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Characterization of a Thromboxane A_2/Prostaglandin H_2 Receptor in Guinea Pig Lung Membranes Using a Radioiodinated Thromboxane Mimetic

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SUMMARY

Thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGH_2) are potent constrictors of airway smooth muscle and may mediate some of the pulmonary effects of leukotrienes. To date, the TXA_2/PGH_2 receptor in lung has not been well characterized. In this report, we describe the evaluation of the TXA_2/PGH_2 receptor in guinea pig lung membranes using the new radiolabeled TXA_2 mimetic [1*S*(1α,2β(5*Z*),3α(1*E*,3*S**),4α)]-7-[3-(3-hydroxy-4-(4′-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1]heptan-2-yl]-5-heptenoic acid (IBOP). IBOP elicited a dose-dependent contraction of guinea pig lung parenchymal strips (EC_{50} = 3.03 ± 0.97 nM, three experiments), which was blocked by the TXA_2/PGH_2 antagonists SQ29548 (pK_B = 7.44 ± 0.2, three experiments), BM13505 (pK_B = 6.29 ± 0.26, three experiments), and I-PTA-OH (pK_B = 5.82 ± 0.36, three experiments). In radioligand binding studies, the binding of [^{125}I]IBOP to guinea pig lung membranes prepared from perfused lungs was saturable, displaceable, and dependent upon protein concentration. Binding was optimal at pH 6.5 and was enhanced by the addition of mono- and divalent cations. The standard assay buffer was 25 mM 3-(*N*-morpholino)propanesulfonic acid, pH 6.5, 100 mM NaCl, 5 mM $MgCl_2$. Binding was inhibited by pretreatment with dithiothreitol, *N*-ethylmaleimide, or β-mercaptoethanol. Binding was unaffected by the addition of guanine nucleotide analogs at concentrations up to 300 μM. Analysis of the time course of binding of [^{125}I]IBOP at 30° yielded k_{-1} = 0.0447 min^{-1}, k_1 = 2.49 × 10^8 M^{-1} min^{-1}, and $K_d = k_{-1}/k_1$ = 180 pM. Computer analysis of equilibrium binding studies using nonlinear methods (LUNDON-1) revealed a single class of noninteracting binding sites with a K_d of 86.9 ± 11.9 pM and a B_{max} of 81.8 ± 7.7 fmol/mg of protein (three experiments). [^{125}I]IBOP binding to guinea pig lung membranes was inhibited by a series of TXA_2/PGH_2 receptor agonists and antagonists, with a rank order different from that previously determined for washed guinea pig platelets (Spearman's r = 0.686, $p > 0.05$). [^{125}I]IBOP binding to guinea pig lung membranes was also inhibited by the prostanoids prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 9α,11β-prostaglandin F_2, all of which have been proposed to act at the TXA_2/PGH_2 receptor in lung.

The unstable arachidonic acid metabolites PGH_2 and TXA_2 are potent stimulators of airway smooth muscle contraction (1–3), presumably acting via a TXA_2/PGH_2 receptor, as has been described for platelets and vascular smooth muscle (4). TXA_2/PGH_2 receptors in platelets and vascular smooth muscle from a number of species have been characterized pharmacologically, and differences in rank orders of potency for inhibition of TXA_2 mimetic-induced platelet aggregation or vascular smooth muscle contraction by a series of antagonists suggest that the platelet and vascular smooth muscle TXA_2/PGH_2 receptors are different (5, 6), although other investigators have suggested homogeneity of TXA_2/PGH_2 receptors (7, 8). TXA_2/PGH_2 receptors from platelets and vascular smooth muscle have been characterized using radioligand binding studies (for review see Ref. 4), and the receptor from human platelets has recently been purified (9). The putative TXA_2/PGH_2 receptor from lung has not been as well characterized. There is some evidence that it may be different from the receptor in vasculature (10–12)

D.E.Magee was a Charles Dana Scholar of DePauw University.

ABBREVIATIONS: PG, prostaglandin; IBOP, [1*S*(1α,2β(5*Z*),3α(1*E*,3*S**),4α)]-7-[3-(3-hydroxy-4-(4′-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1]heptan-2-yl]5-heptanoic acid; U46619, 15(*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid; SQ29548, [1*S*-[1α,2β(5*Z*),3β,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; SQ26655, [1*S*-(1α,2β(5*Z*),3α(1*E*,3*S**),4α)]-7-[3-(3-hydroxy-1-octenyl)-7-oxabicyclo[2.2.1]heptan-2-yl]-5-heptenoic acid; BM13505, 4-((((4-chlorophenyl)sulfonyl)amino)ethyl)benzeneacetic acid; EC_{50}, concentration of agonist giving a response 50% of the maximum response to that agonist; IC_{50}, concentration of inhibitor giving 50% decrease of binding relative to control; GPLM, guinea pig lung membranes; G protein, guanine nucleotide-binding regulatory protein; MOPS, 3-(*N*-morpholino)propanesulfonic acid; LT, leukotriene; TX, thromboxane; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; GDPβS, guanosine-5′-*O*-(2-thio)diphosphate; Gpp(NH)p, guanosine-5′-($\beta\gamma$-imido)triphosphate; I-PTA-OH, 9,11-dimethylmethano-11,12-methano-16-(3-iodo-4-hydroxyphenyl)-13,14-dihydro-13-aza-15$\alpha\beta$-ω-tetranor-TXA_2.

and platelet (13), but these observations are on tissues from different species (canine saphenous vein, guinea pig trachea or parenchymal strips, and human platelets), weakening the conclusion that the receptors on the various tissues are different. Based on observations of the effects of various prostanoids on guinea pig trachea or parenchymal strip and human bronchial smooth muscle, it has been proposed that there is a single receptor for the contractile prostanoids (PGD_2, $PGF_{2\alpha}$, PGH_2, TXA_2) in airways (14–16), which may be the TXA_2/PGH_2 receptor.

