Effects of the Adenosine A1-Receptor Antagonist on Defecation, Small Intestinal Propulsion and Gastric Emptying in Rats

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ABSTRACT—We examined the effects of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and (R)-7,8-dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1H-imidazo[2,1-i]purin-5(4H)-one (KF20274), selective adenosine A1-receptor antagonists, on the gastrointestinal propulsion in rats, as compared with those of the laxative bisacodyl. DPCPX and KF20274 (p.o.) dose-dependently increased the fecal pellet output, whereas these drugs at the dose that increased defecation did not affect small intestinal propulsion or gastric emptying. Bisacodyl increased defecation and slowed gastric emptying without any influence on small intestinal propulsion. Bisacodyl, but not DPCPX or KF20274, induced diarrhea at the dose inducing defecation. The present results suggest that the adenosine A1-receptor antagonist selectively enhances the lower gastrointestinal propulsion, resulting in defecation without diarrhea.

Keywords: Adenosine A1-receptor antagonist, Gastrointestinal propulsion

Adenosine has been shown to modulate a variety of neurotransmission processes in the gastrointestinal (GI) tract (1–3). Adenosine was found to inhibit the release of such major contractile neurotransmitters as acetylcholine and tachykinins from the synaptosomal fraction of guinea pig ileum via adenosine A1 receptors (2, 4, 5). In the longitudinal muscle-myenteric plexus (LMMP) preparation of guinea pig ileum, the contraction induced by electrical transmural stimulation was suppressed by the adenosine A1-receptor agonist cyclopentyladenosine (CPA) (6). This suppression was antagonized by the adenosine A1-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (6). These in vitro observations suggest that the lower GI tract has adenosine A1 receptors that modulate contraction and propulsion.

Recently, we showed that the adenosine A1-receptor agonists (2S)-N6-(2-endo-norbornyl) adenosine ((S)-ENBA) and N6-cyclohexyladenosine abolish the giant migrating contractions induced by glycerol enema in rats (7) and that the adenosine A1-receptor antagonist DPCPX (0.1–0.3 mg/kg, i.p.) significantly increases defection in rats (8). These observations indicate that not only exogenous but also endogenous adenosine controls the colonic propulsion in vivo via adenosine A1 receptors. However, the in vivo effect of the adenosine A1-receptor antagonist on the upper GI propulsion has not been known prior to the present study. In the present study, we examined the effects of the adenosine A1-receptor antagonists DPCPX and (R)-7,8-dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1H-imidazo[2,1-i]purin-5(4H)-one (KF20274) (8) on defection, small intestinal propulsion and gastric emptying in rats; and we compared them with those of the laxative bisacodyl, which has been reported to promote colonic motility as well as colonic secretion, resulting in defection (9).

Male Sprague-Dawley rats (Japan SLC, Inc., Hamamatsu), weighing 150–250 g, were used for the experiments. All animals were maintained on ordinary laboratory chow and tap water ad libitum under a constant 12-hr light-dark cycle.

In the experiment examining the drug effect on defection, each animal was placed in an individual cage 3 hr before the oral administration of a test drug. Fecal pellet output during the 3-hr period after drug administration was determined in each rat. The evacuated feces were collected on an aluminum foil sheet every 20 min. When the animal passed urine, urine was carefully removed with blotting paper. Evacuation of unformed feces was regarded as diarrhea. The wet weight of the fecal pellet output was determined immediately after the collection of the feces. The dry weight was determined after the feces were dried for 6 hr at 100°C. The water content of the feces was
calculated according to the following formula:

Water content of fecal pellet output [%] = \(1 - \frac{(\text{Dry weight of fecal pellet output [g]})}{(\text{Wet weight of fecal pellet output [g]})}\) \times 100

The small intestinal propulsion was determined according to the reported procedure (10). The animals were deprived of food 24 hr prior to the experiment but allowed free access to water until 3 hr before the experiment. A suspension of 10% (w/v) charcoal in aqueous gum arabic (5% w/v) was used as a test meal. Ten minutes after the test meal was given, the animals were sacrificed by cervical dislocation. The small intestine was then exposed by laparotomy, and the percentage traverse of the charcoal meal in the small intestine was determined.

