has shown that, although all acidic membrane lipids bind calcium, sulfatide is the most potent [16]. Sulfatide, as a result, has been suggested as a possible membrane calcium ionophore [16].

Acute administration of ethanol reduced the sulfatide content of hypothalamus (although no other brain region), but it was without effect on brain regional phospholipid content. Chronic ethanol administration increased the sulfatide content of whole brain, but no such action was observed when brain regions were analyzed for sulfatide. One would expect that an increased sulfatide content in whole brain would be reflected in a similar change in at least some of the brain regions. Since this was not the case, sulfatide involvement in the actions of ethanol must be considered questionable.

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Inhibition of antigen-induced histamine release and thromboxane synthase by FPL 55712, a specific SRA-A antagonist?

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FPL 55712, sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid, has been described to be a selective antagonist for SRS-A, *in vitro* [1]. For this reason it has been commonly used to ascertain the involvement of SRS-A in anaphylactic reactions in various animal model systems [2–6]. In this communication we describe two other properties of FPL 55712 that should be considered when interpreting such studies: (1) FPL 55712 is a potent inhibitor of antigen-induced histamine release *in vitro*, and (2) FPL 55712 inhibits thromboxane synthase.

For assessment of the ability of this drug to inhibit antigen-induced histamine release, two model systems were employed. One was an IgE-dependent system utilizing passively sensitized rat peritoneal cells. The second was an IgG-dependent system employing chopped guinea pig lung fragments from actively sensitized animals. The IgE-dependent passively sentized rat peritoneal system was similar to that described by Herzig and Kusner [7]. Reaginic antiserum was prepared by injecting (i.p.) male OLA-1, Hooded Lister rats (Oxfordshire Laboratories, Oxfordshire, England), weighing 150–200 g, with 10 μg egg albumin (Nutritional Biochemical Corp., Cleveland, OH) in 0.5 ml of Pertussis Vaccine (10 ou of heat-killed bacteria, Cannaught Laboratories, Willowdale, Toronto, Canada). On day 30 the animals were boosted with a second injection of 1 μg egg albumin in 0.5 ml saline (i.p.), and on day 34 they were anesthetized with CO_2 and exsanguinated by cardiac puncture. The sera from 150 rats were inidividually tested by passive cutaneous anaphylaxis (PCA) in rats, using a 48-hr sensitization period [8]. Sera with a titer of at least 1:50 were combined. The titer of the resulting combination was 1:256. The pooled serum was stored at −70°. The reaginic nature of the antiserum was demonstrated by heating for 4 hr at 56°. This reduced the titer to less than 1:4 in the PCA test. For passive sensitization, a mixed population of peritoneal cells (containing 5–10% mast cells) was harvested by lavage from eight to twelve male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), weighing between 180 and 220 g, which were fasted overnight. The buffer used was Hanks' balanced salt solution containing 50 μg/ml sodium heparin (Sigma Chemical Co, St. Louis, MO) buffered to pH 6.9 with 5% (v/v) of 0.1 M sodium phosphate. The cells were isolated by centrifugation at 350 *g* for 5 min (0–4°) and resuspended in 2 ml of the reaginic serum. After sensitization for 2 hr at 37°, 1 ml of buffer was added and the mixture was recentrifuged as described above. The sensitized cells were then resuspended in buffer to a concentration of approximately 1×10^6 cells/ml, and triplicate

Table 1. Concentration-dependent inhibition of anaphylactic histamine release from actively sensitized guinea pig lung fragments*

Concn of FPL 55712 (M)	% Inhibition of histamine release Experiment 1	Experiment 2
10^{-7}		0.4 ± 3.7
10^{-6}		11.5 ± 1.7†
10^{-5}	37.4 ± 1.5‡	24.2 ± 2.3‡
10^{-4}	45.0 ± 1.5‡	34.0 ± 2.3‡

* Assays were performed in triplicate on pooled lung fragments from three animals. Values are mean ± S.E. In Experiment 1, antigen induced release of 33.6 ± 0.6 per cent (corrected for a spontaneous release of 0.3 per cent), while in Experiment 2, a release of 34.7 ± 1.0 per cent (corrected for a spontaneous release of 0.9 per cent) was obtained.

