Effect of KW-5092, Novel Gastroprokinetic Agent, on the Peristalsis in the Isolated Guinea Pig Ileum

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Received October 11, 1996 Accepted February 28, 1997

ABSTRACT—We examined the effect of KW-5092, a gastroprokinetic agent with acetylcholinesterase inhibitory and acetylcholine release facilitatory activities, on the peristalsis of isolated guinea pig ileum. KW-5092 (10^-9 - 3 \times 10^{-6} \text{ M}) increased the frequency of the peristaltic wave without changing its amplitude. Neostigmine increased the frequency at 10^{-7} \text{ M}, but domperidone (10^{-8} - 3 \times 10^{-6} \text{ M}) had no effect on the peristalsis. The present results suggest that KW-5092 enhances the peristalsis via the inhibition of acetylcholinesterase, resulting in the intestinal propulsion.

Keywords: Peristalsis, Ileum, Acetylcholine

Peristalsis is important for gastrointestinal propulsion (1). Peristalsis is observed even in an isolated segment of intestine (2). When fluid is infused continuously into the lumen of the isolated segment, the intestine gradually distends until it reaches the threshold distention (3); thereafter, the circular muscle at the oral end of the intestine contracts, and the contraction propagates aborally along the intestine. Once the intestine is emptied, the cycle starts again during the infusion of fluid (4).

KW-5092 ([1-[2-[[5-(piperidinomethyl)2-furanyl]amino]ethyl]-2-imidazolidinylidene]propanedinitrile fumarate) is a newly synthesized gastroprokinetic agent that stimulates gastrointestinal motility in conscious dogs (5). KW-5092 enhances gastric emptying, small intestinal propulsion, colonic propulsion and defecation in rats (6, 7). In in vitro studies, KW-5092 was shown to enhance the release of acetylcholine (ACh) from enteric neurons (8) and to inhibit the activity of acetylcholinesterase (AChE) (9). Moreover, this drug stimulates spontaneous contraction of guinea pig ileum (8). In the present study, we examined the effects of KW-5092 on the peristalsis of isolated guinea pig ileum, in comparison with those of neostigmine and domperidone. The purpose of the study was to investigate the mechanism of action by which KW-5092 enhances the intestinal propulsion in vivo (6).

The experiment was performed according to the previous method (10). Male guinea pigs (250–500 g; Japan SLC, Inc., Hamamatsu) were sacrificed with ether, and 20-cm segments of ileum approximately 15-cm proximal to the ileocal junction were isolated. A portion of the ileum (3–4 cm) without lymphoid tissue was horizontally placed in an organ bath (diameter: 8 cm, height: 4 cm) filled with Tyrode's solution continuously bubbled with 95% O_2 + 5% CO_2 and maintained at 37°C. The oral end of the ileum was connected to the inflow silicon tube that was looped to allow the preparation to freely change its length. The anal end of the ileum was connected to the outflow glass cannula, in which the three-forked cock was installed: one end was connected to a pressure transducer (DX-300; Nihon Kohden Co., Tokyo) to record the intraluminal pressure, and the other end was used as the outlet of perfusate. The end of the outlet was held 2 cm above the preparation. After completion of the preparation setup, the intestine was intraluminally perfused with Tyrode's solution, bubbled with 95% O_2 + 5% CO_2 and maintained at 37°C, at the rate of 1–6 ml/hr, which induced a peristalsis of 5–6 cycles/10 min. The bathing solution was continuously exchanged. In the present study, the peristalsis induced by intraluminal perfusion was exemplified as coordinated contraction and relaxation of circular and longitudinal smooth muscles, which paralleled the change in intraluminal pressure (peristaltic wave). Thus, we analyzed the frequency and the amplitude of the peristaltic wave.

In the experiment for serosal drug administration, a drug solution was applied to the organ bath and the bathing solution. In the experiment for intraluminal administration, a drug solution was applied to the inflow...
Fig. 1. Peristalsis in isolated guinea pig ileum. When fluid is infused continuously into the lumen, the ileum gradually distends (a, b) until, at a threshold distention, the circular muscle at the oral end of the ileum contracts (c), and a wave of contraction propagates aborally along the ileum (d). Once the ileum has emptied, the cycle starts again if the infusion of fluid continues.
tube (intraluminal perfusate). Drugs used were: KW-5092 (Kyowa Hakko Kogyo, Co., Ltd., Shizuoka), neostigmine methylsulfate (Sigma Chemical Co., St. Louis, MO, USA), domperidone (Janssen Pharmaceutica, Beerse, Belgium) and atropine sulfate (Nacalai Tesque, Kyoto).

Results were expressed as the ratio of the peristalsis after drug administration to that of the spontaneous peristalsis. Statistical analyses of frequency and amplitude of peristalsis were performed by the paired t-test between the values before (spontaneous peristalsis) and after drug treatment. The criterion for statistical significance was set at P < 0.05.

