Kreb's solution for five times between each part within the sets of experiments. All drugs were added to the organ bath in volumes not exceeding 100 µl (10% organ bath volume).

4.5. Data analysis

The amplitude, frequency andmotility index of the contraction induced by DA-9701 was expressed as percentage of the control. Results are expressed as means ± S.E.M. of different experiments. Statistical significance of differences between data was determined using the two-tailed Student's t-test for paired observations or one-way analysis of variance, as appropriate. Differences were considered to be statistically significant when $P < 0.05$.

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