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INHIBITORY EFFECT OF TRANILAST ON PROSTAGLANDIN D SYNTHETASE

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Abstract—The effect of Tranilast [*N*-(3,4-dimethoxycinnamoyl) anthranilic acid] on the synthesis of prostaglandin D_2 (PGD_2) by homogenates of rat peritoneal mast cells was investigated. The major cyclooxygenase product formed by mast cell homogenates was PGD_2, smaller quantities of PGE_2 and $PGF_{2\alpha}$ were also formed. Tranilast suppressed the production of PGD_2 in a dose-dependent manner with an IC_{50} of 0.1 mM. This suppression was due to inhibition of PGD synthetase, but not cyclooxygenase, since the formation of PGE_2 and $PGF_{2\alpha}$ were unchanged at a 0.1 mM concentration. In addition, the glutathione-dependent conversion of [^{14}C]PGH_2 to PGD_2 by PGD synthetase (PGH-D isomerase, EC 5.3.99.2) was inhibited by Tranilast, with 50% inhibition achieved at 0.08 mM in broken cell preparations of rat peritoneal mast cells. Tranilast also inhibited purified rat spleen and brain PGD synthetases. Furthermore, Tranilast prevented the PGD_2 generation from intact mast cells stimulated by the calcium ionophore A23187. These results suggest that Tranilast exerts some of its therapeutic effects by prevention of PGD_2 generation in mast cells and some other tissues.

Tranilast [*N*-(3,4-dimethoxycinnamoyl) anthranilic acid] has been used therapeutically for a variety of disorders in which mast cell participation has been demonstrated [1, 2]. The mechanism of action of Tranilast is unknown, but is potentially related to inhibition of mast cell activation and prevention of generation and/or release of mast cell mediators.

Prostaglandin (PG) D_2 is a major prostanoid formed in mast cells obtained from rats and humans [3–5], and is actively produced *in vitro* during stimulation with calcium ionophore or anti-IgE antibody [5–8]. *In vivo*, PGD_2 also shows various kinds of pharmacological activities which differ from those of other prostanoids, and has resulted in life-threatening hypotensive episodes in several patients with systemic mastocytosis [9]. In addition to the vascular effects and the ability to enhance vascular permeability, PGD_2 can modulate the release of other substances by cells, especially mast cells [10]. Although PGD_2 has no effect on the release of histamine from rat and human cells [6], Peters *et al.* [11] reported that PGD_2 enhanced histamine release at concentrations of 1–100 nM in human basophils. Recently, Kozuka *et al.* [12] reported that the release of PGD_2 and histamine from abdominal skin of ovalbumin-sensitized guinea pig is increased, and they suggested that acute anaphylactic reaction in the skin is partly mediated by PGD_2, acting either directly or in synergism with other mediators produced during the inflammatory reaction. Therefore, PGD_2 produced in mast cells is thought to be significantly involved in the immunologic or allergic process. In this study, we examined the effect of Tranilast on the synthesis of PGD_2 in rat peritoneal mast cells to clarify the action mechanism of this unique drug.

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MATERIALS AND METHODS

Materials. [1-^{14}C]Arachidonic acid (56.0 mCi/mmol) was purchased from New England Nuclear. [1-^{14}C]PGH_2 was prepared as described previously [13]. Synthetic PGD_2, PGE_2 and $PGF_{2\alpha}$ were gifts from the Ono Pharmaceutical Co. (Osaka, Japan). Tranilast was provided by the Kissei Pharmaceutical Co., Ltd (Matsumoto, Japan). All other chemicals used were of analytical grade.

Animals. Sprague–Dawley rats weighing 250–300 g were killed in each experiment.

Cell preparation. The collection and bovine serum albumin density gradient purification of mast cells were performed by the method of Kennerly *et al.* [14]. Mast cell preparations were at least 95% pure, and at least 98% of these cells were viable as assessed by trypan blue exclusion. Cell suspension was adjusted to a concentration of 1×10^6 mast cells/ml of phosphate-buffered saline (PBS) and pooled at 4°. Mast cell homogenates were prepared by sonication on ice.

Metabolism of exogenous arachidonic acid. Mast cell homogenates were incubated with 3.6 μM [1-^{14}C]arachidonic acid in 0.1 M Tris–HCl, pH 8.0, containing 1 mM glutathione (GSH) in a total reaction volume of 50 μl. The reaction proceeded for 10 min at 37°. The reaction was terminated by the addition of 0.3 ml of a mixture of solvents; diethyl ether/methanol/0.5 M citric acid (30:4:1, by vol.). Extraction from the reaction mixture, thin-layer chromatography, and quantitative measurements were performed as described previously [15]. The effect of Tranilast was assessed by preincubating mast cell homogenates for 10 min at 25° with various concentrations of Tranilast. The drug was dissolved in 1% sodium bicarbonate solution and diluted to the desired concentration with buffer, as described previously [2].