The gastric emptying was determined with a modification of the reported procedure (11). The animals were deprived of food 24 hr prior to the experiment but allowed free access to water until 3 hr before the experiment. A solution of 0.05% (w/v) phenol red in aqueous sodium carboxymethylcellulose (1.5% w/v) was used as a test meal. The test drug was orally administered 1 hr before the test meal was given. Fifteen minutes after administration of the meal, the animal was sacrificed by cervical dislocation. The stomach was then exposed by laparotomy and removed. In each experiment, 4 animals treated with the vehicle were sacrificed immediately after administration of the meal, and the phenol red content in the stomach was considered as the standard (100%) to avoid the errors associated with terminal convulsions of the animal. The removed stomach was incised in 40 ml of NaOH solution (0.1 N) and its content was dissolved. A 1-ml aliquot of the supernatant was added to 2 ml of trichloroacetic acid (7.5% w/v) to precipitate the proteins. After centrifugation (2500 \( \times \) g for 15 min), 1 ml of the supernatant was added to 1 ml of NaOH (1 N) to develop the maximum intensity of the color. The absorbance at 560 nm of the solution was then measured with a spectrophotometer (U-1080; Hitachi, Ltd., Tokyo). The gastric emptying (G.E.) for each rat was calculated according to the following formula:

\[ \text{G.E.} \% = \frac{1 - (\text{Amount of phenol red recovered from the test stomach})}{(\text{Average amount of phenol red recovered from the standard stomach})} \times 100 \]

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**Table 1. Effects of DPCPX, KF20274, CGS 15943, CP 66713 and bisacodyl on defecation in rats**

| Drugs          | Wet weight (g) | Dry weight (g) | Water content (%) | Incidence of diarrhea |
|----------------|----------------|----------------|-------------------|-----------------------|
| Control        | 0.36 ± 0.10    | 0.19 ± 0.05    | 46 ± 4            | 2/12                  |
| DPCPX 0.01     | 0.53 ± 0.09    | 0.25 ± 0.04    | 51 ± 3            | 0/12                  |
| DPCPX 0.03     | 0.68 ± 0.13    | 0.31 ± 0.05    | 53 ± 3            | 2/12                  |
| DPCPX 0.1      | 0.83 ± 0.14    | 0.37 ± 0.06    | 53 ± 3            | 0/12                  |
| DPCPX 0.3      | 0.90 ± 0.11*   | 0.39 ± 0.05*   | 56 ± 2            | 0/12                  |
| Control 0.0005 | 0.27 ± 0.09    | 0.14 ± 0.05    | 52 ± 2            | 0/10                  |
| KF20274 0.001  | 0.31 ± 0.09    | 0.14 ± 0.04    | 54 ± 3            | 0/10                  |
| KF20274 0.003  | 0.40 ± 0.08    | 0.19 ± 0.04    | 51 ± 2            | 0/10                  |
| KF20274 0.01   | 0.59 ± 0.06    | 0.27 ± 0.03    | 55 ± 2            | 0/10                  |
| KF20274 0.03   | 0.64 ± 0.10*   | 0.36 ± 0.06    | 53 ± 1            | 0/10                  |
| Control 0.03   | 0.78 ± 0.10*   | 0.37 ± 0.06*   | 54 ± 1            | 0/10                  |
| CGS 15943 1    | 0.35 ± 0.11    | 0.15 ± 0.05    | 57 ± 2            | 0/12                  |
| CGS 15943 10   | 0.39 ± 0.11    | 0.15 ± 0.04    | 59 ± 2            | 0/12                  |
| CP 66713 1     | 0.33 ± 0.09    | 0.13 ± 0.03    | 59 ± 2            | 0/12                  |
| CP 66713 10    | 0.51 ± 0.11    | 0.22 ± 0.05    | 58 ± 2            | 0/12                  |
| Control 0.54   | 0.54 ± 0.13    | 0.21 ± 0.04    | 55 ± 3            | 0/12                  |
| Bisacodyl 1    | 0.85 ± 0.32    | 0.32 ± 0.08    | 49 ± 6            | 2/12                  |
| Bisacodyl 3    | 0.73 ± 0.26    | 0.26 ± 0.06    | 50 ± 4            | 2/12                  |
| Bisacodyl 10   | 0.95 ± 0.20    | 0.33 ± 0.06    | 63 ± 3            | 3/12                  |
| Bisacodyl 30   | 2.06 ± 0.29**  | 0.55 ± 0.06**  | 72 ± 3            | 10/12##              |

