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Effects of KW-5092 on Antroduodenal Coordination and Gastric Emptying in Guinea-pigs

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Abstract

KW-5092 (2-[1-[2-[[5-(piperidinomethyl)-2-furanyl]methylamino]ethyl]imidazonylidin-2-ylidenene]malononitrile fumarate) is a novel gastroprokinetic agent with both acetylcholine release facilitatory and acetylcholinesterase inhibitory activity. We have investigated the effects of KW-5092 on antroduodenal coordination and gastric emptying in guinea-pigs.

In the guinea-pig isolated gastroduodenal preparation, KW-5092 at 3×10^{-7} to 3×10^{-6} M significantly increased antroduodenal coordination. The effect of KW-5092 was almost completely inhibited by atropine or tetrodotoxin. Cisapride, a gastroprokinetic agent with acetylcholine release facilitatory activity, also increased coordination whereas neither acetylcholine nor the acetylcholinesterase inhibitor neostigmine affected it. In-vivo, KW-5092 and cisapride enhanced gastric emptying whereas neostigmine delayed it.

These results suggest that acetylcholine release facilitation, but not acetylcholinesterase inhibition, is involved in the enhancement by KW-5092 of antroduodenal coordination and gastric emptying.

Cisapride is a gastroprokinetic agent with acetylcholine release facilitatory activity (Taniyama et al 1991). It has been reported that the compound also enhances antroduodenal coordination in guinea-pig gastroduodenal preparations (Schuurkes et al 1985), and stimulates gastric emptying in man (Baeyens et al 1984) and rats (Megens et al 1986). It is believed that enhanced antroduodenal coordination is involved in the stimulation of gastric emptying by cisapride.

KW-5092 (2-[1-[2-[[5-(piperidinomethyl)-2-furanyl]methylamino]ethyl]imidazonylidin-2-ylidenene]-malononitrile fumarate) is a novel gastroprokinetic agent with both acetylcholine release facilitatory (Kishibayashi et al 1994a) and acetylcholinesterase inhibitory activity (Kishibayashi et al 1994b). It enhances a wide range of gastrointestinal motility in anaesthetized rabbits (Sasho et al 1993) and in anaesthetized or conscious dogs (Kishibayashi et al 1994c).

Because KW-5092 enhances the motility of the stomach, it seemed of interest to examine the effect of this drug on gastric propulsion. In this study we have investigated the effects of KW-5092 on antroduodenal coordination and gastric emptying in guinea-pigs and examined the effects of cisapride and the acetylcholinesterase inhibitor neostigmine to determine whether acetylcholine release facilitation or acetylcholinesterase inhibition correlates with the stimulation of antroduodenal coordination and gastric emptying.

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Materials and Methods

Drugs

KW-5092 (2-[1-[2-[[5-(piperidinomethyl)-2-furanyl]-methylamino]ethyl] imidazonylidin-2-ylidenene]-malononitrile fumarate) was synthesized in our laboratories. Cisapride was a kind gift from Janssen-Kyowa (Tokyo, Japan). Neostigmine methylsulphate, tetrodotoxin and phenol red were purchased from Sigma (St Louis, MO), acetylcholine chloride from Nacalai Tesque (Kyoto, Japan) and trichloroacetic acid from Wako (Osaka, Japan).

Animals

Male Hartley guinea-pigs, 250–500 g, were purchased from Japan SLC (Hamamatsu, Japan). The

animals had free access to ordinary chow and tap water under a constant 12-h light–dark cycle. All animals received care in compliance with the Guiding Principles for the Care and Use of Laboratory Animals formulated by the Bioethical Committee of Pharmaceutical Research Institute, Kyowa Hakko Kogyo.

