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Thromboxane A_2-mediated Cl^- secretion induced by platelet-activating factor in isolated rat colon

Tomoyuki Suzuki [1], Hideki Sakai [*,1], Noriaki Takeguchi

Department of Pharmaceutical Physiology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

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Abstract

Thromboxane A_2 is a novel endogenous secretagogue of Cl^- secretion in the distal colon. Here, we examined if the Cl^- secretion caused by platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is mediated by thromboxane A_2 production using isolated mucosae of the rat colon. Furosemide (100 μM) and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB; 300 μM) completely inhibited PAF (10 μM)-induced increase in short-circuit current (Isc) across the mucosa, indicating that PAF caused a Cl^- secretion in the rat colon. A selective thromboxane A_2 receptor antagonist (sodium(*E*)-11-[2-(5,6-dimethyl-1-benzimidazolyl)-ethylidene]-6,11-dihydrobenz[*b*,*e*]oxepine-2-carboxylate monohydrate; KW-3635), and a selective thromboxane synthase inhibitor (sodium 4-[α-hydroxy-5-(1-imidazolyl)-2-methylbenzyl]-3,5-dimethylbenzoate dihydrate; Y-20811) inhibited the PAF-induced Cl^- current in a concentration-dependent manner. The IC_{50} values of KW-3635 and Y-20811 were 2.1 and 0.5 μM, respectively. 30 μM KW-3635 and 1 μM Y-20811 inhibited the PAF response by 92% and 83%, respectively. These inhibitors did not affect the prostaglandin E_2-induced increase in Isc. A 5-lipoxygenase-activating protein inhibitor (3-[1-(*p*-chlorobenzyl)-5-(isopropyl)-3-*t*-butylthioindol-2-yl]-2,2-dimethyl-propanoic acid sodium; MK-886) (5 μM) did not affect the PAF-induced Cl^- current. The present study suggests that the PAF-induced Cl^- secretion in the rat colonic mucosa is mainly mediated by a release of thromboxane A_2. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Colon, rat; PAF (platelet-activating factor); Thromboxane A_2; Cl^- secretion

1. Introduction

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is an endogenous phospholipid which is produced by a wide variety of cells. PAF is associated with a number of pathophysiological conditions including arterial thrombosis, acute inflammation, endotoxic shock, and acute allergic disease (Braquet et al., 1987).

In the human colon, PAF has been suggested as a mediator of pathogenesis of inflammatory bowel disease such as ulcerative colitis (Eliakim et al., 1988; Wardle et al., 1996) and Crohn's disease (Kald et al., 1990; Denizot et al., 1992; Sobhani et al., 1992). In fact, PAF levels in the inflamed colonic mucosa from patients with active ulcerative colitis (Eliakim et al., 1988; Appleyard and Hillier, 1995) and Crohn's disease (Kald et al., 1990; Sobhani et al., 1992) were found to be much higher than those in the non-inflamed mucosa. Increased PAF formation was also found in experimental animal models of colitis (Longo et al., 1994; Mascolo et al., 1995).

PAF has been reported to stimulate Cl^- secretion in the distal colon of human (Borman et al., 1998), rat (Bern et al., 1989; Buckley and Hoult, 1989) and rabbit (Travis and Jewell, 1992). Stimulation of Cl^- secretion by PAF may contribute to the production of diarrhea in inflammatory bowel disease (Wardle et al., 1996; Borman et al., 1998). The PAF-induced Cl^- secretion is apparently mediated by production of the arachidonic acid metabolites (Bern et al., 1989; Travis et al., 1995; Wardle et al., 1996; Borman et al., 1998), although the final metabolites that are involved in the Cl^- secretion have not been specified.

Recently, we found that thromboxane A_2, one of the arachidonic acid metabolites, stimulated Cl^- secretion in isolated rat colonic mucosa (Sakai et al., 1995, 1997). In

* Corresponding author. Tel.: +81-76-434-7581; fax: +81-76-434-5051.

E-mail address: sakaih@ms.toyama-mpu.ac.jp (H. Sakai).

[1] These authors contributed equally to this work.

