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Short communication

KW-5092, a novel gastrokinetic agent, facilitates luminal serotonin release from the guinea-pig colon

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Abstract

The present study was designed to determine the influence of KW-5092 ((1-[2-[[[5-(piperidinomethyl)-2-furanyl]methyl]amino]ethyl]-2-imidazolidinylidene) propanedinitrile fumarate), a novel gastroprokinetic agent on intraluminal serotonin (5-hydroxytryptamine, 5-HT) release which reflects the release of 5-HT from enterochromaffin cells, using the luminally perfused isolated guinea-pig proximal colon in vitro. 5-HT was determined by high-performance liquid chromatography with electrochemical detection. KW-5092 (1–10 μM) concentration-dependently caused an increase in the luminal 5-HT outflow. In the presence of atropine (0.2 μM) or tetrodotoxin (0.3 μM), the stimulatory action of KW-5092 (10 μM) was inhibited by 94% and 74%, respectively. These results suggest that KW-5092 stimulates intraluminal 5-HT release from luminally perfused proximal colon of the guinea-pig via the stimulation of cholinergic neurons. Because 5-HT is recognized as an important messenger substance in the control of intestinal motility, this stimulatory effect could be considered as an indirect action of KW-5092 that may contribute to its prokinetic effects.

Keywords: 5-HT (5-hydroxytryptamine, serotonin) release; KW-5092; Colon, guinea-pig

1. Introduction

KW-5092 ((1-[2-[[[5-(piperidinomethyl)-2-furanyl]-methyl]amino]-ethyl]-2-imidazolidinylidene) propanedinitrile fumarate) is a newly synthesized gastroprokinetic agent, which leads to increased gastric emptying and to accelerated transit through the small and large intestines (Kishibayashi and Karasawa, 1995). The exact mechanism by which KW-5092 increases gastrointestinal motility is still not completely understood. However, KW-5092 has been shown to evoke the facilitation of cholinergic excitatory neurotransmission in the guinea-pig ileum, and this has been put forward as the mechanism by which motility is enhanced (Kishibayashi et al., 1994).

Serotonin (5-hydroxytryptamine, 5-HT) is recognized as an important messenger substance in the control of intestinal motility (Goyal and Hirano, 1996), and the majority of 5-HT are located in mucosal enterochromaffin cells (Erspamer and Asero, 1952). More recently, we have shown that the isolated luminally prefused colon of the guinea-pig is a useful preparation to study the luminal release of 5-HT from the enterochromaffin cells (Kojima and Ikeda, 1998). It was thus of interest to test whether KW-5092 might affect the luminal 5-HT release. The aim of this study was to determine the influence of KW-5092 on intraluminal spontaneous 5-HT release from the luminally perfused colon of the guinea-pig in vitro.

2. Materials and methods

2.1. Experimental set-up

Male Dunkin–Hartley guinea-pigs, weighing 270–400 g, were anaesthetized with enflurane and bled. The preparation of the luminally perfused proximal colon and its set-up were done as described in a previous study (Kojima and Ikeda, 1998). The oral and aboral ends of the colon were cannulated and perfused with a physiological salt solution (PSS, mM; NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.56, ascorbic acid 0.12, Na_2EDTA 0.03, pH 7.4) at a constant

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Table 1
The spontaneous luminal outflow (mean outflow) of 5-HT and KW-5092 (KW, 1–10 μM)-evoked maximal 5-HT outflow in the absence and in the presence of atropine (ATR, 0.2 μM) or tetrodotoxin (TTX, 0.3 μM). Values are mean ± S.E.M; *n*, number of animals studied

Compounds	5-HT outflow (pmol/g tissue/3 min)	*n*
Control	33 ± 5.2	10
ATR	8.2 ± 1.7[a]	9
TTX	18 ± 3.6	8
KW 1 μM	70 ± 12	6
KW 10 μM	150 ± 38[b]	6
KW 10/ATR	15 ± 2.5[c]	4
KW 10/TTX	49 ± 7.4[c]	4

[a] $P < 0.05$; [b] $P < 0.01$ vs. the control outflow; [c] $P < 0.01$ vs. the KW 10 μM alone (Dunnett's test).

rate of 0.7 ml/min by a peristaltic pump. The luminally perfused colon was then suspended in a longitudinal direction under a 3.2-g load in a 25-ml tissue bath filled with PSS at 37°C and oxygenated with 5% CO_2–95% O_2. After set-up, the luminally perfused colon was allowed to equilibrate for 70 min with renewal of the bathing solution every 14 min.

2.2. Measurement of intraluminal 5-HT

After equilibration for 70 min, the experiments were carried out by collecting the luminal fluid every 3 min. The collected luminal fluid was lyophilized, dissolved in 0.4 M perchloric acid (200 μl) and passed through a 0.45-μm filter (chromatodisc 4A, GL Sciences). 5-HT in the filtrate was measured by high-performance liquid chromatography (LCEC II system, BAS) with electrochemical detection (LC-4B, BAS) as described previously (Kojima and Ikeda, 1998). The separation of 5-HT was achieved by a reverse-phase capillary column (length 150 mm, inner diameter 1.0 mm, BAS), using a mobile phase consisting of 0.1 M monochloroacetic acid, 1 mM Na_2EDTA, 60 mg/l sodium octylsulfate and 5% acetonitrile (pH 3.2) at a flow rate of 0.1 ml/min.