Androduodenal coordination (in-vitro study)

Antroduodenal coordination was determined by a method described elsewhere (Schuurkes et al 1985). The animals were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.), killed, and the stomach with the adjacent 10 cm of duodenum was immediately isolated. After ligation of the oesophagus the stomach was filled with saline (20 mL) and suspended in an organ bath containing 200 mL oxygenated (95% O_2–5% CO_2) Tyrode's solution (136 mM NaCl, 2·7 mM KCl, 1·0 mM $MgCl_2$, 0·4 mM NaH_2PO_4, 5·6 mM glucose, 11·9 mM $NaHCO_3$ and 1·8 mM $CaCl_2$, pH 7·4) maintained at 37°C. The duodenum was cannulated and the end of the cannula was connected to a pressure transducer (DX-300, Nihon Kohden, Tokyo, Japan) to record changes in intraluminal volume, and to a bottle of saline to ensure a constant hydrostatic pressure of 6 cm H_2O.

Spontaneous phasic contractions of the stomach were continuously observed and recorded as rhythmic changes in gastric volume. Some of the antral contractions stopped at the pylorus, and others propagated to the duodenum (antroduodenal coordination) (Schuurkes & Van Nueten 1981). Contractions of the stomach and the duodenum were observed, and antroduodenal coordination was quantified as the relative number of antral contractions propagating to the duodenum. Automous duodenal contractions, not linked to the antral contractions, were rarely observed and were not taken into account. After 30-min stabilization different concentrations of the test drug were added to the bath solution. The number of antral contractions and the percentage antroduodenal coordination were determined.

Gastric emptying (in-vivo study)

Gastric emptying was determined by a modification of a reported procedure (Scarpignato et al 1980). The animals were deprived of food 24 h before the experiment but were allowed free access to water until 3 h before the experiment. A solution of 0·05% w/v phenol red in aqueous carmellose sodium (1·5% w/v) was used as the test meal. The test drug was administrated orally 1 h before administration of the test meal. Ten minutes after administration of the test meal (15 mL) the animals were killed by cervical dislocation. The stomach was then exposed by laparotomy and removed. In each experiment, three vehicle-treated animals were killed immediately after being given the meal and the phenol red content in the stomach was considered as the standard (100%) to avoid possible errors associated with terminal convulsions of the animal.

The removed stomach was incised, its contents were dissolved in NaOH solution (0·1 M, 40 mL), and the supernatant (1 mL) was added to trichloroacetic acid solution (7·5% w/v, 2 mL) to precipitate proteins. After centrifugation (2500 *g* for 15 min) the supernatant (1 mL) was added to NaOH (1 M, 1 mL) for development of the maximum intensity colour. The absorbance of the solution at 560 nm was then measured with a spectrophotometer (U-1080; Hitachi, Tokyo, Japan).

The gastric emptying (GE) for each animal was calculated according to the formula:

$$GE\ (\%) = [1 - (\text{Amount of phenol red recovered from the test stomach})/(\text{Average amount of phenol red recovered from the standard stomach})] \times 100$$

Statistical analyses

Results are expressed as means ± s.e.m. Statistical significance in each period was estimated either by the Kruskal–Wallis test followed by the Steel multiple comparison test, or by Wilcoxon rank sum test. A P value < 0.05 was considered indicative of statistical significance.

Results

Effects of drugs on antroduodenal coordination

Figure 1 is a typical photograph of the preparation showing the effects of KW-5092 (10^{-6} M). As shown in Figure 2, KW-5092 (10^{-6} M) evoked transient duodenal contractions, without eliciting antroduodenal coordination, thereafter followed by antral contractions which propagated to the duodenum (antroduodenal coordination) lasting for 30–40 min. KW-5092 at 3×10^{-7} M evoked transient and minimal duodenal contractions in four out of six preparations. As summarized in Figure 3, KW-5092 at 3×10^{-7} to 3×10^{-6} M induced antroduodenal coordination without affecting the frequency of contractions. Atropine (10^{-7} M) or tetrodotoxin (10^{-7} M), which did not themselves affect basal antroduodenal coordination, almost completely inhibited KW-5092-induced antroduodenal coordination (Figure 4).