Previous studies indicate that the lung TXA_2/PGH_2 receptor may play an important role in the bronchoconstrictor response to allergen challenge and its chemical mediators. LTD_4 stimulates the production of TXA_2 in minced guinea pig lung (17), and a role for TXA_2 in the responses to LTD_4 *in vitro* and *in vivo* has been suggested (18, 19). In guinea pigs, TXA_2/PGH_2 antagonists have also been shown to inhibit the *in vivo* bronchoconstrictor responses to inhaled LTD_4, platelet-activating factor, and IgG_1, suggesting a mediator role for TXA_2 in pulmonary disease (20). In clinical trials, an orally active TXA_2/PGH_2 antagonist has been demonstrated to inhibit PGD_2 and allergen-induced bronchoconstriction in allergic asthmatics (21). Thus, it is important to further characterize the lung TXA_2/PGH_2 receptor to facilitate investigations into the role of TXA_2 in airway disease and to determine its similarity to TXA_2/PGH_2 receptors in other tissues. Before this communication, no characterization of the TXA_2/PGH_2 receptor in lung using radioligand binding assays has been reported. Recently, the synthesis of a new radioiodinated TXA_2 analog, IBOP, that acts as a TXA_2 mimetic in human platelets was described, and its use in radioligand binding studies in washed human platelets was reported (22). We describe the characterization of IBOP as a TXA_2/PGH_2 receptor agonist in guinea pig lung parenchymal strips and the results of radioligand binding studies in membranes prepared from guinea pig lung parenchyma.

Experimental Procedures

Materials. IBOP, its amine precursor, and I-PTA-OH were synthesized as previously described (22). [^{125}I]IBOP was prepared by chloramine T iodination of its amine precursor, using carrier-free Na^{125}I from Amersham (Chicago, IL), followed by deamination as previously described (22). Because the high performance liquid chromatography techniques used to purify [^{125}I]IBOP gave complete separation from the starting materials, the specific activity of [^{125}I]IBOP was taken to be that of ^{125}I (2175 Ci/mmol). U46619 was purchased from Upjohn Diagnostics (Kalamazoo, MI). SQ26655 and SQ29548 were gifts from the Squibb Institute for Medical Research (Princeton, NJ). BM13505 was prepared by Dr. Fariborz Mohamadi at Lilly Research Laboratories. PGs were obtained from Biomol (Plymouth Meeting, PA). Guanine nucleotides and analogs were obtained from Boehringer Mannheim (Indianapolis, IN). All other chemicals were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

Guinea pig lung parenchyma contractile responses. Male Hartley guinea pigs (500–900 g), obtained from Charles River (Portage, MI), were anesthetized using a mixture of halothane/O_2/N_2O. The lungs were excised through a sternal thoracotomy and immediately perfused with a warmed (37°) perfusion buffer (126.9 mM NaCl, 4.7 mM KCl, 1.6 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.18 mM KH_2PO_4, 18.0 mM NaH_2CO_3, 11 mM dextrose, 10 μM indomethacin). Perfusion was maintained until the effluent was clear. Strips of lung parenchyma (2 × 2 × 15 mm) were prepared from lobes that were completely blanched, indicating thorough removal of red blood cells. Parenchymal strips were mounted isometrically in organ chambers containing perfusion buffer warmed to 37° and equilibrated with O_2/CO_2 (95:5), pH 7.35. Parenchymal strip tension was monitored continuously with a force transducer and polygraph. Each strip was equilibrated under a 1-g preload tension for 1 hr before being challenged with histamine (10 μM) to assess functional integrity. Cumulative concentration-response curves were generated for the TXA_2/PGH_2 mimetic IBOP. EC_{50} values for IBOP were determined for individual tissues, using a nonlinear iterative regression program, and averaged with all animals weighted equally. The dose-dependent inhibition by SQ29548 on IBOP-contracted strips was determined by addition of SQ29548 to strips previously contracted with 10 nM IBOP. In addition, relative potencies of BM13505, I-PTA-OH, and SQ29548 as antagonists of IBOP-induced contractions were determined by generation of pK_B values for each antagonist. Concentration-response curves for IBOP in the presence of vehicle or antagonist were evaluated side by side, such that control and treatment tissues for all of the antagonists were prepared from the same lung.