2.3. Drugs

The following drugs were used: atropine sulphate (Wako, Osaka, Japan); tetrodotoxin (Sankyo, Tokyo, Japan); KW-5092 ((1-[2-[[[5-(piperidinomethyl)-2-furanyl]-methyl]amino]ethyl]-2-imidazolidinylidene) propanedinitrile fumarate), gift from Kyowa Hakko Kogyo, Shizuoka, Japan). The reported concentrations are the calculated final concentrations in the bath medium.

2.4. Statistical analysis

The intraluminal outflow of 5-HT is expressed as picomole per gram wet weight of tissue and per collection period (pmol/g tissue/3 min). Data are presented as means ± standard error of the mean (S.E.M.) from 4 to 10 experiments with proximal colons from different animals. The significance of differences was evaluated by Dunnett's multiple-comparison test. Probability values < 0.05 were considered significant.

3. Results

After an equilibration period of 70 min, the mean spontaneous luminal outflow (measured between 70 and 94 min of incubation) of 5-HT was 33 ± 5.2 pmol/g tissue/3 min ($n = 10$, Table 1). In control experiments, the luminal outflow of 5-HT remained constant during the observation period (Fig. 1). The luminal outflow of 5-HT was significantly reduced when atropine (0.2 μM, $n = 9$) was present from the start of incubation (Table 1), as has already been reported (Kojima and Ikeda, 1998). Tetrodotoxin (0.3 μM, $n = 8$) showed a tendency to reduce the mean 5-HT outflow, but these changes did not reach statistical significance (Table 1). Addition of KW-5092 ((1-[2-[[[5-(piperidinomethyl)-2-furanyl]methyl]-amino]ethyl]-2-imidazolidinylidene) propanedinitrile fumarate, 1–10 μM, $n = 6$) to the bath medium caused an increase in the outflow of 5-HT in a concentration-dependent manner (Fig. 1; Table 1). In the presence of atropine (0.2 μM, $n = 4$) or tetrodotoxin (0.3 μM, $n = 4$) (from the start of incubation), the KW-5092 (10 μM)-evoked maximal 5-HT outflow was significantly reduced by 94% and 74%, respectively (Table 1).

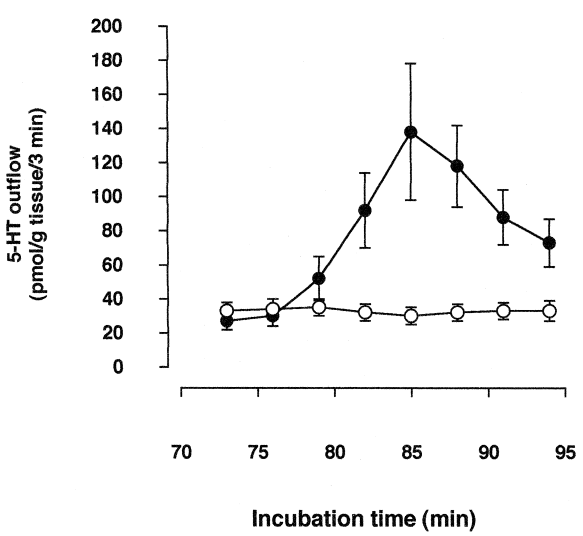

Fig. 1. Effect of KW-5092 (10 μM, ●) on the spontaneous luminal outflow of 5-HT from isolated luminally perfused proximal colon of the guinea-pig (control, ○). KW-5092 was present from 76 to 95 min of incubation. Ordinate scale: outflow of 5-HT, expressed as picomole per gram tissue per 3 min. Each point represents the mean ± S.E.M. (vertical bars) from 6 to 10 experiments.

4. Discussion

The isolated luminally perfused intestine has been shown to be a useful preparation to study the luminal release of serotonin (5-HT) from enterochromaffin cells (Fujimiya et al., 1997; Kojima and Ikeda, 1998). The present study revealed a stimulatory effect of KW-5092 ((1-[2-[[[5-(piperidinomethyl)-2-furanyl]methyl]amino]ethyl]-2-imidazolidinylidene) propanedinitrile fumarate) on spontaneous luminal 5-HT outflow from the luminally perfused isolated guinea-pig proximal colon. The inhibition of the stimulatory action of KW-5092 by atropine or tetrodotoxin strongly suggests that KW-5092 exerts a stimulatory effect through the release of endogenous acetylcholine from cholinergic neurons. The result is consistent with our previous work indicating that the luminal 5-HT release from the isolated guinea-pig proximal colon is stimulated via cholinergic pathways (Kojima and Ikeda, 1998). However, tetrodotoxin did not completely abolish the stimulatory action of KW-5092 on 5-HT outflow. Hence, a non-neuronal cholinergic system might be partially involved in the KW-5092-evoked 5-HT outflow. Although the exact nature of the non-neuronal cholinergic system remains unknown, recent findings demonstrate the existence of the non-neuronal cholinergic system in rat and human intestinal epithelium (Klapproth et al., 1997; Reinheimer et al., 1998).

5. Conclusion

These results showed that KW-5092 stimulates intraluminal 5-HT release from the guinea-pig proximal colon via the stimulation of cholinergic neurons. Because 5-HT is recognized as an important messenger substance in the control of intestinal motility (see Section 1), this stimulatory effect on 5-HT release could be considered as an indirect action of KW-5092 that may contribute to its prokinetic effects.

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