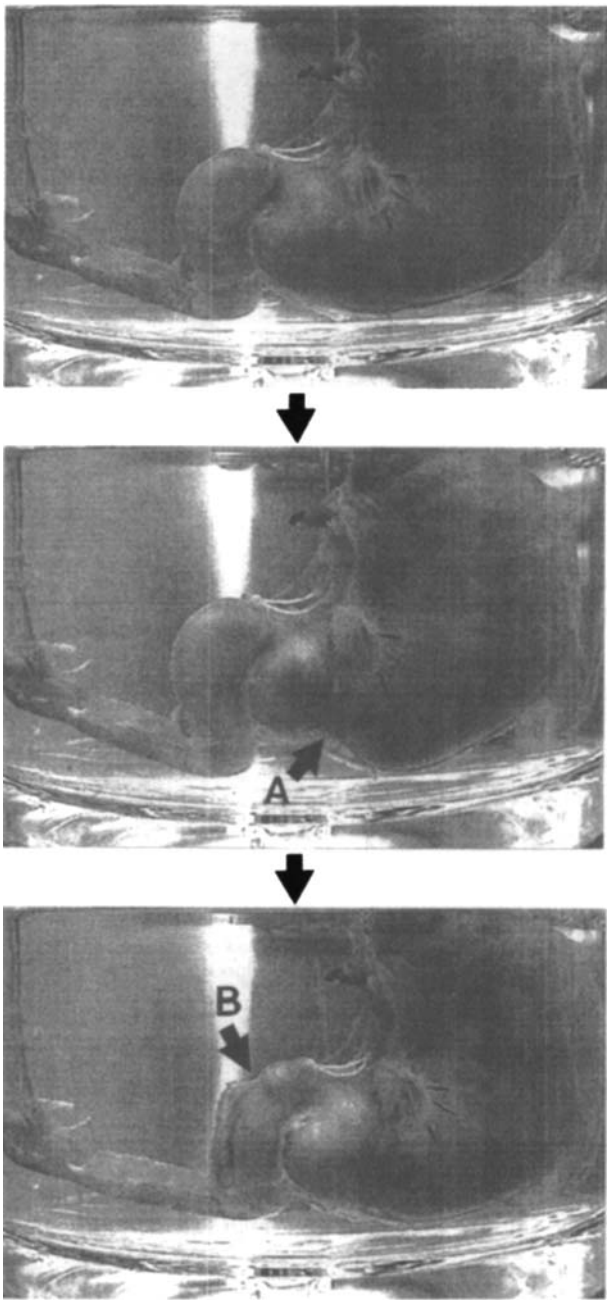

Figure 1. Typical photographs of the gastroduodenal preparation showing the antroduodenal coordination induced by KW-5092 (10^{-6} M) in the guinea-pig. A, antral contraction; B, duodenal contraction linked to antral contraction.

Figure 2 shows typical traces obtained from the preparation showing the effects of cisapride and neostigmine. Cisapride (10^{-6} M) evoked antroduodenal coordination and did not evoke transient duodenal contractions without eliciting antroduodenal coordination. Cisapride at 10^{-7} to 3×10^{-6} M induced the coordination without affecting the number of antral contractions (Figure 5) whereas neostigmine at 10^{-6} M and 3×10^{-6} M evoked continuous duodenal contractions, on which

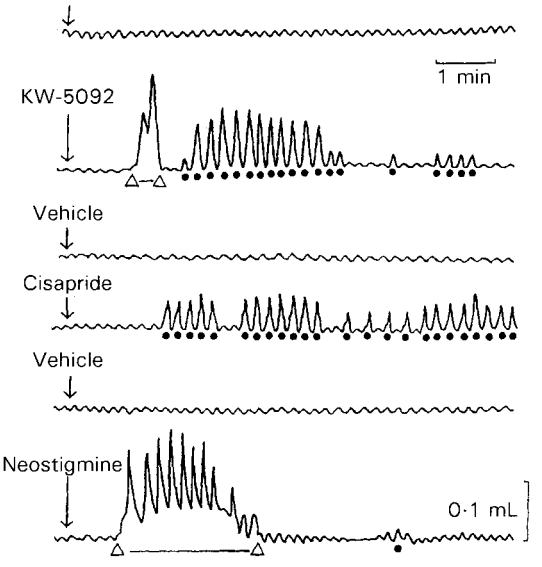

Figure 2. Typical traces of the effects of KW-5092, cisapride and neostigmine on motility in the guinea-pig gastroduodenal preparation. Vehicle, KW-5092 (10^{-6} M), cisapride (10^{-6} M) or neostigmine (10^{-6} M) was applied at the point indicated by the arrow. •, Antroduodenal coordination; △-△, duodenal contraction independent of antroduodenal coordination.