In the present experimental condition, peristalsis of the isolated guinea pig ileum almost constantly persisted during the experimental period. The constriction of the circular muscle initiated from the oral end at the threshold pressure and propagated toward the anal end, thereby ejecting the intraluminal fluid (Fig. 1). Accompanying the fluid ejection, the longitudinal muscle was elongated, and then the next cycle of peristalsis was initiated.

**Fig. 2.** Effects of the serosal administration of KW-5092 (A), neostigmine (B) and domperidone (C) on the peristalsis in isolated guinea pig ileum. Each point and vertical bar represents the mean ± S.E.M. of 4–6 preparations. The frequency (open circle) and amplitude (closed circle) show the ratio for the spontaneous peristalsis. Drugs were given to the serosal side of the ileum. *P < 0.05, **P < 0.01, ***P < 0.001, compared with the value of the spontaneous peristalsis (Paired t-test). V: Vehicle.

**Fig. 3.** Effect of atropine on the peristalsis in isolated guinea pig ileum. Each column and vertical bar represents the mean ± S.E.M. of 3–5 preparations. The frequency shows the ratio for the spontaneous peristalsis. Drugs were given to the serosal side of the ileum. *P < 0.05, compared with the spontaneous peristalsis (paired t-test).

KW-5092 (3 × 10^-7–3 × 10^-6 M), when applied into the serosal side, significantly increased the frequency without affecting the amplitude (Fig. 2A). Neostigmine also increased the frequency at 10^-7 M, while this drug tended to reduce the amplitude (Fig. 2B). We suppose that the latter effect was due to the reduced spontaneous contraction by neostigmine. In fact, neostigmine (10^-7–10^-5 M) is known to cause tonic contraction via a direct effect on smooth muscle (11). KW-5092 does not have such a direct effect because the contraction induced by KW-5092 is almost completely abolished by tetrodotoxin (M. Suzuki et al., unpublished observation). On the other hand, domperidone at concentrations from 10^-8 to 3 × 10^-6 M had no effect on spontaneous peristalsis (Fig. 2C). Atropine at 10^-9 M, which per se did not affect the peristalsis, reduced the increment of frequency by KW-5092 (10^-6 M) (Fig. 3). When given to the mucosal side, KW-5092 (10^-7–3 × 10^-6 M) did not affect the peristalsis (data not shown), suggesting that KW-5092 induces the intestinal propulsion via the blood stream in vivo.

Serosal application of KW-5092 (3 × 10^-7–3 × 10^-6 M) increased the frequency, but not the amplitude, of peristalsis. It is supposed that the increase in frequency is mediated via the decrement of the stretch receptor threshold or the enhancement of contraction (3).
However, KW-5092 is not likely to act on the stretch receptor directly because the mucosal administration of KW-5092 did not affect the peristalsis. Enhancement of contraction by KW-5092 might have contributed to the increased frequency via the activation of the stretch receptor because this drug is known to enhance the spontaneous contraction of isolated guinea pig ileum (EC$_{50}$ = 4.7 x 10$^{-7}$ M) (2). In this study, atropine (10$^{-9}$ M) inhibited the enhancement of peristalsis by KW-5092, suggesting that the effect of KW-5092 was mediated via cholinergic activation. In fact, KW-5092 enhances the release of ACh (EC$_{50}$ = 6.9 x 10$^{-7}$ M) (2) and inhibits the activity of AChE (IC$_{50}$ = 6.8 x 10$^{-8}$ M) (3) in guinea pig ileum. Further studies, however, are required to elucidate the precise mechanism whereby KW-5092 increases the frequency of the peristalsis.

In the present study, KW-5092 and neostigmine, both of which exhibit AChE inhibitory action, enhanced the peristalsis. In contrast, domperidone, a gastroprokinetic agent which is reported to enhance the ACh release from the stomach via the blockade of dopamine D$_2$ receptor (12), did not affect the peristalsis. The stimulation of ACh release may not result in the enhanced peristalsis in the ileum. Alternatively, the inability of domperidone to enhance the peristalsis may be due to the fact that endogenous dopamine is not involved in the ACh release in the ileum. The previous in vivo study demonstrated that neostigmine at the doses of 3 and 10 mg/kg (p.o.) accelerated the small intestinal propulsion (6). KW-5092 also stimulates the small intestinal propulsion in rats in vivo (6), whereas domperidone does not affect the propulsion. These data suggest that the stimulatory effects of KW-5092 and neostigmine on the peristalsis in vitro and the propulsion in vivo were exerted through AChE inhibition. We cannot, however, exclude the possibility that the stimulated ACh release played a role in the enhanced peristalsis of the KW-5092-treated ileum.

The present study showed that KW-5092 enhances the peristalsis of the intestine, presumably resulting in the intestinal propulsion (6). It is thus expected that KW-5092 may be an effective drug for the diseases caused by impaired gastrointestinal transit such as the constipation of irritable bowel syndrome or the non-ulcer dyspepsia.

In summary, the present study demonstrates that KW-5092 stimulates the peristalsis of the ileum through the inhibition of AChE, possibly resulting in the accelerated small intestinal propulsion in vivo.

Acknowledgment
We would like to thank Dr. N. Suzuki of Kanazawa University for his technical advice.

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