KW-5092, a Novel Gastroprokinetic Agent, Reverses the Norepinephrine-Induced Decline of the Gastric Mucosal Blood Flow in Rats

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ABSTRACT—We examined the effects of KW-5092 ([1-[2-[[5-(piperidinomethyl)-2-furanyl]methyl]-amino]ethyl]-2-imidazolidinylidene)propanedinitrile fumarate), a novel gastroprokinetic agent, on gastric mucosal blood flow (GMBF) in anesthetized rats. Intravenous infusion of KW-5092 (0.1 mg/kg/min for 30 min), which did not affect the basal GMBF, reversed the norepinephrine (1 μg/kg/min, i.v. infusion for 30 min)-induced decline of GMBF in the corpus and the antrum. The improvement by KW-5092 of the GMBF was abolished by atropine (0.1 mg/kg/min, i.v. infusion for 30 min). These results suggest that KW-5092, via cholinergic activation, could counteract the decline of GMBF induced by adrenergic activation.

Keywords: KW-5092, Gastric mucosal blood flow, Norepinephrine

Gastric mucosal blood flow (GMBF) is reduced by exogeneous norepinephrine (NE) (1) as well as by sympathetic nerve stimulation (2). In contrast, GMBF is increased by vagal nerve stimulation (3). The increased GMBF following vagal nerve stimulation is counteracted by NE (3), suggesting a counter-regulatory mechanism for the control of GMBF via the sympathetic (adrenergic) and the parasympathetic (cholinergic) nervous system. KW-5092 ([1-[2-[[5-(piperidinomethyl)-2-furanyl]methyl]-amino]ethyl]-2-imidazolidinylidene)propanedinitrile fumarate) is a newly synthesized gastroprokinetic agent, which enhances gastrointestinal motilities in a wide range from the stomach to the colon (4, 5). In the guinea pig ileum, KW-5092 enhances acetylcholine (ACh) release from enteric neurons (6) and inhibits acetylcholinesterase (AChE) (7). Since KW-5092 stimulates cholinergic activity, it seemed of interest to examine the effects of this drug on the GMBF. In the present study, we investigated the effects of KW-5092 on the basal GMBF and on the decreased GMBF induced by NE in anesthetized rats.

Male Sprague-Dawley rats (Japan SLC, Inc., Hamamatsu), weighing 150 to 200 g, were used for the experiment. The animals were maintained on ordinary laboratory chow and tap water ad libitum under a constant 12-hr light-dark cycle. The drugs used were KW-5092, NE and atropine. KW-5092 was synthesized in our laboratories. NE and atropine sulfate were purchased from Sankyo Co., Ltd. (Tokyo) and Nacalai Tesque, Inc. (Kyoto), respectively. The test drugs were dissolved in saline and intravenously infused to rats at a volume of 0.1 ml/kg/min.

GMBF was determined according to the reported procedure (8). The animals were deprived of food 24 hr prior to the experiment but allowed free access to water. Each animal was anesthetized by urethane (1.25 g/kg, s.c.), and a polyethylene tube was placed into the trachea for the maintenance of respiration. For the measurement of blood pressure and the i.v. infusion of drugs, polyethylene tubes were placed into the carotid artery and the jugular vein, respectively. The body temperature was maintained at 37±1°C with a temperature controller (CMA/150; Carnegie Medicine, Stockholm, Sweden). After a midline incision, the stomach was exposed, and the great curvature was incised. Thereafter, the fiber-optic probe of the laser flowmetry system (ALF21; Advance Co., Ltd., Tokyo) was gently placed on the mucosa of the corpus or the antrum of the stomach. One hour after the administration of urethane, the GMBF and systemic blood pressure were measured for 90 min. The GMBF was measured by the laser flowmetry system, and the blood pressure was measured by a pressure transducer (DX-300; Nihon Kohden Co., Tokyo) connected to the tube placed into the carotid artery. After a stabilization period of 30 min, KW-5092 (0.1 mg/kg/min), NE (1 μg/kg/min) and/or atropine (0.1 mg/kg/min) (or the vehicle) were infused to the jugular vein for 30 min. In the
present study, the dose of KW-5092, 0.1 mg/kg/min for 30 min, was examined since KW-5092 at 0.03 mg/kg/min for 30 min did not significantly reverse the NE-induced decline of GMBF in the preliminary study.

The results are expressed as means ± S.E.M. Statistical significance was estimated by Student's t-test. A P value of less than 0.05 was considered to be statistically significant.