phasic contractions were superimposed, in all the preparations examined (data not shown). These evoked contractions were not accompanied by antroduodenal coordination (Figure 2). In contrast with KW-5092 and cisapride, neostigmine at 3×10^{-7} to 3×10^{-6} M did not affect antroduodenal coordination or the number of antral contractions during the experimental period (60 min).

Acetylcholine at 3×10^{-7} to 3×10^{-6} M did not affect antroduodenal coordination or the number of antral contractions. In all the preparations acetylcholine at 10^{-6} M and 3×10^{-6} M evoked continuous duodenal contractions without eliciting antroduodenal coordination (data not shown).

Effects of drugs on gastric emptying

Oral administration of KW-5092 at 10 mg kg^{-1} or cisapride at 0·3 mg kg^{-1} significantly enhanced gastric emptying whereas neostigmine administered orally at 3 mg kg^{-1} significantly delayed gastric emptying (Figure 6).

Discussion

This study has demonstrated that KW-5092, similarly to cisapride, enhances antroduodenal coordination and stimulates gastric emptying in the guinea-pig. The enhancement by KW-5092 of antroduodenal coordination was almost completely abolished by atropine or tetrodotoxin, indicating that the enhancement is mediated via the activation

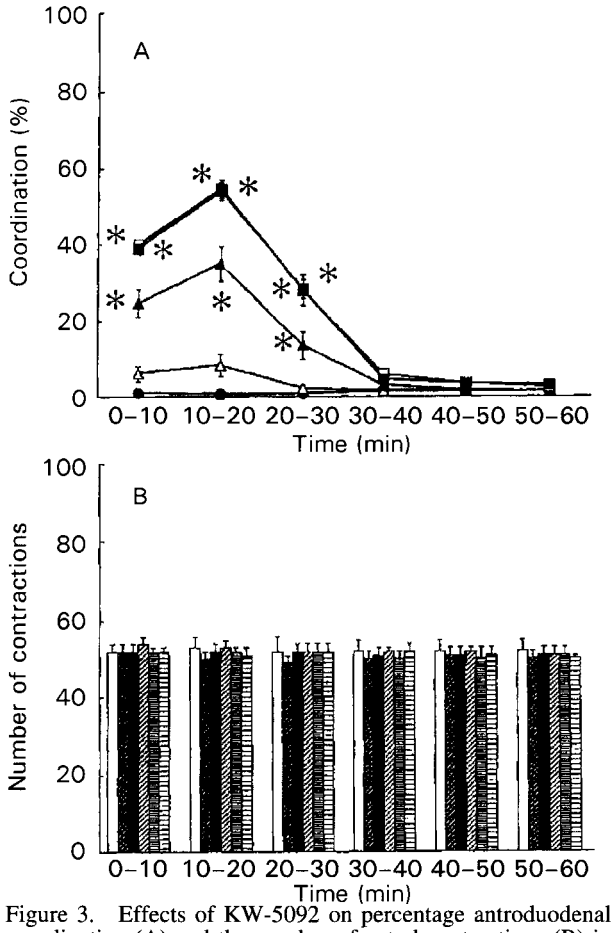

Figure 3. Effects of KW-5092 on percentage antroduodenal coordination (A) and the number of antral contractions (B) in the guinea-pig gastroduodenal preparation. A. Effects of KW-5092 (●, 3×10^{-8} M; △, 10^{-7} M; ▲, 3×10^{-7} M; □, 10^{-6} M; ■, 3×10^{-6} M) and of vehicle (○). B. Effects of KW-5092 (▨, 3×10^{-8} M; ■, 10^{-7} M; ▨, 3×10^{-7} M; ▤, 10^{-6} M; ▤, 3×10^{-6} M) and of vehicle (□). Each point or column represents the mean ± s.e.m. of results from six preparations. *$P < 0.05$, significantly different from the result for the vehicle-treated group (Steel multiple comparison test).