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these studies, 9,11-epithio-11,12-methano-thromboxane A_2 (STA_2), a stable thromboxane A_2 analogue, was shown to mimic the function of thromboxane A_2. Thromboxane A_2 and STA_2 directly act on colonic epithelial cells which contribute to Cl^- secretion (Sakai et al., 1997; Ikari et al., 1999). Similar to the function of PAF in the colon, thromboxane A_2 has been suggested to play a major pathogenic role in inflammatory bowel disease in human and experimental animal models (Rampton and Collins, 1993).

Taken together, we postulate that thromboxane A_2 may be a mediator in the PAF-induced Cl^- secretion. In the present study using isolated rat colonic mucosa, we therefore tested if the PAF-induced Cl^- secretion is inhibited by a specific thromboxane A_2 receptor antagonist and by a specific thromboxane synthase inhibitor.

2. Materials and methods

2.1. Chemicals

Sodium(*E*)-11-[2-(5,6-dimethyl-1-benzimidazolyl)-ethylidene]-6,11-dihydrobenz[*b*,*e*]oxepine-2-carboxylate monohydrate (KW-3635; Kyowa Hakko Kogyo, Shizuoka, Japan), sodium 4-[α-hydroxy-5-(1-imidazolyl)-2-methylbenzyl]-3,5-dimethylbenzoate dihydrate (Y-20811; Yoshitomi Pharmaceutical, Fukuoka, Japan) and prostaglandin E_2 (Toray, Tokyo, Japan) were generous gifts. PAF (1-alkyl-2-acetoyl-*sn*-glycero-3-phosphocholine) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). 5-Nitro-2-(3-phenylpropylamino)-benzoate (NPPB) was from Research Biochemicals International (Natick, MA, USA), 3-[1-(*p*-chlorobenzyl)-5-(isopropyl)-3-*t*-butylthioindol-2-yl]-2,2-dimethyl-propanoic acid, sodium (MK-886) was from BIOMOL Research Laboratories (Plymouth Meeting, PA, USA), and furosemide was from Wako (Osaka, Japan). KW-3635, NPPB and MK-886 were dissolved in dimethyl sulfoxide, and PAF, prostaglandin E_2 and furosemide were dissolved in ethanol. Dimethyl sulfoxide and ethanol concentrations in the final solutions never exceeded 0.5%, at which concentration the vehicle per se did not affect the short-circuit current (Isc), the potential difference across the mucosa (Pd), or the tissue conductance (Gt).

2.2. Tissue preparation

The following procedures were performed in accordance with the guidelines presented by the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University. The mucosa–submucosa preparation (hereafter simply described as the mucosa) was obtained from female Wistar rats (Japan SLC, Shizuoka, Japan) with a weight of 140–200 g. The animals had free access to water and food until the day of the experiment. Animals were killed rapidly by stunning and cervical dislocation.

