Acceleration by KW-5092 of Intestinal Motility Associated With Acetylcholine Release In Vivo

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ABSTRACT—Effect of KW-5092 ({1-[2-[[[5-(piperidinomethyl)-2-furanyl]methyl]amino]ethyl]-2-imidazolidinylidene}propanedinitrile fumarate) on intestinal motility and release of endogenous acetylcholine (ACh) were measured simultaneously in the small intestine of anesthetized dog using the in vivo microdialysis method. Intraarterial and intravenous administrations of KW-5092 accelerated the intestinal motility and increased dialysate ACh concentrations. These KW-5092-induced responses paralleled the increase in blood concentration of KW-5092. Thus, the acceleration of intestinal motility by KW-5092 was found in vivo to be associated with an increase in ACh release from the intestinal cholinergic neurons.

Keywords: Intestinal motility, Acetylcholine release, In vivo microdialysis

KW-5092 ({1-[2-[[[5-(piperidinomethyl)-2-furanyl]methyl]amino]ethyl]-2-imidazolidinylidene}propanedinitrile fumarate), a ranitidine derivative devoid of histamine H₂-receptor blocking property, enhanced gastric emptying and accelerated the intestinal motility (1–3). In vitro studies showed that KW-5092 is a potent inhibitor of acetylcholinesterase (AChE) (4) and potentiates the electrically-stimulated contractions and release of acetylcholine (ACh) in longitudinal muscle preparations with myenteric plexus (5); thus, these effects have been suggested to participate in the mechanism underlying accelerations of the gastrointestinal motility induced by KW-5092. It is important to identify the mechanism underlying the effects of substances in whole body animals, since in vivo findings are not always consistent with in vitro findings. We detected the intestinal motility associated with ACh release from enteric nerves in the whole body of dogs by in vivo microdialysis method (6). We attempted to demonstrate KW-5092-induced acceleration of intestinal motility associated with increase in ACh release by measuring motility and ACh release simultaneously through a microdialysis fiber implanted around the muscle layers including the myenteric plexus of the small intestine of the dog.

The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health,

USA. Experiments were done on healthy, mature, male beagle dogs, weighing between 7 and 11 kg, and surgical procedures were performed as previously described (6). Under anesthesia with pentobarbital Na (25 mg/kg, i.v.), a strain gauge force transducer was sutured to the serosa of a defined area of the small intestine (a region 30- to 50-cm distal from the ligament of Treitz) for measuring contractility in the circular muscle direction. A dialysis probe (O-P-100-10; Eicom, Kyoto) was gently inserted tangentially into the wall of the small intestine; and part of the dialysis membrane (10-mm-long, 0.2-mm diameter) of the probe was positioned within circular muscle layers including the myenteric plexus, approximately at the site of transducer sutured. A catheter connected to an injection-syringe was inserted in the cephalic antebachium vein for intravenous injection of substances and in the intestinal marginal artery for intraarterial injection. The area of arterial supply was defined by flushing with 1 ml of Ringer solution (147 mM Na⁺, 2.3 mM Ca²⁺, 155.6 mM Cl⁻ and 3 mM K⁺). The dialysis probe was continually perfused at a flow rate of 2.0 µl/min with Ringer solution containing 0.2 mM physostigmine. Because it is well known that the enzymatic activity of extracellular cholinesterase is markedly high, physostigmine, a cholinesterase inhibitor, was added to the perfusion solution to block the degradation of ACh, as in the case of the brain microdialysis method (7). The dialysate was collected every 15 min in the sample loop of the automated sample injector, which was set up on line with

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the HPLC-ECD system. The dialysates from the first to 4th fraction after probe implantation were discarded, and ACh concentration in the dialysate from the 5th to 8th fractions were determined as the mean basal concentration of dialysate ACh. Four fractions of 15-min control dialysates were collected. Then KW-5092 (Kyowa Hakko Kogyo, Tokyo) dissolved in saline was infused into the cephalic antebrachium vein (2 ml volume at 2 ml/min) or intestinal marginal artery (0.5 ml volume at 0.5 ml/min). The concentration of dialysate ACh in the presence of the substance was represented as the percentage of the basal concentration of dialysate ACh of each experiment. The tissues around the probe were dissected at the end of experiment, and the correct position of the probe was verified histologically.

One milliliter of blood was collected from the jugular

vein at 7.5, 23.5, 39.5, 55.5, 87.5, 119.5 and 151.5 min after intravenous administration of KW-5092, and then the plasma concentration of KW-5092 at each time was measured by spectrophotometer at 253 nm.

Nonparametric independent group comparisons were made. For multiple comparisons, the Kruskal-Wallis and Dunnett multiple comparison tests were performed. Statistical significance was inferred from a *P* value of <0.05.