Assay of PGD synthetase activity. Activity of PGD

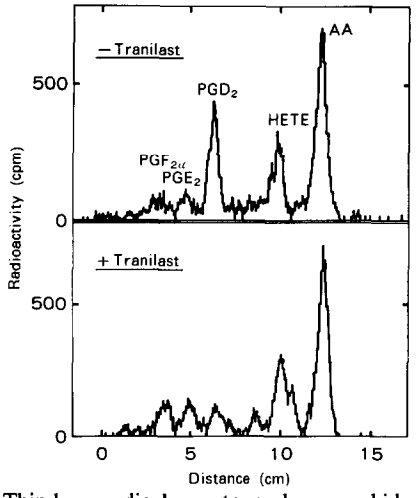

Fig. 1. Thin-layer radiochromatographs or arachidonic acid metabolites in rat peritoneal mast cells in the presence or absence of 1 mM Tranilast.

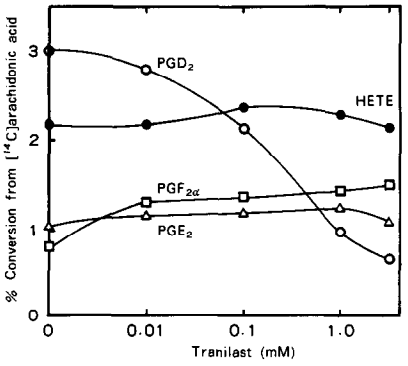

Fig. 2. Effect of various concentrations of Tranilast on the conversion from arachidonic acid.

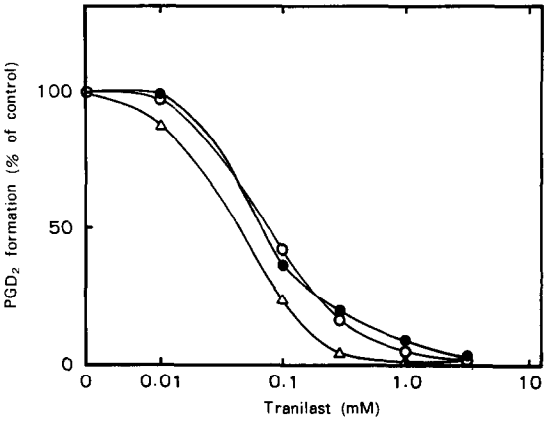

Fig. 3. Effect of various concentrations of Tranilast on the conversion of PGD_2 from $[^{14}C]PGH_2$ by rat mast cell homogenate (●--●), purified rat brain PGD synthetase (△--△) and purified rat spleen PGD synthetase (○--○). The values of PGD synthetase activity are expressed as total amounts of prostaglandins formed minus the amounts of nonenzymatic decay of PGH_2. The values represent percent of enzyme activity without the addition of Tranilast and means of three samples.

synthetase was assayed in a reaction mixture containing 0.1 M Tris–HCl, pH 8.0, 0.5 mM EDTA, 1 mM GSH and mast cell homogenates in a total volume of 50 μl. After preincubation at 24° for 10 min, the reaction was started by the addition of $[1\text{-}^{14}C]PGH_2$ (2 nmol, 117,000 cpm/nmol) dissolved in 2 μl of diethylenglycol dimethyl ether and carried out for 1 min at 24°. Termination of the reaction, extraction and quantification of products were performed, as described previously [13]. The effect of Tranilast was assessed as described above.

PGD_2 *generation from intact mast cells activated by the calcium ionophore* A23187. Purified mast cells (10^6/ml) were suspended in buffered solution (154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 4.0 mM Na_2HPO_4, 2.7 mM KH_2PO_4, pH 7.1) containing 0.1% (w/v) human serum albumin, as described previously [16]. Mast cells were prelabeled with $[^{14}C]$arachidonic acid (5 $\mu Ci/10^6$ cells) for 10 min at 37°. After the addition of Tranilast, the radiolabeled cells were stimulated by exposure of 5 μM A23187 for 60 min at 37°. After terminating the reaction by adding 3 ml of ice-cold chloroform/methanol (1:2, v/v), extraction and quantification of products were performed as described previously [13, 16]. All prostaglandins were presented as percent of total radioactivity detected on thin-layer chromatography.