Preparation of GPLM. Male Hartley guinea pigs (500–700 g) were euthanized by CO_2 asphyxiation. The lungs were excised and perfused through the pulmonary artery with 40 ml of phosphate-buffered saline (120 mM NaCl, 2.7 mM KCl, 10 mM NaH_2PO_4, pH 7.4) supplemented with 5 mM EDTA and 10 μM indomethacin. Necrotic areas of tissue were discarded, and the remainder was quick frozen in liquid nitrogen and stored at −32°. Pooled frozen lungs (25 g) were thawed and resuspended in 5 volumes of Tris·HCl/sucrose/EDTA homogenization buffer (10 mM, pH 7.4/250 mM/5 mM) containing the following protease inhibitors: 10 μg/ml soybean trypsin inhibitor, 100 μg/ml bacitracin, 10 μM phenylmethylsulfonyl fluoride, and 100 μM benzamidine. The lungs were minced well and homogenized with six passes with a Brinkmann Polytron homogenizer (Westbury, NY), using a PTA 20S probe at setting 6. Membranes were prepared from the homogenate by differential centrifugation (1000 × *g*, 10 min; 12,000 × *g*, 20 min; 100,000 × g, 60 min). The 100,000 × *g* pellet was washed once by resuspension in 5 mM Tris·HCl, pH 7.4, and centrifugation at 100,000 × *g* for 30 min. The final pellet was resuspended in 5 mM Tris·HCl, pH 7.4, to a protein concentration of 3–6 mg/ml, frozen in liquid nitrogen, and stored at −70° until used. Protein concentrations were determined using the bicinchoninic acid protein assay (23, 24), in 96-well microtiter plates, using reagents from Pierce (Rockford, IL).

Radioligand binding assays. Binding studies were performed in silanized glass tubes (12 × 75 mm) at 30°. Incubations (222 μl) typically contained 50 μg of GPLM protein, in a buffer consisting of 25 mM MOPS, 100 mM NaCl, 5 mM $MgCl_2$, pH 6.5 (assay buffer), approximately 100,000 cpm of [^{125}I]IBOP (0.11 nM), and displacing ligands or vehicle as appropriate. The reaction was terminated by the addition of 4 ml of ice-cold wash buffer (25 mM Tris·HCl, pH 7.4), followed immediately by rapid vacuum filtration through Whatman GF/C glass fiber filters, using a Brandel 24-place harvester (Gaithersburg, MD). The filters were washed three times with 4 ml of wash buffer. Retained radioactivity was determined by γ counting. Nondisplaceable binding was determined in the presence of 10 μM SQ29548 and was usually ≤10% of total binding.

For the determination of the time course of association of [^{125}I]IBOP to GPLM, the assay mixture, containing 29–329 pM [^{125}I]IBOP and GPLM in assay buffer, was incubated for 1 to 150 min at 30°, and incubation was terminated as described above. Nondisplaceable binding was determined at each time point. The time course of dissociation of [^{125}I]IBOP from its binding site was determined by incubation of the reaction mixture for 120 min at 30° and then addition of SQ29548 to a final concentration of 10 μM to initiate the dissociation of [^{125}I]IBOP. The amount of [^{125}I]IBOP bound was determined by termination of the reaction, as described above, at various intervals for up to 4 hr after the initiation of the dissociation. Nondisplaceable binding was determined in a separate concurrent reaction in the presence of 10 μM SQ29548 (added at the start of the experiment).

Data analysis. The data from kinetic experiments were analyzed, using the nonlinear regression program MultiFit 2.0 (Day Computing,

Cambridge, UK), by fitting to one- or two-component models. Equilibrium binding experiments were analyzed using the nonlinear regression analysis programs LUNDON-1 for saturation experiments and LUNDON-2 (London Software, Inc., Cleveland, OH) for competition experiments (25). *F* test comparison of the sum of squares of residual errors was performed to choose one- or two-component models (25). Analysis of rank orders of potency was performed using Spearman's rank correlation coefficient for the data (26), to compare IC_{50} values obtained in these experiments with those obtained previously for washed guinea pig platelets.

Results

Contraction of guinea pig lung parenchymal strips by IBOP. IBOP induced a concentration-dependent (EC_{50} = 2.38 ± 0.55 nM, six experiments) contraction of guinea pig lung parenchymal strips (Fig. 1), with a maximum tension of 507 ± 48 mg (499 ± 51% of the response to 10 μM histamine, seven experiments). IBOP-induced contractions were inhibited in a concentration-dependent manner by the specific TXA_2/PGH_2 antagonist SQ29548 (27), with complete abolition of the response to IBOP (10 nM) occurring at 3 μM SQ29548 (data not shown). The rank order of potency for three TXA_2/PGH_2 antagonists was determined by calculation of pK_B values from dose-response curves for IBOP produced in control strips and in strips pretreated with either SQ29548 (316 nM), BM13505 (10 μM), or I-PTA-OH (10 μM), as shown in Fig. 1, and was found to be SQ29548 (pK_B = 7.44 ± 0.2, three experiments) > BM13505 (pK_B = 6.29 ± 0.26, three experiments) > I-PTA-OH (pK_B = 5.82 ± 0.36, three experiments).

Binding of [^{125}I]IBOP to GPLM. The binding of [^{125}I]IBOP to GPLM was linear over a protein concentration range of 7.8–62.5 μg/tube (Fig. 2). The effect of pH on binding was examined using several buffer systems over the pH range 5.5–8.5. Displaceable binding increased with decreasing pH and was maximal at pH 6.0–6.5 in MOPS buffer (data not shown). For all further experiments, 25 mM MOPS, pH 6.5, was used as the buffer. The addition of mono- or divalent cations also