† $P < 0.05$.

‡ $P < 0.001$.

samples (2 ml, final volume) were challenged with egg albumin (5 × crystallized, Sigma Chemical Co.) in the presence or absence of test drug for 5 min at 37°. Release reactions were terminated by cooling on ice and centrifugation at 1100 *g* (0–4°). The supernatant fractions were acidified to 0.4 N $HClO_4$ and stored at 0–4° overnight. After recentrifugation of the samples for 10 min at 1800 *g* (0–4°), the supernatant fractions were assayed for histamine content using an automated fluorometric assay [9]. The amount of histamine released into the supernatant fraction was expressed as a percentage of the total histamine content of the cells. The total histamine content of the cells (approximately 3 μg) was determined by heating 2 ml aliquots of sensitized cells in a boiling water bath for 10 min, centrifuging, and assaying supernatant fractions for histamine content as described above.

The data presented in Fig. 1 demonstrate that FPL 55712 was a potent inhibitor of antigen-induced histamine release in the model system described above. The IC_{50} (drug concentration that produced 50 per cent of the maximum obtainable inhibition) for FPL 55712 was 3.5×10^{-7} M in this experiment. In contrast, disodium cromoglycate, the reference drug against which mediator release inhibitor are typically compared, was a less potent inhibitor (IC_{50} was 1.5×10^{-6} M). These results are typical of those obtained in three similar determinations.

The methodology employed to study the effect of FPL 55712 on the IgG-dependent release of histamine from guinea pig lung fragments was similar to that described by Hitchcock [10]. Assays were performed in triplicate by prewarming aliquots of a pool of lung fragments from three actively sensitized animals (150 mg in 5 ml of Tyrode's solution) for 10 min in the presence of various concentrations of FPL 55712 and subsequently challenging the tissue with 40 μg/ml egg albumin (5 × crystallized, Sigma Chemical Co.) for 10 min at 37°. The supernatant fractions were assayed for histamine as described for the peritoneal cell system. Total histamine content was obtained by homogenizing 150 mg portions of lung in 5 ml of Tyrode's and boiling the samples for 10 min prior to centrifugation at 2000 *g* (0–4°) for 10 min and histamine analysis [9].

As demonstrated in Table 1, in two separate experiments FPL 55712 caused a significant inhibition of histamine release in the guinea pig lung test system. Although it was not as potent an inhibitor in this test system as in the IgE-dependent rat peritoneal cell system, it was still more effective than disodium cromoglycate which failed to inhibit histamine release at concentrations as high as 1×10^{-3} M (data not shown). The potency differences observed for FPL 55712 in these two *in vitro* model systems may have been related to the inability of the drug to effectively penetrate the lung tissue. While this manuscript was in preparation, Krell *et al.* [11] reported that FPL 55712 also inhibited the antigen-induced release of histamine from rat, dog and monkey lung tissue, *in vitro*. The effects they observe in these systems were not dose related, however, so potencies also cannot be compared between these lung systems and the IgE-dependent rat peritoneal cell system studied in this paper.