Each value of wet weight, dry weight and water content represents the mean ± S.E.M for 10 or 12 rats. The incidence of diarrhea is expressed as the number of diarrheal rats per 10 or 12 rats examined. *P < 0.05, **P < 0.01, compared with the value in the control group (Steel multiple comparison test). ##P < 0.01, compared with the value in the control group (Fisher's exact probability test).
Fig. 1. Effects of DPCPX (A), KF20274 (B) and bisacodyl (C) on small intestinal propulsion in rats. Each bar represents the mean±S.E.M. of 10 rats.

Fig. 2. Effects of DPCPX (A), KF20274 (B) and bisacodyl (C) on gastric emptying in rats. Each bar represents the mean±S.E.M. of 10 rats. **P<0.01, compared with the value in the control group (Steel multiple comparison test).

DPCPX, KF20274, 9-chloro-2-(2-furyl)-5,6-dihydro-[1,2,4]-triazolo[1,5-c]quinazoline-5-imine (CGS 15943) (12) and 4-amino-8-chloro-l-phenyl-(1,2,4)triazolo(4,3-a)-quinoxaline (CP-66713) (12) were synthesized in our laboratories. Bisacodyl was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Charcoal, phenol red and trichloroacetic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). Gum arabic was purchased from Nacalai Tesque, Inc. (Kyoto). The test drugs were suspended in 0.3% sodium carboxymethylcellulose containing 0.25% polyoxyethylenesorbitan monooleate.

All data are represented as means±S.E.M. Statistical significance was estimated by the Steel multiple comparison test, Dunnett's multiple comparison test or Fisher's exact probability test. A P-value of less than 0.05 was considered statistically significant.

Table 1 shows the drug effect on defecation. DPCPX at 0.01 to 0.3 mg/kg (p.o.) increased both the wet and dry weight of feces in dose-dependent manners, significant increases being observed at 0.3 mg/kg (p.o.). DPCPX at 0.01 to 0.3 mg/kg (p.o.) did not affect the water content or the incidence of diarrhea. This observation is in accordance with the previous one (8) that intraperitoneal administration of DPCPX increased defecation in rats. Moreover, KF20274, another selective adenosine A1-receptor antagonist, at a dose of 0.03 mg/kg (p.o.) significantly increased the wet and dry weight of feces. On the other hand, CGS 15943 and CP 66713, adenosine A2-receptor antagonists, did not affect the defecation. Bisacodyl at 30 mg/kg (p.o.) significantly increased the wet and dry
weight of feces and tended to increase water content. Among the 12 rats examined, bisacodyl at 30 mg/kg (p.o.) induced diarrhea in 10 rats.

Neither DPCPX (0.1–3 mg/kg, p.o.), KF20274 (0.01–3 mg/kg, p.o.) nor bisacodyl (3–30 mg/kg, p.o.) affected small intestinal propulsion (Fig. 1). DPCPX at 0.1 to 3 mg/kg (p.o.) and KF20274 at 0.01 to 3 mg/kg (p.o.) did not affect gastric emptying, whereas bisacodyl at 3 to 30 mg/kg (p.o.) dose-dependently and significantly inhibited it (Fig. 2).