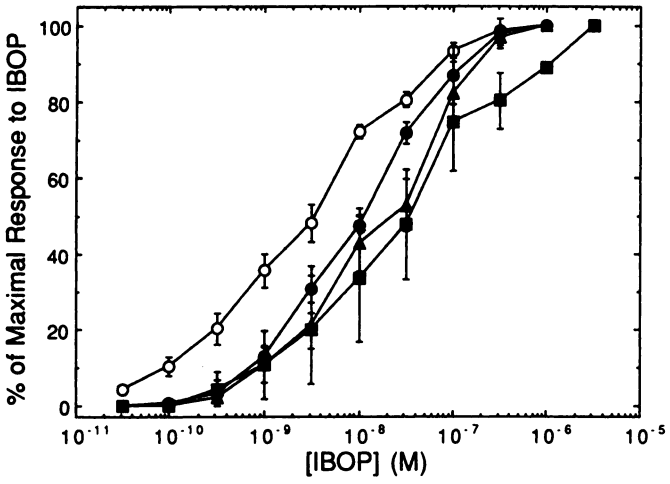

Fig. 1. Concentration dependence of IBOP-induced contractions of guinea pig lung parenchymal strips. The contractile responses of guinea pig lung parenchymal strips to various concentrations of IBOP in control tissues (○) or in tissues pretreated with I-PTA-OH (10 μM) (●), BM13505 (10 μM) (■), or SQ29548 (316 nM) (▲) were determined as described in Experimental Procedures. Data are expressed as a percentage of the maximum response to IBOP and are presented as mean ± standard error of three experiments.

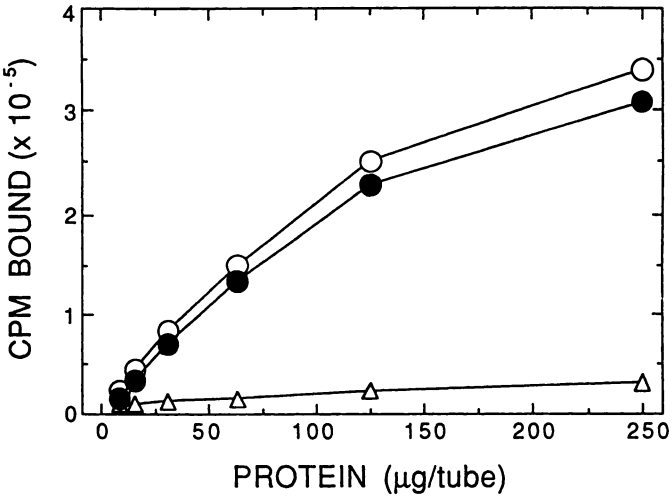

Fig. 2. Protein dependency of [^{125}I]IBOP binding to GPLM. GPLM (0.04–1.12 mg/ml) were incubated with [^{125}I]IBOP (≈100 pM) in assay buffer. Nondisplaceable binding (△) was determined in the presence of 10 μM SQ29548 and was subtracted from total binding (○) to determine displaceable binding (●). Data are presented as the mean of three experiments performed in triplicate. Standard error bars are not shown but were typically less than 5% of the mean. The data between 7.81 and 62.5 μg of protein/tube are fit by the equation $y = 217x - 65.1$, r = 0.999, as determined by linear regression analysis of the means.

enhanced binding, with the addition of 100 mM NaCl and 5 mM $MgCl_2$ giving optimum displaceable binding (data not shown). Pretreatment of membranes with trypsin (10,000 units) or boiling for 10 min resulted in a decrease in displaceable binding of 80 and 91%, respectively. Pretreatment of membranes with 10 mM dithiothreitol or *N*-ethylmaleimide for 30 min resulted in a 30–36% inhibition of displaceable binding. Addition of 1% β-mercaptoethanol to the assay inhibited displaceable binding by 83%. The effect of the addition of GTP or the nonhydrolyzable GTP analogs GTPγS and Gpp(NH)p to the assay on binding of [^{125}I]IBOP to GPLM was also examined. Under the assay conditions used, there was no apparent inhibition of binding of [^{125}I]IBOP to GPLM by any of the guanine nucleotides examined, over a concentration range of 1 nM to 300 μM.

The kinetics of the formation of the ligand-binding site complex were determined from the time course for binding of various concentrations of [^{125}I]IBOP to GPLM. Fig. 3 shows the time course of association from five experiments run over a concentration range of 29–329 pM [^{125}I]IBOP. Values for K_{obs} were determined by nonlinear regression methods and, at each concentration of [^{125}I]IBOP examined, the data were best fit by a one-component model. From a plot of K_{obs} versus [^{125}I]IBOP concentration (Fig. 3, *inset*), values for the rate constants of association (k_1) and dissociation (k_{-1}) were determined from the slope (2.49×10^8 M^{-1} min^{-1}) and *y*-intercept (0.0447 min^{-1}), respectively. The kinetically determined dissociation constant determined from the equation $K_d = k_{-1}/k_1$ was 180 pM. Nonlinear regression analysis of the time course of dissociation of [^{125}I]IBOP from its binding site, initiated by the addition of 10 μM SQ29548 (Fig. 4), gave a best fit of the data by a four-parameter model, suggesting two components of binding. The more rapidly dissociating component comprised 30% of the sites, with a rate constant of 0.114 min^{-1}, whereas the slower component comprised 70% of the sites, with a rate constant of 0.008 min^{-1}. These data are suggestive of two classes of binding