For the assessment of the ability of FPL 55712 to inhibit thromboxane synthase activity, an assay system was employed which followed the conversion of [1-^{14}C]PGH_2 to [1-^{14}C]thromboxane B_2 and [1-^{14}C]HHT, using human platelet microsomes as the enzyme source [12].* Radiolabeled [1-^{14}C]PGH_2 was prepared as follows. The acetone pentane powder of sheep vesicular gland microsomes (225 mg, HilRan Chemicals, Tel Aviv, Israel) was homogenized in 4.5 ml of 0.1 M tris buffer, pH 8.5, containing 0.67 mM phenol and 3 mM para-chloromercuribenzoate (PCMB). This mixture was allowed to equilibrate for 0.5 hr at 25°. An aliquot of [1-^{14}C]arachidonic acid (62.5 μCi in 250 μl benzene, sp. act. 60 mCi/mmole, Rosechem Products, Los Angeles, CA) was evaporated nearly to dryness under a nitrogen stream and was diluted to a final specific activity of 6.7 mC/mmole with unlabeled arachidonic acid in ethanol (1 mg/ml, Supelco, Bellefonte, PA). To each of twenty-four, 2 × 15 cm, screwcap tubes were added aliquots of the sheep vesicular gland microsomal homogenate (167 μl), 0.1 M Tris buffer (9.5 ml, containing 0.67 mM phenol and 3 mM PCMB), and hemin (222 μl of a 0.1 mM solution). After incubation at 37° for 5min, an aliquot of the [1-^{14}C]arachidonate ethanolic solution (100 μl containing 2.5 μCi) was added. After further incubation at 37° for 90 sec, ether (15 ml, −78°) was added, followed by citric acid (750 μl of a 1 mM solution). The tubes were tightly capped and mixed vigorously for 20 sec, centrifuged (1000 *g*, 5 min) to break the resultant emulsion, and placed in an acetone/dry ice bath. This procedure froze the aqueous layer but left the ether extracts in a liquid state for pooling. The ether extracts were dried over anhydrous magnesium sulfate (10 g) at 5° for 10 min. The magnesium sulfate was removed by vacuum filtration using Whatman no. 1 filter paper, and the extract was reduced *in vacuo* to near dryness. The residue was resuspended in 10 ml of hexane and was applied to a 1 × 15 cm silica gel column (Mallinckrodt CC-4 Silicar). The column was eluted with increasing amounts of ether in hexane. [1-^{14}C]Arachidonic acid appeared in the 10% ether fraction, and [1-^{14}C]PGH_2 typically appeared in the 40% ether fraction. The yield, based on recovery of radiolabel, was on the order of 15 per cent. The purity of the radiolabeled PGH_2 was determined to be >90 per cent on the basis of thin-layer chromatography using an isooctane–methyl ethyl ketone–acetic acid (100:9:1) system [13].

* Abbreviations: PG, prostaglandin; TX, thromboxane; and HHT, 12-L-hydroxy-5,8,10-heptadecatrienoic acid.

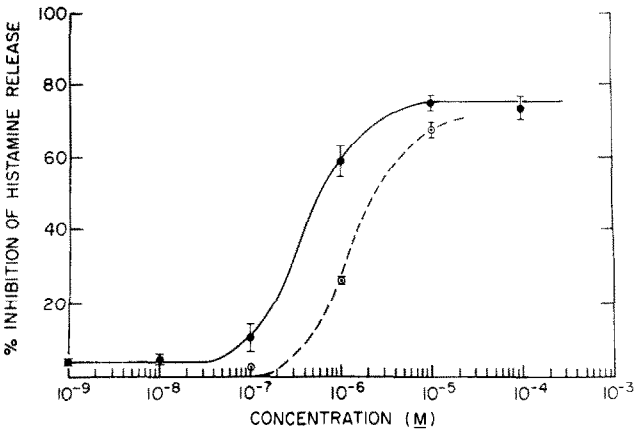

Fig. 1. Inhibition of antigen-induced histamine release from rat peritoneal cells by FPL 55712 and disodium cromoglycate. Passively sensitized cells were challenged with antigen (10 μg egg albumin/ml) in the presence or absence of various concentrations of FPL 55712(●——●) or disodium cromoglycate (○——○), added at the same time as antigen. In the absence of drug, antigen-induced histamine release was 30.6 ± 0.7 per cent (after correcting for a spontaneous release of 7.6 ± 0.8 per cent). The results are presented as the per cent inhibition of this histamine release. Each point is the mean ± S.E. of triplicate determinations.