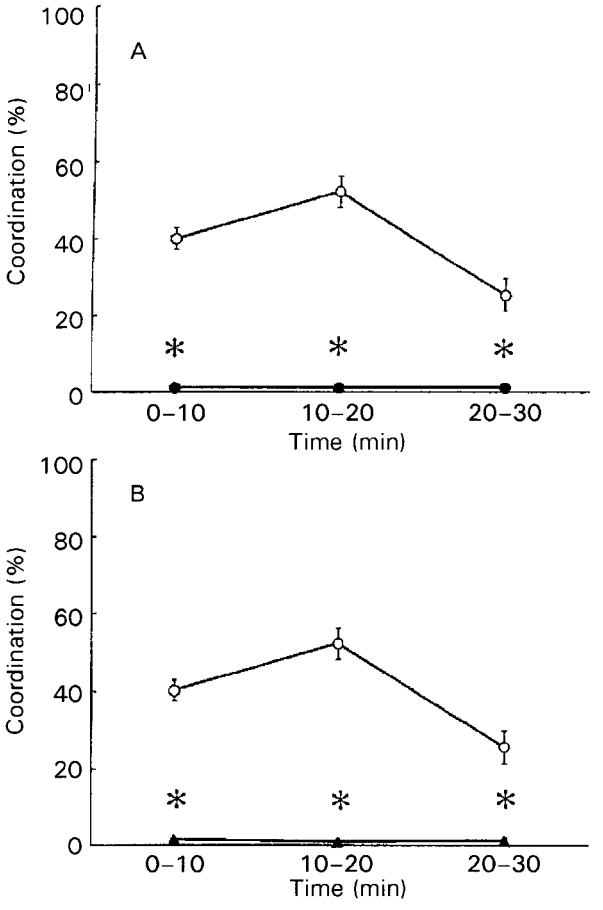

Figure 4. Effects of atropine (A) and tetrodotoxin (B) on the percentage increase of antroduodenal coordination induced by KW-5092 in the guinea-pig gastroduodenal preparation. ○, KW-5092 (10^{-6} M); ●, KW-5092 (10^{-6} M) + atropine (10^{-7} M); ▲, KW-5092 (10^{-6} M) + tetrodotoxin (10^{-7} M). Each point represents the mean ± s.e.m. of results from four preparations. *$P < 0.05$, significantly different from the result for the KW-5092-treated group (Wilcoxon rank sum test).

of cholinergic neurons. These results are in accordance with our previous observations that KW-5092 stimulates the motility of the stomach and the duodenum in dogs (Kishibayashi et al 1994c) and stimulates gastric emptying in rats (Kishibayashi & Karasawa 1995). It is reported that cisapride enhances antroduodenal coordination in guinea-pig isolated gastroduodenal preparations (Schuurkes et al 1985) and stimulates gastric emptying in man (Baeyens et al 1984) and in rats (Megens et al 1986). The current study using guinea-pigs has confirmed these previous observations. Schuurkes et al (1985) reported that the enhancement of antroduodenal coordination by cisapride was almost completely abolished by atropine or tetrodotoxin, suggesting that the enhanced antroduodenal coordination is because it facilitates acetylcholine release. This study with guinea-pigs has confirmed a previous report (Kishibayashi & Karasawa 1995) that neostigmine delays gastric emptying in rats, and has also shown that neostigmine evoked continuous duodenal contractions without eliciting antroduodenal coordination, observations which suggest that eliciting duodenal contractions without antroduodenal coordination could lead to delayed gastric emptying.