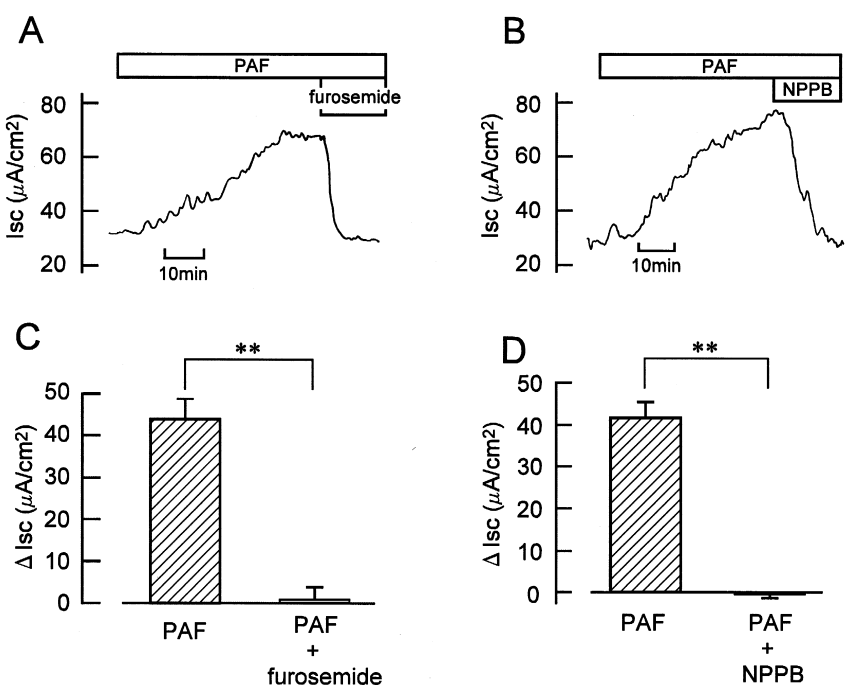

Fig. 1. Inhibition of the PAF-elicited current by furosemide and NPPB. (A and B) At the plateau phases observed after the addition of PAF (10 μM at the serosal side), furosemide (100 μM at the serosal side; A) or NPPB (300 μM at the mucosal side; B) was added. Typical traces are shown. (C and D) The values of Isc just before addition of furosemide or NPPB were read, and data are expressed as net increases from the Isc just before addition of PAF (ΔIsc) (left columns). When the effect of furosemide (C) or NPPB (D) had become steady, the Isc values were read, and data are expressed as ΔIsc (right columns). Data are means ± S.E.M. from 4–5 experiments. ** Significantly different ($P < 0.01$).

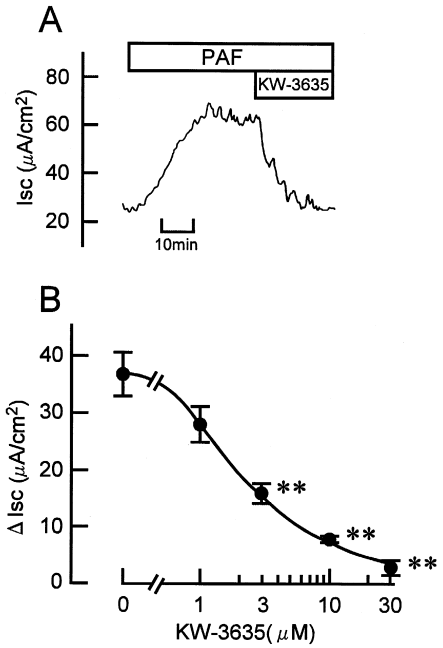

Fig. 2. Inhibitory effect of KW-3635 on the PAF-induced Cl^- current. (A) 30 μM KW-3635 was added at the serosal side after the PAF (10 μM)-elicited plateau phase was observed. A typical trace is shown. (B) KW-3635 was added cumulatively at the serosal side when the plateau phase of Isc was observed after the addition of PAF (10 μM at the serosal side). The values of Isc were read when the effect of KW-3635 had become steady, and data are expressed as net increases from the Isc just before addition of PAF (ΔIsc). Data are means ± S.E.M. from four experiments. ** Significantly different from the value in the absence of KW-3635 ($P < 0.01$).

The serosa and muscularis propria were stripped away by hand to obtain the mucosa–submucosa preparation of distal part of the colon descendens. The Parsons solution for tissue preparation and Ussing chamber experiments consisted of (in mM): 107 NaCl, 4.5 KCl, 25 $NaHCO_3$, 1.8 Na_2HPO_4, 0.2 NaH_2PO_4, 1.25 $CaCl_2$, 1 $MgSO_4$ and 12 glucose. The solution was gassed with carbogen (5% CO_2–95% O_2) at a pH of 7.4.

2.3. Ussing chamber experiments

The tissue was fixed in a modified Ussing chamber and bathed with 4 ml of the Parsons solution incubated at 37°C on each side of the mucosa. The exposed surface of the tissue was 0.3 cm^2. Short-circuit current (Isc) was continuously measured at zero voltage difference with an amplifier (CEZ-9100, Nihon Kohden, Tokyo, Japan). The fluid resistance was compensated. The direction of Isc from the mucosal to serosal side was expressed as positive: that is, an increase in Cl^- movement from the serosal to mucosal side (Cl^- secretion) corresponded to an increase in Isc. The transepithelial potential difference (Pd) under open-circuit conditions was measured in the current clamp mode of the amplifier, and the reference was taken on the serosal side. Tissue conductance (Gt) was determined from the deviation of Isc in response to the command voltage pulse of 0.5 mV (its duration was 100 ms).

2.4. Statistical analysis

Results are presented as the means ± S.E.M. Difference between groups were analyzed by one-way analysis of variance (ANOVA), and correction for multiple comparisons was made by using Dunnett's multiple comparison test. Comparison between the two groups was made using Student's *t* test. Statistically significant differences were assumed at $P < 0.05$.