The present study demonstrated that the adenosine A₁-receptor antagonist increased defecation without any influence on gastric emptying or small intestinal propulsion in rats. In addition, DPCPX (0.3 mg/kg, p.o.) significantly enhanced colonic motility in anesthetized rats (M. Suzuki et al., unpublished data). The site-specific action of the adenosine A₁-receptor antagonist might be attributed to the possible difference of the amount of endogenous adenosine in different parts of the gut. In other words, the amount of endogenous adenosine may be large enough in the colon to provide a sustained inhibition of defecation via adenosine A₁ receptors, whereas the amount of endogenous adenosine may be insufficient in the stomach and the small intestine to modulate the propulsion.

Another possible mechanism for the site-specific action of the adenosine A₁-receptor antagonist may be the absence of adenosine A₁ receptors in the stomach or the small intestine. However, this mechanism is unlikely in light of the following observations: Gullikson et al. (13) reported the suppression by adenosine A₁-receptor agonists of the gastric emptying in rats, suggesting the presence of inhibitory adenosine A₁ receptors in the rat stomach. In addition, Christofi and Cook (14) reported that adenosine A₁-receptor agonists inhibited the electrically-stimulated contractions of the isolated guinea pig ileum, suggesting the presence of inhibitory adenosine A₁ receptors in the ileum. Moreover, the finding that adenosine A₁-receptor agonists suppressed the interdigestive migrating complex in the rat small intestine (15) favors the presence of adenosine A₁ receptors involved in the inhibitory modulation in the rat ileum.

Finally, the site-specific action of the adenosine A₁-receptor antagonist may be due to the presence of endogenous inhibitory neuromodulators other than adenosine in the stomach and small intestine. Adenosine may play less important roles in the sustained inhibition of gastric emptying or small intestinal propulsion. Further studies must be carried out to clarify the mechanism for the site-specific action of the adenosine A₁-receptor antagonist.

In the present study, we observed the site-specific effect of the adenosine A₁-receptor antagonists on the GI propulsion in the in vivo experiment. In contrast, DPCPX is reported to give rise to a small but significant increase in ACh-mediated contraction of the isolated guinea pig LMMP preparation (6). Thus, the adenosine A₁-receptor antagonist may elicit some functional effect on the isolated small intestine or stomach preparation, although the effect was not observed in the present in vivo study.

The present study demonstrated that the adenosine A₁-receptor antagonist increased defecation without diarrhea, suggesting that the adenosine A₁-receptor antagonist has little influence, if any, on fluid secretion. In contrast, bisacodyl increased defecation and induced diarrhea. The occurrence of diarrhea induced by bisacodyl seems to be due to the laxative action of this agent. In fact, bisacodyl promotes not only motility but also fluid secretion (9). In the present study, bisacodyl suppressed gastric emptying at a dose lower than that inducing defecation. Clinically, slowed gastric emptying is well-associated with upper abdominal discomfort such as bloating and nausea. Since the adenosine A₁-receptor antagonist increased defecation without diarrhea or slowed gastric emptying, the abdominal adverse events of the adenosine A₁-receptor antagonist are expected to be fewer than those of bisacodyl when used for the treatment of constipation.

In summary, the adenosine A₁-receptor antagonists DPCPX and KF20274 increased defecation without any influence on gastric emptying or small intestinal propulsion in rats. These observations suggest that the adenosine A₁-receptor antagonist selectively enhances the lower gastrointestinal propulsion. The laxative bisacodyl, but not DPCPX or KF20274, induced diarrhea and slowed gastric emptying. The present observation postulates the therapeutic potential of the adenosine A₁-receptor antagonist for the treatment of constipation.

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