The effects of KW-5092 on the GMBF in the corpus and the antrum are shown in Figs. 1 and 2, respectively. KW-5092 (0.1 mg/kg/min, i.v.) did not affect the basal GMBF in either the corpus or the antrum and did not affect the systemic blood pressure. NE (1 μg/kg/min, i.v.) decreased the GMBF in the corpus and the antrum by 30 ± 7% and 38 ± 4% (mean ± S.E.M., n = 5), respectively, and it elevated the systemic blood pressure by 22 ± 3% (mean ± S.E.M., n = 10). KW-5092 (0.1 mg/kg/min, i.v.) reversed the NE (1 μg/kg/min, i.v.)-induced decline of the GMBF in the corpus and the antrum without affecting the NE-induced elevation of the systemic blood pressure.

These effects of KW-5092 and/or NE on the GMBF were confirmed by measuring the GMBF in anesthetized rats by the hydrogen gas clearance method according to the reported procedure (9) (data not shown).

Atropine (0.1 mg/kg/min, i.v.) per se did not affect the basal GMBF or the NE-induced decline of the GMBF in the corpus and the antrum (data not shown). The improvement by KW-5092 of the GMBF was abolished by atropine (Fig. 3), suggesting that the effect of KW-5092 is mediated via cholinergic activation.

In the previous study using anesthetized rats (2), sympathetic stimulation decreased the GMBF through a constricting effect on the gastric submucosal arterioles, while parasympathetic stimulation increased the GMBF through a vasodilating effect on the gastric submucosal arterioles. In the present study, the reversal by KW-5092 of the NE-induced decline of the GMBF was inhibited by atropine, while the NE-induced elevation of the systemic blood pressure was unaffected. We suppose that the local GMBF is increased by KW-5092 through a vasodilator
effect of ACh, presumably mediated via the ACh release facilitatory and/or the AChE inhibitory action of this drug (6, 7). Moreover, the present observations support the notion that the GMBF is dually regulated via the sympathetic (adrenergic) and the parasympathetic (cholinergic) nervous system.

In the previous study, cisapride, a gastroprokinetic agent, at the gastroprokinetic doses enhanced the basal GMBF in the antrum via cholinergic actions without affecting the systemic blood pressure in anesthetized dogs (10). Moreover, cisapride reversed the indomethacin-induced decline of the GMBF in the corpus in anesthetized rats (11). In the present study, KW-5092 also reversed the NE-induced decline of the GMBF via cholinergic action. These results suggest that the activation of cholinergic actions may play some roles in the improvement of the GMBF dysfunction by certain gastroprokinetic agents.

It is reported that ischemia in the gastric mucosa is the main cause of the acute gastric mucosal lesions in rats with hemorrhagic shock (12) and in patients with thermal or head injury (13). Moreover, the hemorrhagic shock stress-induced decline of the GMBF in the miniature swine is reported to be due to the vasoconstrictor action of NE, which is released from sympathetic nerve endings (14). These observations indicate that the continued decline of the GMBF could lead to the formation of gastric mucosal lesions, one of the mediators of which is NE. It is thus suggested that KW-5092 could ameliorate gastric mucosal lesions in patients suffering from depressed GMBF.

In the previous study, oral administration of KW-5092 at 10 and 30 mg/kg significantly enhanced gastric emptying in rats (5). On the other hand, KW-5092 at 10 mg/kg (p.o.) also reversed the NE-induced decline of the GMBF in anesthetized rats (N. Kishibayashi et al., unpublished observation), indicating that KW-5092 at the gastroprokinetic dose reverses the NE-induced decline of GMBF. Yamaguchi (15) reported that in rats, hemorrhagic hypotension by blood withdrawal decreased GMBF.

![Fig. 2. Effects of KW-5092 on the basal gastric mucosal blood flow (GMBF) (A), the basal systemic blood pressure (B), the NE-induced decline of GMBF (C) and the NE-induced increase of systemic blood pressure (D) in the gastric antrum of anesthetized rats. KW-5092 (0.1 mg/kg/min) (●), norepinephrine (NE, 1 μg/kg/min) (△), the combination of KW-5092 (0.1 mg/kg/min) and NE (1 μg/kg/min) (▲), or the vehicle (○) was intravenously infused during 0–30 min. Each bar represents the mean±S.E.M. of 5 rats. *P<0.05, **P<0.01, compared with the value in the rat treated with vehicle (Student's t-test). †P<0.05, ‡P<0.01, compared with the value in the rat treated with NE (Student's t-test).]
without affecting gastric motility while water-immersion restraint decreased gastric motility without affecting GMBF, suggesting that gastric motility and GMBF may not affect each other. Thus, it is not likely that the gastroprokinetic action of KW-5092 contributed to the enhancement of GMBF by this drug. However, further study is necessary to clarify this point.

In conclusion, the present study using anesthetized rats demonstrated that KW-5092, via the cholinergic action, reversed the NE-induced decline of the GMBF in the corpus and the antrum. The result suggests that cholinergic activation could counteract the decline of GMBF induced by adrenergic activation. The presently clarified protective effect of KW-5092 against the obstruction of GMBF could contribute to the amelioration of the GMBF dysfunction in humans.

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