3. Results

3.1. PAF-induced Cl^- current in isolated rat colonic mucosa

First, we tested the effects of PAF on Isc of the isolated colonic mucosa set in Ussing chambers. We used 10 μM PAF in all the experiments because this concentration of PAF was known to give a maximal response in the rat

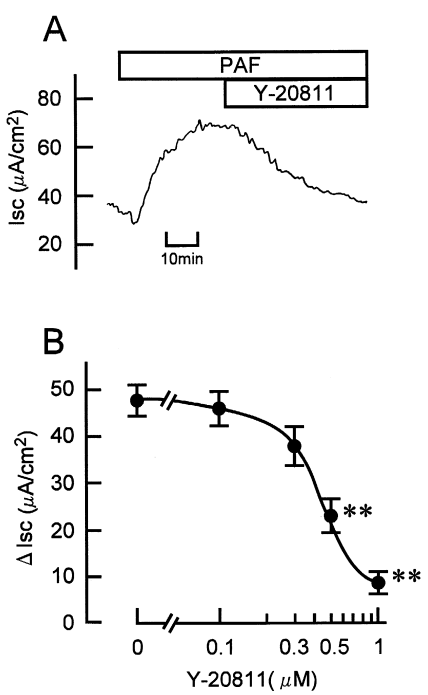

Fig. 3. Inhibitory effect of Y-20811 on the PAF-induced Cl^- current. (A) 1 μM Y-20811 was added at both the serosal and mucosal sides after the PAF (10 μM)-elicited plateau phase was observed. A typical trace is shown. (B) Y-20811 was added cumulatively at both the serosal and mucosal sides when the plateau phase of Isc was observed after the addition of PAF (10 μM at the serosal side). The values of Isc were read when the effect of Y-20811 had become steady, and data are expressed as net increases from the Isc just before addition of PAF (ΔIsc). Data are means ± S.E.M. from four experiments. ** Significantly different from the value in the absence of Y-20811 ($P < 0.01$).

colon (Wardle et al., 1996). PAF (10 μM at the serosal side) significantly increased Isc from 28.9 ± 2.7 to 69.4 ± 3.5 $\mu A/cm^2$ at a sustained plateau phase ($P < 0.01$, $n = 18$). The plateau phase started at 30 ± 2 min ($n = 18$) after the addition of PAF. PAF also increased Pd (from 2.7 ± 0.2 to 5.6 ± 0.6 mV at the plateau phase; $P < 0.01$, $n = 10$) and Gt (from 8.4 ± 0.5 to 10.3 ± 0.6 mS/cm^2 at the plateau phase; $P < 0.05$, $n = 10$).

Fig. 1 shows that the PAF-induced increase in Isc was completely inhibited by furosemide (100 μM at the serosal side), an inhibitor of basolateral Na^+–K^+–$2Cl^-$ cotransporter (Chipperfield, 1986), and NPPB (300 μM at the mucosal side), a blocker of apical Cl^- channel in the rat colon (Diener and Rummel, 1989; Sakai et al., 1995, 1997). These results indicate that the PAF-response is caused by secretion of Cl^- ions.

3.2. Inhibition of the PAF-induced Cl^- current by a thromboxane A_2 receptor antagonist

Here, we used a specific thromboxane A_2 receptor antagonist, KW-3635 (Miki et al., 1992). Previous reports showed that KW-3635 (10 μM) did not antagonize various receptors of prostaglandin E_2, prostaglandin I_2, leukotriene D_4 and neurotransmitters, and did not inhibit cyclooxygenase, thromboxane synthase or prostaglandin I_2 synthase (Karasawa et al., 1991; Miki et al., 1992). In addition, we confirmed previously that KW-3635 (100 μM) did not affect the baseline Isc (in the absence of secretagogues) in the rat colon (Sakai et al., 1997).

The PAF (10 μM)-induced Cl^- secretion was inhibited in a concentration-dependent manner by KW-3635 added at the serosal side (Fig. 2). Its IC_{50} value was 2.1 μM. 30 μM KW-3635 inhibited the PAF response by 92 ± 3% ($n = 5$).

3.3. Inhibition of the PAF-induced Cl^- current by a thromboxane synthase inhibitor

To confirm further the involvement of thromboxane A_2 production in the PAF-induced Cl^- secretion, we used Y-20811 (Mikashima et al., 1986), a specific thromboxane synthase inhibitor. Y-20811 (100 μM) was shown to have no effect on cyclooxygenase or prostaglandin I_2 synthase (Mikashima et al., 1986). The baseline Isc was not affected by 1 μM Y-20811 in the rat colon (Sakai et al., 1997).

