Characterization of the Vascular Thromboxane A₂/Prostaglandin Endoperoxide Receptor in Rabbit Aorta
Regulation by Dexamethasone

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Recently, we have shown that dexamethasone treatment of rabbits specifically reduces vascular smooth muscle responsiveness to agonists that interact with the vascular thromboxane A₂/prostaglandin H₂ (TXA₂/PGH₂) receptor. One potential site at which dexamethasone can influence prostanoid-mediated vasoconstriction may be at the level of the vascular TXA₂/PGH₂ receptor. Therefore, we characterized the vascular TXA₂/PGH₂ receptor in rabbit aortic membranes and examined the influence of dexamethasone treatment on vascular TXA₂/PGH₂ receptor affinity and number. The binding of \(^{125}\)I\([15S-(1α,2βSZ),3α(1E,3R)4α]^{-}7-[3-(3-hydroxy-4-(p-i odophen oxy)-1-butenyl)-7-oxabicyclo[2.2.1]heptan-2-yl]-5-heptanoic acid ([\(^{125}\)I] BOP), a potent TXA₂/PGH₂ receptor agonist, to rabbit aortic membranes was saturable, displaceable, and dependent on protein concentration. Scatchard analysis of equilibrium binding data disclosed one class of high affinity binding sites with a \(K_d\) of 0.44±0.13 nM and a \(B_{max}\) of 114.4±5.2 fmol/mg protein (n=7). Removal of the endothelium before membrane preparation did not significantly alter the affinity or number of binding sites for \([^{125}\text{I}]\) BOP. Kinetic analysis of the rates of \([^{125}\text{I}]\) BOP association/dissociation yielded a \(K_d\) of 0.62 nM. The ability of various agonists at the contractile potencies in rabbit aortic rings. Moreover, stereospecific displacement of \([^{125}\text{I}]\) BOP binding in aortic membranes and inhibition of U46619-mediated aortic contractions were obtained with the stereoisomers L657925\((-\) and L657926\(\) ). Collectively, these data suggest that this binding site represents the functionally relevant vascular TXA₂/PGH₂ receptor. In functional experiments, \([^{125}\text{I}]\) BOP induced concentration-dependent contractions of the rabbit aorta, which were reduced by 52% in vessels from dexamethasone-treated rabbits. Binding experiments performed in aortic membranes from dexamethasone-treated rabbits revealed a 25% reduction in vascular TXA₂/PGH₂ receptor number with no change in affinity. Thus, the dexamethasone-induced decrease in TXA₂/PGH₂ receptor number in aortic membranes from dexamethasone-treated rabbits may contribute to the accompanying decrease in vascular responsiveness to TXA₂/PGH₂ receptor agonists. (Circulation Research 1990; 67:1562-1569)

Thromboxane (TXA₂), prostaglandin endoperoxide H₂ (PGH₂), and synthetic TXA₂/PGH₂ mimetics such as 15S-hydroxy-11α, 9α[epoxymethano]prosta-5Z-dienoic acid (U46619) contract vascular smooth muscle via interaction with a common vascular receptor.\(^{1-4}\) Pharmacological studies comparing data on vascular TXA₂/PGH₂ receptors in human and canine saphenous veins and in rabbit aortas suggest the presence of a homogenous population of vascular receptors, which may be different from the TXA₂/PGH₂ receptor found in platelets.\(^{5-7}\) Information on the functional and biochemical characterization of the vascular TXA₂/PGH₂ receptor is limited,\(^{8-10}\) and there are no reports addressing the regulatory mechanisms that may influence its function.

Recently, we found that treatment of rabbits with dexamethasone attenuates specifically contractions of aortic and carotid arterial rings elicited by U46619 without affecting the constrictor responses to potassium chloride, phenylephrine, histamine, and endothelin.\(^{11}\)
One potential site at which dexamethasone can interfere with prostanooid-induced vasoconstriction may be at the level of the vascular TXA \textsubscript{2}/PGH \textsubscript{2} receptor(s). Therefore, the purpose of the present study is to characterize the vascular TXA \textsubscript{2}/PGH \textsubscript{2} receptor in rabbit aortic membranes by using the newly synthesized ligand [\textit{S}-\textit{[1\alpha,2\beta(5Z),3\alpha(1E,3R),4\alpha]-7-[3-(3-hydroxy-4-(p-iódophenoy)-5-heptanoic acid ([\textit{125I}]BOP), a potent TXA \textsubscript{2}/PGH \textsubscript{2} receptor agonist,\textsuperscript{12} and to examine the influence of dexamethasone on vascular TXA \textsubscript{2}/PGH \textsubscript{2} receptor affinity and number.