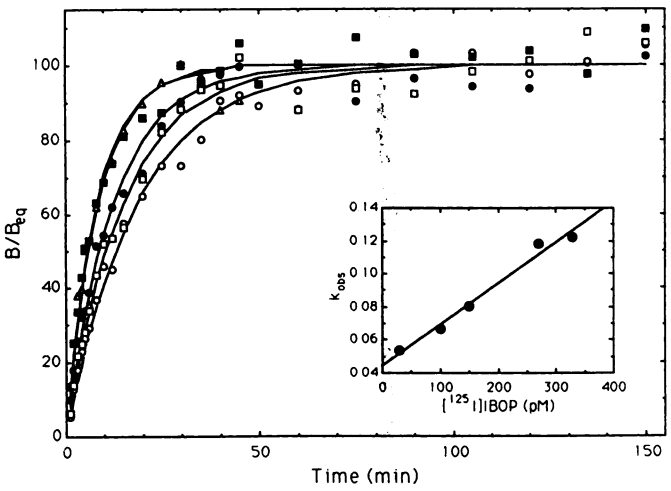

Fig. 3. Time course of [^{125}I]IBOP binding to GPLM. GPLM (25 μg of protein) were incubated at 30° with either 29 pM (○), 101 pM (□), 150 pM (●), 270 pM (△), or 329 pM (■) [^{125}I]IBOP and either vehicle for determination of total binding or 10 μM SQ29548 for estimation of nondisplaceable binding, for various times as indicated. Data are presented as the mean displaceable binding from experiments performed in triplicate. The lines fitting the data were obtained by nonlinear regression analysis, which indicated a best fit of the data in all cases by a one-component model with the equation $y = \lim \cdot (1 - \exp(-K \cdot T))$. *Inset*, plot of k_{obs} (obtained by nonlinear regression analysis of each experiment) versus [^{125}I]IBOP concentration. The equation for the line obtained from linear regression analysis is $y = 2.49 \times 10^{-4}x + 0.045$, $r = 0.990$.

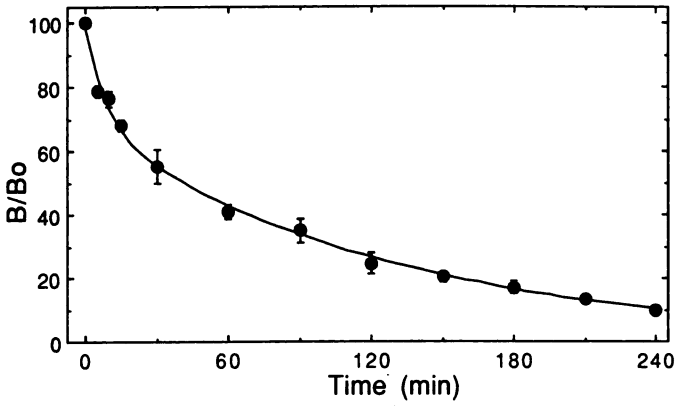

Fig. 4. Time course of dissociation of [^{125}I]IBOP from its binding site on GPLM. GPLM (50 μg of protein) were incubated with [^{125}I]IBOP (≈100 pM) and 10 μM SQ29548 (nondisplaceable binding) or vehicle (total binding) for 120 min at 30°, to allow the reaction to attain equilibrium. At this time (designated time 0), SQ29548 (10 μM) was added to the total binding tubes to initiate the dissociation of [^{125}I]IBOP, and the reaction was stopped at various times as indicated. Data are presented as the mean ± standard error of percentage of control displaceable binding (B/B_o) from three experiments, each performed in triplicate. Nonlinear regression analysis of the data yielded a best fit by a two-component/four-parameter model [$y = \text{lim1} \cdot (1 - \exp(-K_1 \cdot T)) + \text{lim2} \cdot (1 - \exp(-K_2 \cdot T))$].

sites for [^{125}I]IBOP but may also be due to alteration of the binding site over the 6-hr time course of these experiments.

Results of equilibrium binding studies are shown in Fig. 5. Analysis of the data using the nonlinear curve fitting program LUNDON-1 yielded a best fit of the data by a one-site model, with a K_d of 86.9 ± 11.9 pM and a B_{max} of 81.8 ± 7.7 fmol/mg of protein (three experiments).

Inhibition of [^{125}I]IBOP binding to GPLM by TXA_2/PGH_2 receptor ligands. A number of structurally dissimilar

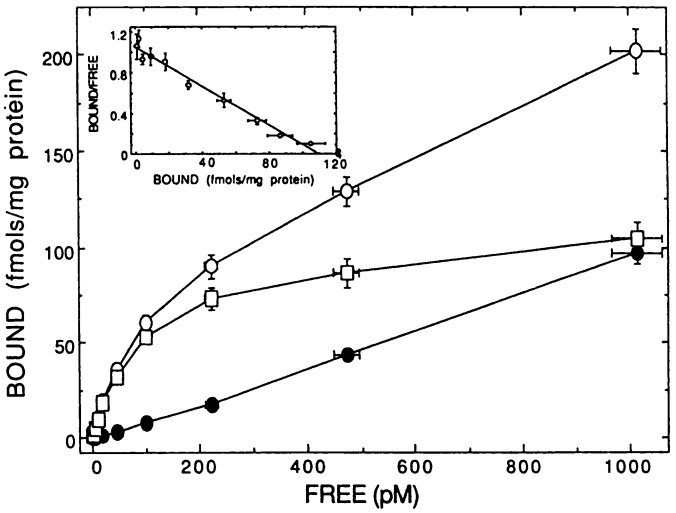

Fig. 5. Saturation of binding of [^{125}I]IBOP to GPLM. GPLM (50 μg of protein) were incubated with various concentrations of [^{125}I]IBOP and vehicle (total binding) (○) or 10 μM SQ29548 (nondisplaceable binding) (●) for 120 min at 30°. Displaceable binding (□) was determined by subtraction of nondisplaceable binding from total binding. Data are presented as the mean ± standard error of displaceable binding from three experiments, each performed in triplicate. *Inset*, Scatchard plot of displaceable binding. The equation of the line determined by linear regression analysis of mean values is $y = -0.0097x + 1.055$, $r = 0.988$.