In a typical thromboxane synthase assay, an aliquot of human platelet microsomes (50 μl, containing ~150 μg protein) was added to a 12 × 75 mm silanized test tube containing [1-^{14}C]PGH_2 (0.5 μg, 0.01 μCi) in 200 μl of phosphate-buffered saline (the final PGH_2 concentration was 10 μM). The solution was incubated in a 25° water bath for 2 min. Ether (2 ml) and citric acid (50 μmoles) were subsequently added with vigorous agitation, and the solution was placed in a dry ice/acetone bath. The ether extract was separated from the frozen aqueous layer and transferred in stages to a 1.0 ml reacti-vial (Pierce Chemical Co., Rockford, IL), and the volume was reduced under nitrogen to 50–100 μl. The extract was applied to an i.t.l.c., type SG, glass fiber sheet (Gelman Instrument Co., Ann Arbor, MI) and developed in isooctane–methyl ethyl ketone–acetic acid (100:19:1). The sheets were air dried and scanned using a Berthold LB radiochromatographic scanner. Assay conditions were adjusted such that three major radioactive peaks were noted in radiochromatographic profiles of extracts (Fig. 2b). These were the products of the enzymatic reaction, [1-^{14}C]TXB_2, [1-^{14}C]HHT and the substrate, [1-^{14}C]PGH_2 (under the assay conditions employed, 85–95 per cent was converted to the assay products). In the absence of enzyme or the presence of heat-denatured enzyme (Fig. 2a) only 10–15 per cent of the [1-^{14}C]PGH_2 decomposed to nonenzymatic products.

The ability of FPL 55712 or other test drugs to inhibit the enzymatic reaction was quantitated by adding various concentrations of the drug (in 10 μl ethanol) to assay mixtures. After assay, extraction, and chromatographic separation of the products (no new products were formed in the presence of FPL 55712 or other test drugs), the percentage of [1-^{14}C]PGH_2 remaining was determined by cutting the appropriate areas from the glass fiber sheets and scintillation counting in 10 ml of LSC mixture (Yorktown Research, Hackensack, NJ). The IC_{50} values were determined by linear regression analysis of the percentage of PGH_2 remaining plotted versus the log of the inhibitor concentration. As can be seen in Table 2, using the described test system, FPL 55712 was a relatively potent inhibitor of thromboxane synthase, being 100-fold more active than imidazole, a standard inhibitor of thromboxane synthase in human platelets [14]. The inhibitory activity observed with FPL 55712 was nearly equivalent to that displayed by several 1-substituted imidazole derivatives that are among the most potent thromboxane synthase inhibitors yet described [15–17].

Table 2. Comparison of the inhibitory potency of FPL 55712 and 1-substituted imidazoles on thromboxane synthase from human platelet microsomes

Compound	IC_{50} (μM)
FPL 55712	6.5
Imidazole	600
4′-(1H-Imidazol-1-yl) acetophenone	10
p-(1H-Imidazol-1-yl) phenol	1
1-Nonyl imidazole	10

The data presented in Table 2 differ quantitatively from the data presented by Tai and Yuan [15] for imidazole and its 1-substituted analogs. This may be due, in part, to the difference in substrate concentrations employed (5 μM for Tai vs 10 μM in the experiments reported herein). Qualitatively, using the assay conditions employed in this report, there is essentially no difference in the rank order of inhibition of various 1-substituted imidazoles (unpublished results) and the rank order reported by Tai and Yuan [15].

The results reported herein suggest that caution should be used in interpreting the results of experiments in which FPL 55712 is utilized to define a role for SRS-A in anaphylactic reactions. When used at concentrations greater than 1×10^{-6} M, FPL 55712 has the potential for acting through mechanisms other than simply SRS-A antagonism. For example, numerous reports have appeared recently in which this drug has been utilized to define a role for SRS-A in *in vitro* model systems designed to study antigen-induced bronchospasm in guinea pig parenchyma and trachea strip preparations. In most of these studies, FPL 55712 prevented or reversed bronchospasm when utilized at concentrations greater than 10^{-5} M [3–5], concentrations at which we have shown the drug to be effective at inhibiting both histamine release and thromboxane production. Both histamine and thromboxane are known to be bronchoconstrictive agents in the guinea pig which are either directly released (histamine) or newly synthesized and released (thromboxane A_2) in response to antigen challenge [18, 19]. Therefore, at the concentrations used in these studies, one