Materials and Methods

Materials

Optically active \textsuperscript{[\textit{125I}]BOP and \textsuperscript{[\textit{127I}]BOP were synthesized by Morinelli et al.\textsuperscript{12} Prostaglandin F\textsubscript{2\alpha} (PGF\textsubscript{2\alpha}), thromboxane B\textsubscript{2}, and U46619 were purchased from Cayman Chemical Co., Ann Arbor, Mich. 9,11-Epiphtio-11,12-methano-TXA\textsubscript{2} (ONO 11113) was a gift from ONO Pharmaceutical Co., Osaka, Japan. [\textit{S}]-[1\alpha,2\beta(5Z),3\beta,4\alpha]-7-[3-[2-[(Phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]heptan-2-yl]-5-heptanoic acid (SQ29548) was a gift from Dr. Martin Ogletree of the Squibb Institute for Medical Research, Princeton, N.J. L657,925(\textit{–}) and L657,926(+)\textsuperscript{12} were gifts from Dr. John Gillard of Merck-Frost Canada Inc., Point Claire-Dorval, Quebec, Canada. CGS 13080 was provided by CIBA-GEIGY Corp., Summit, N.J.

Animals

Male New Zealand White rabbits (2.0–2.5 kg) were treated for 6 consecutive days with daily subcutaneous injections of dexamethasone 21-acetate (2.5 mg/kg) suspended in sesame oil. Control rabbits received sesame oil only (0.5 ml) during the treatment period. On day 6, control and dexamethasone-treated rabbits were anesthetized by an intramuscular injection of ketamine HCl (Ketaset, 50 mg/kg, Aveco Co., Fort Dodge, Iowa) and xylazine (Rompun, 8 mg/kg, Mobay Co., Shawnee, Kan.), and the thoracic aortas were excised. For characterization of the vascular TXA\textsubscript{2}/PGH\textsubscript{2} receptor, only aortas from control rabbits were used.

Preparation of Vascular Membranes

After excision, thoracic aortas were rapidly placed into ice-cold phosphate-buffered saline (10 mM phosphate buffer with 140 mM NaCl, pH 7.4) containing indomethacin (10 \mu M) to inhibit endogenous prostaglandin synthesis, and the periadventitial fat was removed. In some experiments, the vascular endothelium was disrupted by rubbing the intimal surface of the vessel with a cotton-tipped swab. Aortas were blotted dry, immediately frozen in liquid nitrogen, and stored at −70°C until use. Routinely, 10–20 aortas were pooled for each membrane preparation. For preparation of aortic membranes, frozen tissues were pulverized and placed into 25 mM Tris buffer (1 g/10 ml, pH 7.4) containing sucrose (0.25 M), indomethacin (10 \mu M), EDTA (1 mM), phenylmethylsulfonylfluoride (1 mM), soybean trypsin inhibitor (10 \mu g/ml), leupeptin (1 \mu g/ml), and pepstatin (1 \mu g/ml). After homogenization with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.) (three bursts at setting 5, each burst for 20 seconds), the aortic homogenate was centrifuged at 1,000g for 15 minutes, and the resulting supernatant was subsequently centrifuged at 10,000g for 15 minutes. Vascular membranes were obtained by centrifugation of the 10,000g supernatant at 100,000g for 90 minutes. The membrane pellet was resuspended in 25 mM HEPES buffer (pH 7.4) containing indomethacin (10 \mu M) and was frozen in liquid nitrogen and stored at −70°C until use. The specific activity of the plasma membrane marker enzyme, 5-nucleotidase, measured in the 100,000g pellet was similarly increased in aortic preparations from vehicle-treated (27.7-fold) and dexamethasone-treated (23.9-fold) rabbits.

Protein concentrations were determined by the method of Bradford,\textsuperscript{14} using bovine serum albumin as a protein standard.