We have previously demonstrated that KW-5092 facilitated acetylcholine release (Kishibayashi et al 1994a) and inhibited acetylcholinesterase activity (Kishibayashi et al 1994b). In the current study cisapride, a gastroprokinetic agent which facilitates acetylcholine release, enhanced antroduodenal coordination whereas the acetylcholinesterase inhibitor neostigmine did not, suggesting that enhanced antroduodenal coordination is more related to acetylcholine release than to acetylcholinesterase inhibition. It is thus likely that KW-5092 enhanced antroduodenal coordination by

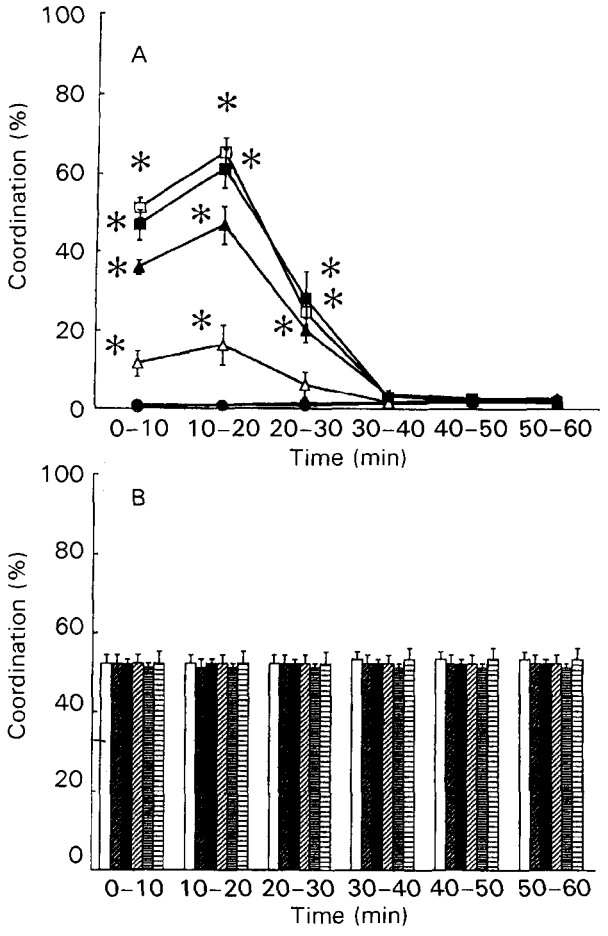

Figure 5. Effects of cisapride on percentage antroduodenal coordination (A) and the number of antral contractions (B) in the guinea-pig gastroduodenal preparation. A, Effects of cisapride (●, 3×10^{-8} M; △, 10^{-7} M; ▲, 3×10^{-7} M; □, 10^{-6} M; ■, 3×10^{-6} M) and of vehicle (○). B, Effects of cisapride (▨, 3×10^{-8} M; ■, 10^{-7} M; ▨, 3×10^{-7} M; ▤, 10^{-6} M; ▤, 3×10^{-6} M) and of vehicle (□). Each point or column represents the mean ± s.e.m. of results from six preparations. *$P < 0.05$, significantly different from the result for the vehicle-treated group (Steel multiple comparison test).