Fig. 3 shows that the PAF (10 μM)-induced Cl^- secretion was inhibited in a concentration-dependent man-

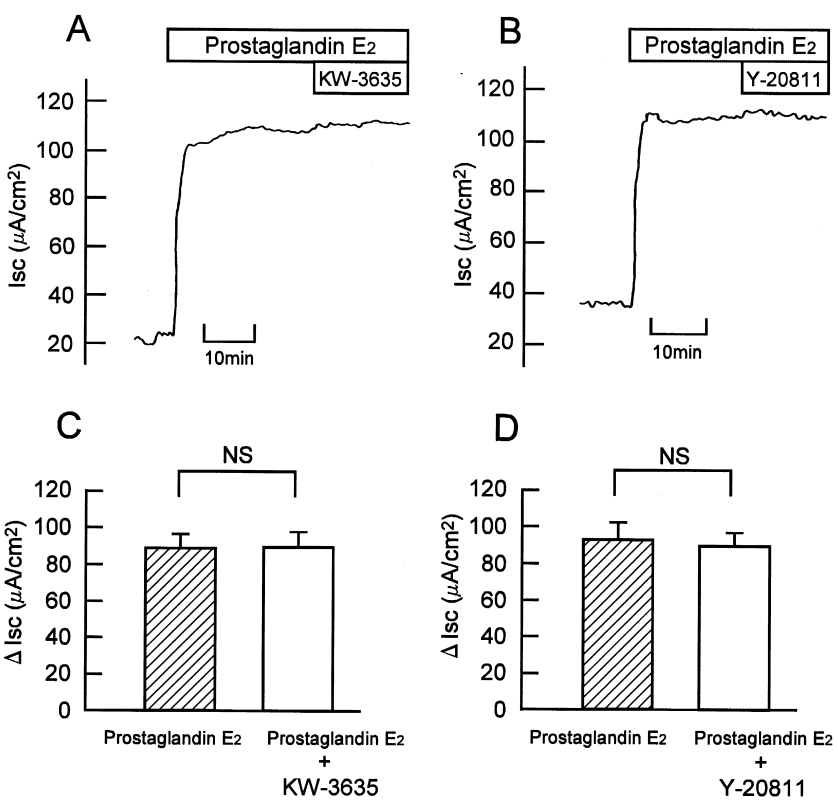

Fig. 4. Effects of KW-3635 and Y-20811 on the prostaglandin E_2-elicited current. (A and B) At the plateau phases observed after the addition of prostaglandin E_2 (0.5 μM at the serosal side), KW-3635 (30 μM at the serosal side; A) or Y-20811 (1 μM at both the serosal and mucosal sides; B) was added. Typical traces are shown. (C and D) The values of Isc were read just before addition of KW-3635 or Y-20811, and data are expressed as net increases from the Isc just before addition of prostaglandin E_2 (ΔIsc) (left columns). At 15 min after the addition of KW-3635 (C) or Y-20811 (D), the Isc values were read, and data are expressed as ΔIsc (right columns). Data are means ± S.E.M. from 4–5 experiments. NS, not significantly different ($P > 0.05$).

ner by Y-20811 added at both the serosal and mucosal sides. Its IC_{50} value was 0.5 μM. 1 μM Y-20811 inhibited the PAF response by 83 ± 4% ($n = 5$).

3.4. Effects of KW-3635 and Y-20811 on the prostaglandin E_2-induced increase in Isc

KW-3635 (30 μM) did not significantly affect the prostaglandin E_2 (0.5 μM)-induced increase in Isc (Fig. 4A and C), indicating that it does not antagonize prostaglandin EP receptors present in the rat colonic mucosa. Y-20811 (1 μM) also did not affect the prostaglandin E_2-induced increase in Isc (Fig. 4B and D), indicating that it does not block the synthesis of eicosanoids other than thromboxane A_2.