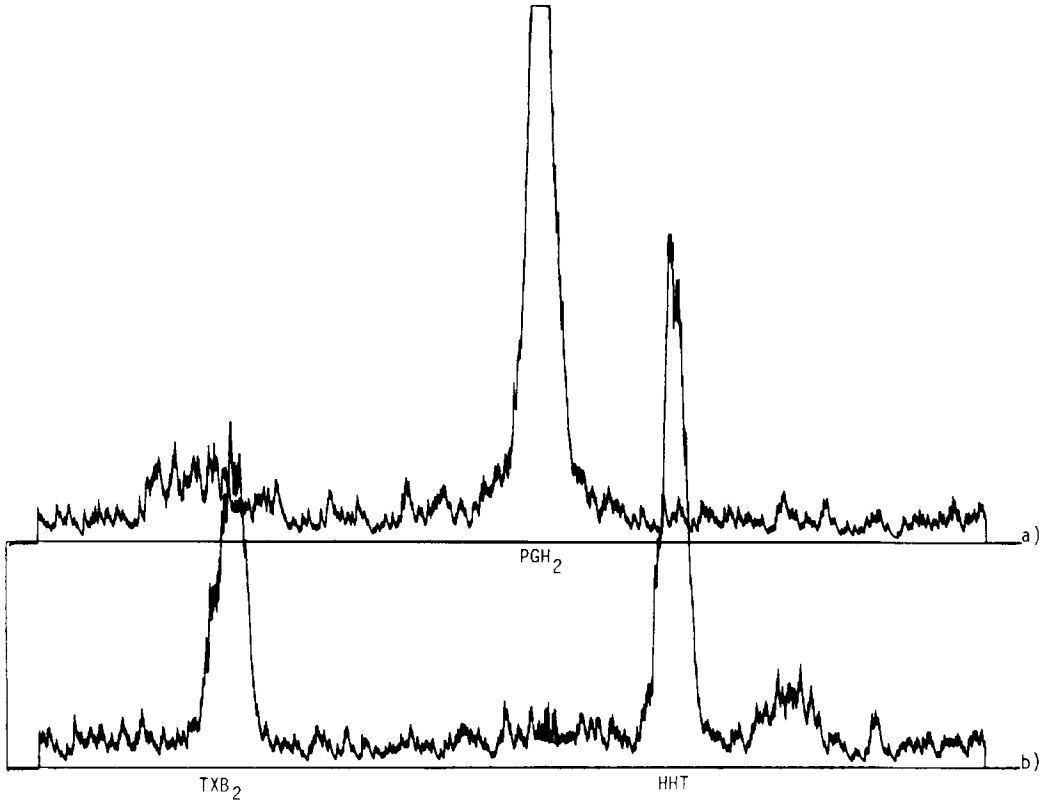

Fig. 2. Radiochromatographic profile of the products noted in the thromboxane synthase assay. (A) Nonenzymatic products derived from [1-^{14}C]-GH_2 after incubation with heat-denatured human microsomes. (B). Enzymatic products derived from [1-^{14}C]PGH_2 after incubation with human platelet microsomes.

should also consider the effects which FPL 55712 might have on thromboxane production or histamine release, before a role for SRS-A in these model systems can be definitely ascertained. An effect of FPL 55712 on histamine release or thromboxane production might also explain, in part, its previously described inhibitory activity on passive lung anaphylaxis in the rat [2] and its role in the inhibition of the Schultz–Dale response in the guinea pig ileum [6].

In summary, FPL 55712 was originally described to be a selective antagonist of SRS-A. We feel this description still holds true as long as the drug is utilized at concentrations between 10^{-8} and 10^{-6} M. When used at higher concentrations, other activities of this drug must be considered. These include its ability to antagonize $PGF_{2\alpha}$ and PGE_1 [1], to inhibit cyclic nucleotide phosphodiesterase [20] and, as presently described in this paper, to inhibit allergic mediator release and thromboxane synthase.

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