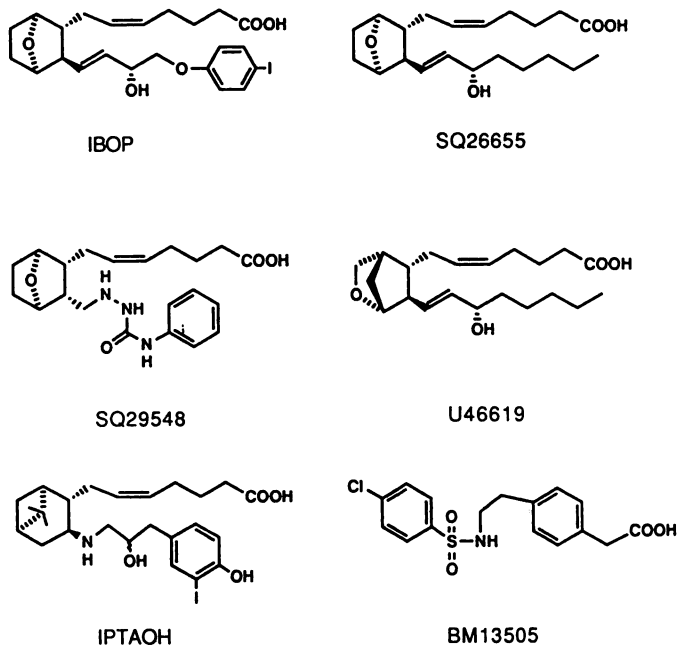

Fig. 6. Structures of TXA_2/PGH_2 receptor ligands used in competition experiments.

compounds (shown in Fig. 6) that act as either mimetics or antagonists at the TXA_2/PGH_2 receptor in other tissues were examined for their ability to inhibit the binding of [^{125}I]IBOP to GPLM. Fig. 7 shows the displacement curves for these compounds. A summary of the data is presented in Table 1. Nonlinear regression analysis of the data using the program LUNDON-2, in which one- and two-site models were examined with an F test comparison of the sum of squares of residual errors (25), yielded best fits by a one-site model for all of the compounds tested, except the PG endoperoxide analog U46619. The data for U46619 were best fit by a two-site model, with 56

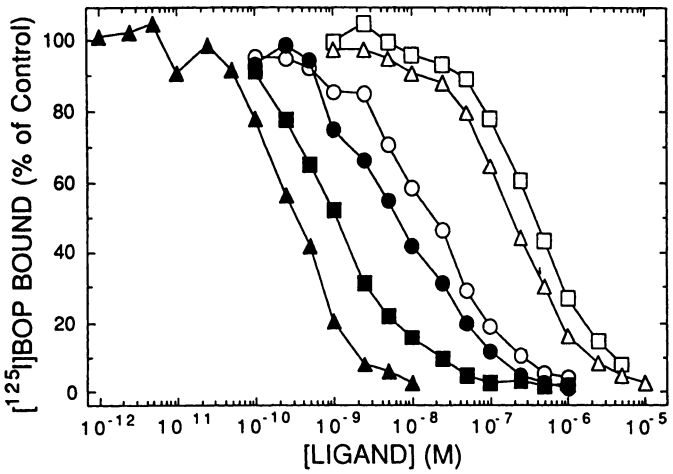

Fig. 7. Inhibition of binding of [^{125}I]IBOP to GPLM by TXA_2/PGH_2 receptor ligands. GPLM (50 μg of protein) were incubated for 120 min at 30° with [^{125}I]IBOP (≈100 pM) and vehicle (control) or various concentrations of IBOP (▲), SQ26655 (■), U46619 (●), SQ29548 (○), BM13505 (△), or I-PTA-OH (□). Data are expressed as percentage of control binding and are plotted as mean of three experiments, each performed in duplicate. Standard errors are omitted for clarity but were typically less than 5% of the mean.

TABLE 1

Parameters of a series of TXA_2/PGH_2 receptor ligands competing with [^{125}I]IBOP binding to GPLM

IC_{50} values and pseudo-Hill numbers (n_H) were obtained by linear regression of log-logit plots of competition curves. K_i values were obtained from nonlinear regression analysis using LUNDON-2. Values are expressed as mean ± standard error of data obtained from three experiments, except for SQ29548 (12 experiments).

Ligand	IC_{50}	Pseudo n_H	K_i
	nM		nM
SQ29548	20.9 ± 1.9	0.83 ± 0.04	8.4 ± 1.2
BM13505	184 ± 22.0	0.87 ± 0.04	105.6 ± 12.2
I-PTA-OH	384 ± 33.9	0.97 ± 0.06	199.3 ± 21.7
IBOP	0.322 ± 0.030	1.10 ± 0.04	0.189 ± 0.015
SQ26655	1.12 ± 0.06	0.79 ± 0.02	0.639 ± 0.026
U46619	6.19 ± 0.3	0.67 ± 0.02	0.96 ± 0.08 (High)
			16.51 ± 1.06 (Low)

TABLE 2

Comparison of affinities of TXA_2/PGH_2 receptor ligands for TXA_2/PGH_2 receptors in washed guinea pig platelets and GPLM

Ligand	Affinity	
	Lung[a]	Platelet
	nM	
IBOP	0.322	7.9[b]
SQ26655	1.12	3.0[c]
U46619	6.19	16.0[c]
SQ29548	20.9	9.8[b]
BM13505	184	24.3[b]
I-PTA-OH	384	14.5[c]

[a] IC_{50} values taken from Table 1.
[b] D. E. Mais, unpublished observations, using [^{125}I]IBOP.
[c] IC_{50} values determined for inhibition of [^{125}I]I-PTA-OH binding (taken from Ref. 37).