3.5. Effects of a 5-lipoxygenase-activating protein inhibitor and a prostaglandin EP_1 receptor antagonist on the PAF-induced Cl^- current

To check whether the PAF-induced Cl^- secretion is mediated by leukotrienes, we used MK-886, an inhibitor of 5-lipoxygenase-activating protein (Dixon et al., 1990). Fig. 5 shows that MK-886 (5 μM at both the serosal and mucosal sides) did not significantly affect the PAF (10 μM)-induced Cl^- current, indicating that leukotrienes are not involved in the PAF response in the rat colon.

It may be important to test the effects of prostaglandin EP receptor antagonists on the PAF response. The EP receptor in the colonic crypt is an EP_2 subtype (Homaidan et al., 1995). To our knowledge, however, there is currently no specific antagonist for the EP_2 receptor. Here, we checked effect of an EP_1 receptor antagonist, AH6809 (Coleman et al., 1994), on the PAF-induced Cl^- current. As expected, we could not observe any effect of AH6809: that is, PAF (10 μM at the serosal side) induced a steady increase in Isc ($\Delta Isc = 48.3 \pm 7.6$ μA/cm^2), and the additional incubation with AH6809 (5 μM at the serosal side) for 20 min did not induce any significant change in Isc ($\Delta Isc = 48.9 \pm 8.9$ μA/cm^2) ($P > 0.05$; $n = 3$).

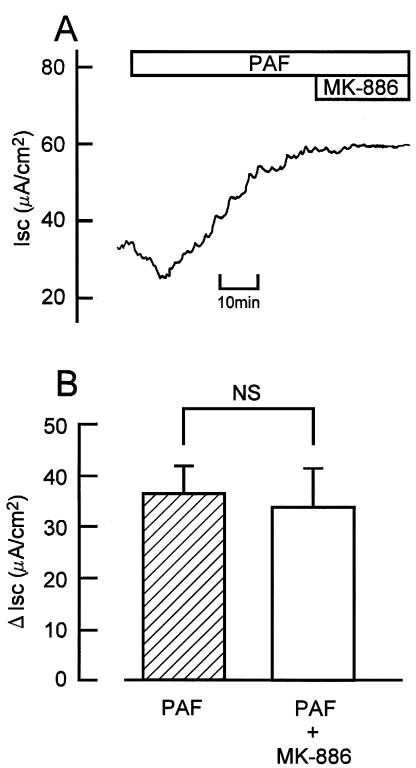

Fig. 5. Effect of MK-886 on the PAF-induced Cl^- current. (A) 5 μM MK-886 was added at both the serosal and mucosal sides after the PAF (10 μM)-elicited plateau phase was observed. A typical trace is shown. (B) The values of Isc just before addition of MK-886 were read, and data are expressed as net increases from the Isc just before addition of PAF (ΔIsc) (left column). At 20 min after the addition of MK-886, the Isc values were read, and data are expressed as ΔIsc (right column). Data are means ± S.E.M. from four experiments. NS, not significantly different ($P > 0.05$).

4. Discussion

We found previously that irinotecan, an anti-tumor drug, induces Cl^- secretion in the rat colon (Sakai et al., 1995). Recently we found that the irinotecan-induced Cl^- secretion in the colon is mainly mediated by release of thromboxane A_2, and that STA_2, a stable thromboxane A_2 analogue (Katsura et al., 1983), mimics the effect of irinotecan (Sakai et al., 1997). Both endogenous thromboxane A_2 and exogenous STA_2 act on the thromboxane A_2 receptor in the epithelial crypt cells (Sakai et al., 1997; Ikari et al., 1999). These findings confirmed, for the first time, that thromboxane A_2 is a secretagogue in the animal model of colon. In the present study, we tested if the PAF-induced Cl^- secretion in the rat colon is mediated by thromboxane A_2 production.

So far, cyclooxygenase inhibitors have been reported to inhibit the PAF-induced Cl^- secretion in the rat colon (Bern et al., 1989), rabbit colon (Travis et al., 1995) and human colon (Borman et al., 1998). In contrast, lipoxygenase inhibitors were much less effective (Bern et al., 1989; Travis et al., 1995). Our present results also confirmed that leukotrienes are not involved in the PAF response (Fig. 5). Bern et al. (1989) showed that PAF increased the release of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$, a metabolite of prostaglandin I_2, from the rat colon. Longo et al. (1995) showed that PAF increased the release of prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2, a metabolite of thromboxane A_2, but not leukotriene D_4 from the rabbit colon. PAF-induced releases of prostaglandin E_2 and leukotriene D_4, but not 6-oxo-prostaglandin $F_{1\alpha}$, were observed in the human colon (Wardle et al., 1996; Borman et al., 1998). Exogenously administrated prostaglandin E_2 (Diener et al., 1988; Calderaro et al., 1991), prostaglandin $F_{2\alpha}$ (Calderaro et al.,