Basal concentrations of ACh measured in the dialysate were 0.733 ± 0.380 pmol/15 min ($n=15$), a value that remained constant until 240 min from 60 min after perfusion and showed little variation with each animal. The dialysis fiber was implanted into the circular muscle layer including myenteric plexus of the intestine; therefore, concentrations of ACh detected in this system may originate

Fig. 1. Effect of intravenous administration of KW-5092 on the intestinal motility (A), dialysate ACh concentration (B) and plasma KW-5092 concentration (C). A: Representative pattern of motility. B: Each point represents the mean $\pm$ S.E.M. from 4 animals administered 2 ml of saline ($\bullet$ — $\bullet$), 4 animals administered 0.3 mg/kg of KW-5092 ($\circ$ — $\circ$) and 6 animals administered 1 mg/kg of KW-5092 ($\bullet$ — $\bullet$). Dialysate ACh concentration in one each fraction (15-min dialysate) was calculated relative to the basal dialysate ACh concentration (before administration of agent) as 100%, and each value was represented at the center of 15-min intervals. *Significant difference from the basal dialysate ACh concentration (0.733 ± 0.380 pmol/15 min) ($P < 0.05$). C: Each point represents the mean $\pm$ S.E.M. of plasma KW-5092 concentration from 4 animals administered 0.3 mg/kg of KW-5092 ($\circ$ — $\circ$) and 6 animals administered 1 mg/kg of KW-5092 ($\bullet$ — $\bullet$).

from nerve terminals of preganglionic and postganglionic cholinergic neurons. Intravenous administration of saline (2 ml volume at 2 ml/min) did not affect the spontaneous motility and basal concentration of dialysate ACh (Fig. 1: A and B). Intravenous administration of KW-5092 at 1 mg/kg remarkably accelerated the motility and significantly increased the concentration of dialysate ACh (Fig. 1: A and B). Thus, KW-5092 was observed to accelerate the intestinal motility associated with an increase in ACh release, in the whole body animal, as shown in the *in vitro* experiment (5). The plasma concentration of KW-5092 also increased after intravenous administration of KW-5092 at 1 mg/kg (Fig. 1C), thereby indicating that the KW-5092-induced acceleration of motility associated with increase in ACh release paralleled the increase in plasma concentration of KW-5092. Intravenous administration of KW-5092 at 0.3 mg/kg maximally increased the plasma concentrations of the substance in the 7.5-min sample to approximately 130 ng/ml, which was the same level of that in the 23.5-min sample after intravenous administration of the substance at 1 mg/kg, while the motility and concentration of dialysate ACh did not change. Such a plasma concentration of the substance may not be enough to increase the release of ACh from the cholinergic nerve terminals.

Intraarterial administration of saline (0.5 ml volume at 0.5 ml/min) did not affect the spontaneous motility and basal concentration of dialysate ACh. Intraarterial local administration of KW-5092 at 0.01 mg accelerated the intestinal motility and increased the concentration of dialysate ACh (Fig. 2). Since locally administered KW-5092 increased the release of ACh and accelerated motility, KW-5092-induced effects may be expressed by direct action at the enteric neurons, not via effects on various organs of the whole system. Furthermore, this result indicates that the increase in dialysate ACh concentration is not attributed to the inhibition of AChE activity by KW-5092 (4), because AChE activity neighboring the active site of the microdialysis probe had been inhibited by the perfusion solution containing high concentration of physostigmine.

The motility accelerated after administration of KW-5092, while the dialysate ACh concentration significantly increased from the second fraction after administration of KW-5092, but not significantly at the first fraction. Since the ACh concentration was the concentration in the dialysate collected for 15 min after administration of the substance, the dialysate surveyed was probably diluted by the dialysate collected before expression of the substance effect. There is also a possibility that a small increase in ACh release accelerates the motility.

The present study demonstrated in the whole body animal that KW-5092 administered intravenously accelerated intestinal motility due to increase in ACh release from the cholinergic nerve terminals. Ranitidine, a lead com-

Fig. 2. Effect of intraarterial administration of KW-5092 on the intestinal motility (A) and dialysate ACh concentration (B). Dialysate ACh concentration was calculated relative to the basal dialysate ACh concentration (before administration of KW-5092 at 0.01 mg) as 100%. Each column represents the mean $\pm$ S.E.M. from 4 animals. *Significant difference from the basal dialysate ACh concentration (0.733 ± 0.380 pmol/15 min) ($P < 0.05$).

pound of KW-5092, has been reported to possess anticholinesterase and antimuscarinic activity (8, 9). Increase in ACh release and acceleration of motility by KW-5092 were detected under the condition of complete inhibition of cholinesterase; therefore, the effect of KW-5092 may be produced by inherent activity of KW-5092 other than that of ranitidine. The present study could not elucidate the mechanism underlying the increase in ACh release by KW-5092. Indirect action of KW-5092 has been shown in the guinea pig colon, in which the substance stimulates intraluminal 5-hydroxytryptamine release due to the stimulation of cholinergic neurons (10).

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