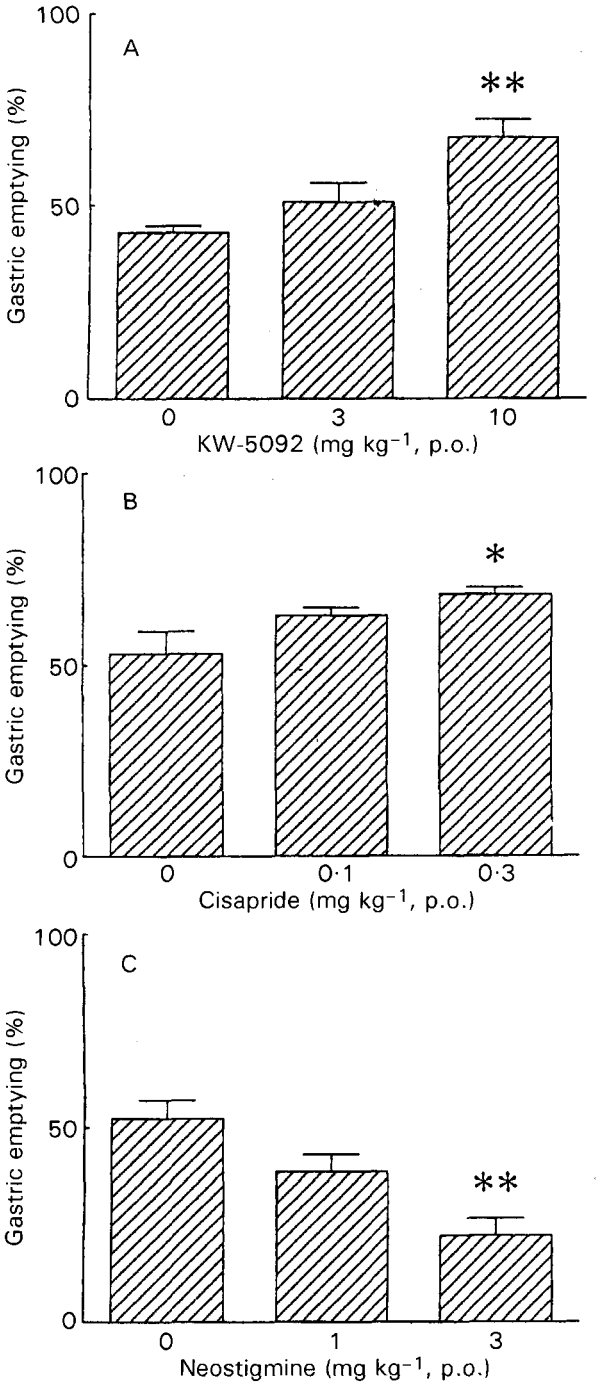

Figure 6. Effects of KW-5092 (A), cisapride (B) and neostigmine (C) on gastric emptying in the guinea-pig. Each column with bar represents the mean ± s.e.m. of results from six animals. *$P < 0.05$, **$P < 0.01$, significantly different from the control result (0 mg kg^{-1}, p.o.; Steel multiple comparison test).

facilitating release of acetylcholine and not by inhibiting acetylcholinesterase. The inability of exogenous acetylcholine to enhance antroduodenal coordination also suggests that the overall stimulation of muscarinic receptors of the antrum or the duodenum might not result in enhanced antroduodenal coordination. We suppose that cisapride and KW-5092 might selectively activate cholinergic neurons, which modulate antroduodenal coordination, resulting in stimulated gastric emptying.

Cisapride is reported to act on the inhibitory 5-HT_1 receptor as an antagonist and on the excitatory 5-HT_4 receptor as an agonist, and thereby enhance ileal motility in guinea-pigs (Taniyama et al 1991). Because KW-5092 at 10^{-5} M has very little affinity for 5-HT_1, 5-HT_2 or 5-HT_3 receptors and does not stimulate or antagonize 5-HT_4 receptors (data not shown), it is unlikely that KW-5092 elicits antroduodenal coordination via a 5-HT-ergic mechanism, as has been suggested for cisapride. Further studies are required to elucidate the precise mechanism of the modulation by KW-5092 of antroduodenal coordination.

In the clinical setting cisapride relieves gastric motility dysfunctions, including gastric stasis and gastroparesis, responsible for delayed gastric emptying (McCallum et al 1988). In man cisapride significantly increases antroduodenal coordination and stimulates digestive and indigestive antroduodenal motility (Lux et al 1994). Fraser et al (1994) suggested that the stimulation of antral pressure waves is involved in the accelerated gastric emptying induced by cisapride in patients with gastroparesis and in normal subjects. In the current study KW-5092, similarly to cisapride, enhanced antroduodenal coordination and gastric emptying in guinea-pigs and our previous study showed that the drug enhanced small intestinal propulsion, colonic propulsion and gastric emptying in rats (Kishibayashi & Karasawa 1995). These observations suggest that KW-5092 might relieve not only dysfunctions of gastric motility but also the non-ulcer dyspepsia which overlaps with irritable bowel syndrome (Colin-Jones 1988).

In conclusion, this study has demonstrated that KW-5092 enhances antroduodenal coordination and gastric emptying presumably by facilitating acetylcholine release. KW-5092 is likely to be a useful drug for the treatment of the patients with gastric motility dysfunction.

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