1991), leukotriene D_4 (Hyun and Binder, 1993), iloprost, a prostaglandin I_2 derivative (Diener et al., 1988), and STA_2, a stable thromboxane A_2 analogue (Sakai et al., 1997), stimulated Cl^- secretion in the rat and/or rabbit colon. Based on these results, contribution of several eicosanoids to the PAF-induced Cl^- secretion has been considered.

However, our present results clearly have shown that the PAF-induced Cl^- secretion in the rat colon is mainly mediated by a release of thromboxane A_2, because KW-3635, a selective thromboxane A_2 receptor antagonist, and Y-20811, a selective thromboxane synthase inhibitor, at their highest concentrations inhibited the PAF response by 83–92% (Figs. 2 and 3). On the other hand, KW-3635 and Y-20811 did not significantly affect the prostaglandin E_2 response (Fig. 4). The prostaglandin EP receptor in the colonic crypt has been reported to be an EP_2 subtype (Homaidan et al., 1995). It is important to confirm that an EP_2 receptor antagonist does not affect the PAF-induced Cl^- secretion in our preparation. However, there is currently no specific antagonist for the EP_2 receptor. Apparently, AH6809, an EP_1 receptor antagonist, did not affect the PAF response.

Why is thromboxane A_2 only an effective secretagogue among several possible eicosanoids for the PAF-induced Cl^- secretion? At present, we do not have a clear answer to this question. But the following explanation may be plausible. This may be due to the different localization of different types of PAF-sensitive cells that release each of eicosanoids. Ferraris et al. (1993) reported that PAF activity was present in extracts of both epithelial cells and lamina propria mononuclear cells. Izzo (1996) suggested that the origin of PAF in the digestive tract might be mast cells, platelets, monocytes, macropharges, eosinophils, neutrophils and basophils, all of which can produce PAF under several experimental conditions. Eosinophils are one of possible candidates for the PAF-targeting cells that selectively release thromboxane A_2 in the colon, because Giembycz et al. (1990) found that PAF stimulated the release of thromboxane B_2 and prostaglandin E_1/E_2 from purified guinea-pig eosinophils at a relative molar ratio of 30:1 (thromboxane B_2:prostaglandin E_1/E_2). We suggest that the cells releasing thromboxane A_2 may be located close to the epithelial crypt cell that is responsible for Cl^- secretion. Apparently, further studies are necessary to clarify these points.

Pharmacological characteristics of the Cl^- secretion induced by PAF (Figs. 1 and 2) were similar to those induced by the thromboxane A_2 analogue, STA_2 (Sakai et al., 1997). Furosemide and NPPB inhibited both the PAF- and STA_2-induced Cl^- secretion. The IC_{50} values of KW-3635 for the PAF- and STA_2-responses were in the same range.

Interestingly, both PAF (Nassif et al., 1996) and thromboxane A_2 (Rampton and Collins, 1993) are suggested to be a pathophysiological mediator of inflammatory bowel disease. In fact, PAF antagonists (Longo et al., 1995; Meenan et al., 1996), thromboxane synthase inhibitors (Vilaseca et al., 1990; Tozaki et al., 1999), and a thromboxane A_2 receptor antagonist (Taniguchi et al., 1997) significantly ameliorated mucosal inflammation in animal models of colitis. Casellas et al. (1995) reported that oral administration of ridogrel, a thromboxane synthase inhibitor, for patients with active ulcerative colitis showed clinical and colonoscopic improvement, and that the inhibitor significantly reduced the release of thromboxane B_2 while the release of prostaglandin E_2 and leukotriene D_4 was not affected.

In conclusion, we found that PAF-induced Cl^- secretion in the rat colon was mainly mediated by a release of thromboxane A_2. Stimulation of Cl^- secretion by PAF-thromboxane A_2 pathway may be, at least, partly associated with diarrhea in inflammatory bowel disease. Inhibition of thromboxane synthesis may be found to be clinically effective in treating inflammatory bowel disease in which excess PAF is generated.

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