± 1% of the sites having high affinity (0.96 ± 0.08 nM) and 44 ± 1% of the sites having lower affinity (16.51 ± 1.06 nM) for U46619 (three experiments).

A comparison of binding data obtained in these experiments with those obtained in other studies using washed guinea pig platelets is presented in Table 2. Analysis of the rank orders of potency gives a Spearman's rank correlation coefficient value of 0.4286 ($p > 0.05$), suggesting that lung and platelet TXA_2/PGH_2 receptors are different.

Inhibition of [^{125}I]IBOP binding to GPLM by TXB_2 and other prostanoids. It has been proposed that the TXA_2/PGH_2 receptor mediates the contractile responses to PGD_2, PGE_2, and $PGF_{2\alpha}$ in guinea pig lung parenchyma (14, 28, 29) and to PGD_2, $PGF_{2\alpha}$, and the PGD_2 metabolite 9α,11β-PGF_2 in guinea pig trachea (30, 31). We thus examined the ability of these prostanoids, as well as that of the inactive TXA_2 metabolite TXB_2, to inhibit binding of [^{125}I]IBOP to GPLM. Fig. 8 shows the displacement curves for those compounds and SQ29548, which was included as a positive control. All of the compounds tested inhibited [^{125}I]IBOP binding to GPLM with the rank order of potency (K_i values given in parentheses, three experiments) PGD_2 (687 ± 108 nM) = 9α,11β-PGF_2 (779 ± 129 nM) > $PGF_{2\alpha}$ (1.39 ± 0.09 μM) > PGE_2 (4.00 ± 0.67 μM) > TXB_2 (>10 μM). In these assays, SQ29548 had a K_i of 8.0 ± 1.0 nM. Comparison of these values with those in the literature for contractile responses in guinea pig lung parenchyma or trachea is difficult, because there is no report of the responses for all of the prostanoids in a single type of tissue. With this caveat in mind, however, the values for inhibition of [^{125}I]IBOP binding to GPLM do compare favorably with EC_{50} values for PGD_2, $PGF_{2\alpha}$, and 9α,11β-PGF_2 in guinea pig trachea (30, 31) and for PGE_2 and $PGF_{2\alpha}$ in guinea pig lung strip (28, 29).

Discussion

This report describes the first analysis of TXA_2/PGH_2 receptors from lung tissue using radioligand binding studies. The TXA_2 analog IBOP has been previously characterized as a TXA_2/PGH_2 receptor agonist in washed human platelets (22), where the utility of [^{125}I]IBOP for radioligand binding studies was also described. IBOP induces a concentration-dependent contraction of guinea pig lung parenchymal strips, with an EC_{50} value of 3.03 nM. The inhibition of the contractile response to IBOP by the specific TXA_2/PGH_2 antagonists SQ29548, BM13505, and I-PTA-OH indicates that the pharmacologic activity of IBOP in the lung is due to interaction with a $TXA_2/$

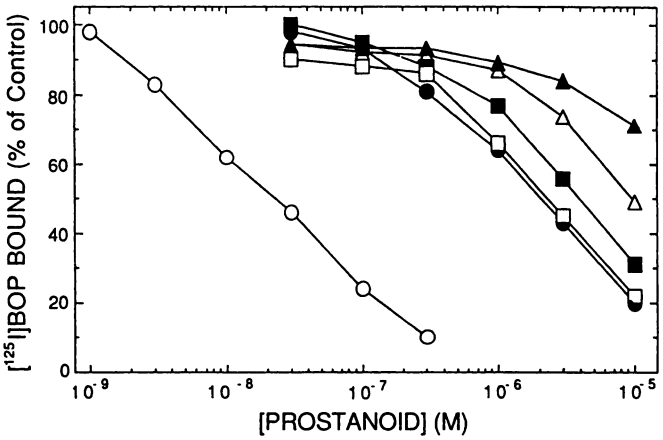

Fig. 8. Inhibition of binding of [^{125}I]IBOP to GPLM by TXB_2 and various prostanoids. GPLM were incubated for 120 min at 30° with [^{125}I]IBOP (≈100 pM) and vehicle (control) or various concentrations of 9α,11β-PGF_2 (□), PGD_2 (●), $PGF_{2\alpha}$ (■), PGE_2 (△), or TXB_2 (▲). SQ29548 (○) was also run as a positive control. Data are expressed as percentage of control binding and are plotted as mean of three experiments, each performed in triplicate. Standard errors are omitted for clarity but were typically less than 5% of the mean.