RESULTS

When mast cell homogenates of rat peritoneal fluid were incubated with $[^{14}C]$arachidonic acid, the major reaction product was PGD_2, followed by PGE_2 and $PGF_{2\alpha}$ in small quantities (Fig. 1). Hydroxyeicosatetraenoic acid (HETE) was also formed in a small quantity as a lipoxygenase product. When 1 mM Tranilast was added to the reaction, the formation of PGD_2 was reduced markedly, but the synthesis of HETE, PGE_2 and $PGF_{2\alpha}$ was unchanged, as shown in Fig. 1. In contrast, when 1 mM indomethacin was added instead of Tranilast, the formation of not only PGD_2, but also PGE_2 and PGF_2 was suppressed. The formation of HETE was unchanged in the presence of indomethacin (data not shown). When the concentration of Tranilast was changed to 0.1 mM, the synthesis of PGD_2 was reduced to approximately 50%, but the formation of HETE, PGE_2 and $PGF_{2\alpha}$ was not changed (Fig. 2).

When $[^{14}C]PGH_2$ was used as a subtrate, PGD_2 was also the major prostanoid in rat mast cell homogenates. Tranilast also inhibited rat mast cell PGD synthetase activity in a dose-dependent manner, as shown in Fig. 3. Tranilast at a 1 mM concentration almost completely inhibited enzyme activity, and a 0.08 mM concentration inhibited PGD synthetase activity by 50% (Fig. 3).

Tranilast also inhibits PGD synthetase purified from rat spleen [15] in a manner similar to that from mast cells, and purified rat brain PGD synthetase [17] was also suppressed by Tranilast with a lower concentration (Fig. 3).

Table 1. Effect of Tranilast on the production of prostaglandins from [^{14}C]arachidonic acid by rat peritoneal mast cells*

Tranilast (0.1 mM)	PGD_2	PGE_2	$PGF_{2\alpha}$
	(% of total radioactivity)		
(−)	4.14 ± 1.87	2.85 ± 1.21	0.89 ± 0.33
(+)	0.74 ± 0.03†	0.69 ± 0.18‡	0.77 ± 0.22§

* Intact mast cells were activated by 5 μM A23187. All prostaglandins are presented as percent of total radioactivity detected on thin-layer chromatography. The remaining percent of total radioactivity was recovered as [^{14}C]arachidonic acid. Values shown are the means of triplicate determinations ± SE. Experimental procedures were described in Materials and Methods.

† $P < 0.01$, compared with controls.

‡ $P < 0.02$, compared with controls.

§ Not significantly different from controls.

Furthermore, 0.1 mM Tranilast markedly prevented the PGD_2 generation from intact mast cells activated by 5 μM A23187 (Table 1). In contrast to the *in vitro* experiments shown in Figs 1 and 2, PGE_2 production was also inhibited by Tranilast, but $PGF_{2\alpha}$ production was not affected. When 1 mM Tranilast was added to intact mast cells, the PGD_2 generation was suppressed, but to a lesser extent than in the case of 0.1 mM. This is because a high concentration of Tranilast impaired mast cells and caused the release of arachidonic acid from cell membrane (data not shown).

DISCUSSION

Tranilast has been used therapeutically for various allergic diseases including asthma, allergic rhinitis and atopic dermatitis. It has been reported that Tranilast inhibits the release of chemical mediators such as histamine and slow reactive substances from mast cells caused by antigen–antibody reactions. Although it has been noted that the inhibitory mechanism of the chemical mediator release of Tranilast is the inhibition of the energy-requiring system and/or Ca^{2+} influx at the time of mast cell degranulation [18], the exact mechanism of action is unknown at present. In this study, we demonstrated that Tranilast directly inhibits the activity of PGD synthetase in rat peritoneal mast cells. Unlike aspirin, indomethacin or dapsone [19], Tranilast does not inhibit cyclooxygenase activity, but directly suppresses PGD synthetase, because (i) the inhibition was observed when either arachidonic acid or PGH_2 was used as substrate, and (ii) the synthesis of PGE_2 and $PGF_{2\alpha}$ was unchanged. Tranilast similarly inhibited rat spleen PGD synthetase. This finding is very reasonable, because PGD synthetases in rat spleen and peritoneal mast cells are GSH dependent and identical both immunologically and biochemically [20]. Tranilast also suppressed rat brain PGD synthetase with a smaller IC_{50} value, but this type of PGD synthetase is GSH independent and different from rat spleen type enzyme [21].

Furthermore, as shown in Table 1, Tranilast inhibited PGD_2 generation in intact mast cells at 0.1 mM, the concentration used in broken cell preparations. PGD_2 plays an important role in various immunological and allergic responses as a chemical mediator [3–5]. These results, together with previous reports that serum levels of Tranilast rise up to 0.1 mM in patients with bronchial asthma after an oral administration of 5 mg/kg body weight [22], and the fact that Tranilast could be incorporated into rat mast cells [23], suggest that the pharmacological action of Tranilast is expressed at least in part via inhibition of PGD synthetase activity.

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