PGH_2 receptor. Although IBOP and the antagonists were all less potent in the contraction assay than in the binding assay, the rank order of potency for the antagonists as inhibitors of the contractile response to IBOP (SQ29548 > BM13505 > I-PTA-OH) is the same as that found for inhibition of [^{125}I]IBOP binding to GPLM, consistent with the binding site for [^{125}I] IBOP representing the lung TXA_2/PGH_2 receptor. The decrease in potency observed in the contraction assay may be related to the differences in assay conditions, tissue binding, and distribution of ligand in the parenchymal strips or other factors. Nevertheless, the similar relative potencies of the compounds in the two assays are indicative of the same site of action. The potency of IBOP in guinea pig lung contraction is somewhat greater than that found for human platelet aggregation (22) and guinea pig platelet aggregation.[1]

Radioligand binding studies suggest that [^{125}I]IBOP interacts with a single class of high affinity binding sites on GPLM. From a rigorous determination of the rate of formation of the IBOP-binding site complex, it was apparent that the data were best fit by a one-component model, with a K_d of 180 pM. Likewise, analysis of saturation isotherms and competition curves with unlabeled IBOP indicated only a single class of high affinity binding sites, with K_d values of 87 and 189 pM, respectively. Dissociation time course experiments, however, yielded data suggestive of multiple components of binding, with dissociation rate constants of 0.1137 min^{-1} (30% of sites) and 0.008 min^{-1} (70% of sites). Computation of affinities for these sites, using a k_1 of 2.49×10^8 M^{-1} min^{-1}, gives K_d values of 456 and 32 pM, respectively. The appearance of the low affinity site may be an artifact of the extended time of the incubation (6 hr for the entire experiment) and is not supported by equilibrium binding studies conducted for shorter times, in which a site with that affinity would surely have been detected.

The effects of the disulfide-reducing agents dithiothreitol and β-mercaptoethanol and the sulfhydryl-alkylating agent *N*-ethylmaleimide suggest that disulfide bonds may play an important role in maintenance of the active site and that a free sulfhydryl group may be involved in binding. The lack of any inhibition of [^{125}I]IBOP binding by guanine nucleotides suggests that G proteins do not modulate binding of IBOP to GPLM. This result is surprising, given that the TXA_2/PGH_2 receptor in human platelets seems to be coupled to a G protein, based on observations that TXA_2/PGH_2 agonists stimulate a high affinity GTPase activity (32–34) and that the G protein inhibitor GDPβS blocked responses to U46619 (35). The lack of inhibition by guanine nucleotides of [^{125}I]IBOP binding to GPLM may be due to an uncoupling of the receptor from its G protein during preparation of the membranes or may be an artifact of the assay conditions. This seems unlikely, because [^{3}H]LTD_4 binding to GPLM is sensitive to guanine nucleotides in membranes prepared in a similar manner and assayed under similar conditions (36), and in these membranes under the same conditions guanine nucleotides inhibit the binding of [^{3}H] LTB_4 to its binding site.[2] Clearly, further efforts are needed to determine the role of G proteins in regulation of lung TXA_2/PGH_2 receptors.

The affinity of IBOP for the lung TXA_2/PGH_2 receptor (K_d = 87 pM) is greater than that found for washed human platelets (K_d = 2.2 nM) (22) and washed guinea pig platelets (K_d = 7.9 nM).[1] Additionally, there is a large discrepancy between the potency of I-PTA-OH at the guinea pig lung (K_i = 199.3 nM) and platelet (K_i = 14.5 nM) TXA_2/PGH_2 receptors (37), suggesting that there may be a difference between platelet and lung TXA_2/PGH_2 receptors. Further evidence for differences in lung and platelet TXA_2/PGH_2 receptors is given by the observation that PGE_2, PGD_2, 9α,11β-PGF_2, and $PGF_{2\alpha}$ inhibit [^{125}I]IBOP binding to GPLM with potencies similar to those found for their stimulation of trachea or parenchyma contractile responses. These data are consistent with the hypothesis that the TXA_2/PGH_2 receptor in guinea pig lung parenchyma mediates the contractile responses to these prostanoids (14, 28, 29, 31), whereas it appears that, in human platelets at least, PGD_2 and $PGF_{2\alpha}$ interact with receptors other than the TXA_2/PGH_2 receptor (38, 39). Furthermore, in human platelet membranes, IC_{50} values for PGD_2 and $PGF_{2\alpha}$ were greater than 100 μM (40), whereas their K_i values in GPLM were 687 nM and 1.39 μM, respectively. Comparison of affinities for a number of structurally dissimilar TXA_2/PGH_2 receptor ligands at guinea pig lung and platelet receptors (Table 2) reveals a statistically ($p > 0.05$) significant difference between the rank orders of potency in the two tissues. This suggests that the receptors may indeed be different in lung and platelets. It should be noted, however, that the receptor preparations were different (membranes for lung and intact cells for platelet) and that some of the platelet data were obtained using the antagonist [^{125}I]I-PTA-OH as the radioligand. Although these data suggest that the platelet and lung TXA_2/PGH_2 receptors are different, it is clear that further experimentation is needed to evaluate these putative differences and to determine whether the lung TXA_2/PGH_2 receptor is distinct from that described for vascular smooth muscle.

In summary, this report represents the first characterization of lung TXA_2/PGH_2 receptors using radioligand binding studies and describes the utility of [^{125}I]IBOP for such studies. The preparation of lung membranes with a binding site for TXA_2/PGH_2 agonists and antagonists provides the opportunity for further analysis of lung TXA_2/PGH_2 receptors.

[1] D. E. Mais, unpublished observations.
[2] D. L. Saussy, Jr., unpublished observations.

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