Radioligand Binding of \textsuperscript{[\textit{125I}]BOP to Rabbit Aortic Membranes

Membranes were thawed and gently homogenized with a glass–glass Dounce homogenizer at 4°C. Equilibrium binding studies were performed by incubating membrane protein (50 \mu g) suspended in 25 mM HEPES buffer (pH 7.4) containing 10 mM CaCl\textsubscript{2}, 20 mM MgCl\textsubscript{2}, 3 \mu M indomethacin, and approximately 20,000 cpm of 25 pM \textsuperscript{[\textit{125I}]BOP in the presence of increasing concentrations of \textsuperscript{[\textit{127I}]BOP (from 0.01 to 500 nM) or other displacing ligands. Incubations were carried out for 30 minutes at 30°C in a final volume of 0.2 ml. Nonspecific binding was determined in the presence of 50 \mu M SQ29548, the thromboxane receptor antagonist.\textsuperscript{1} The reaction was quenched by the addition of 4 ml ice-cold 50 mM Tris-HCl (pH 7.4), followed by rapid vacuum filtration of the sample through filters (model GF/C, Whatman Inc., Clifton, N.J.). The filters were washed three additional times, each time with 4 ml buffer, and bound radioactivity was counted in a gamma counter (75% efficiency for \textsuperscript{125I}). Specific binding was 75–80% of the total binding, and in all experiments, less than 10% of the radioligand was membrane bound. In competition binding experiments, the data were analyzed for one and two binding sites using the computer program LIGAND.\textsuperscript{15} In displacement studies, the concentration of ligand that displaces 50% of specifically bound \textsuperscript{[\textit{127I}]BOP (IC\textsubscript{50}) for each displacing ligand was calculated from linear regression analysis of log-logit transformations of the displacement data.

Determination of the Kinetic Rate Constants of \textsuperscript{[\textit{125I}]BOP Binding

The kinetic rate constants of \textsuperscript{[\textit{125I}]BOP binding to aortic membranes were determined at 22°C rather than at 30°C, the temperature selected for equilibrium binding studies, because the measurement of...
initial rates of association/dissociation of [125I]BOP to and from aortic membranes was more accurate at the lower temperature.

To determine the rate of association of [125I]BOP, aortic membrane protein (50 μg) suspended in 25 mM HEPES buffer (pH 7.4) containing 10 mM CaCl2, 20 mM MgCl2, 0.1 nM [125I]BOP, and approximately 35,000 cpm (43 pM) of [125I]BOP was incubated for 15 seconds to 50 minutes at 22° C in a final volume of 0.2 ml. Specific binding was determined as the difference between the amount of [125I]BOP bound in the absence and in the presence of SQ29548 (50 μM). At various time points, the samples were filtered as previously described. The specific binding of [125I]BOP versus time of incubation was plotted, the data were linearly transformed, and the pseudo-first-order rate constant (Ko) was determined from the slope of the regression line.

To determine the rate of dissociation of [125I]BOP from its membrane binding site, samples were incubated as described above for 60 minutes, [125I]BOP (500 nM final concentration) was added, and the amount of specifically bound [125I]BOP was measured at various time points. The data were transformed to a linear plot, and the first-order rate constant for dissociation (k1) was determined from the slope of the regression line. The association rate constant (k2) was determined from the equation: k2 = (Ko - k1)/[L], where L is the concentration of ligand; the kinetically determined Ko is calculated from the equation: Ko = k2/k1.

Isometric Tension Measurements in Rabbit Thoracic Aortic Rings

Rabbit thoracic aortas were excised and placed into cold Krebs' bicarbonate buffer (pH 7.4), the periadventitial fat was removed, and the vessels were cut into 2–3-mm-wide rings. A maximum of four aortic rings from each control and dexamethasone-treated rabbit was studied. The composition of the Krebs' bicarbonate solution was (mM) NaCl 118.5, KCl 4.7, CaCl2 2.5, KH2PO4 1.2, MgSO4 · 7H2O 1.1, NaHCO3 25.0, and dextrose 5.6. Arterial rings were mounted in 5-ml water-jacketed organ baths containing Krebs' bicarbonate buffer maintained at 37°C and continuously gassed with 95% O2, 5% CO2. The vascular rings were equilibrated under 2 g of resting tension for 1.5–2.0 hours. The buffer was changed at 15-minute intervals, and basal tension was adjusted as required during the equilibration period. Two grams of basal tone were optimal for concentration–response curves to agonists in aortic rings. Changes in tension were measured using force transducers (model FT03c, Grass Instruments, Quincy, Mass.) coupled to a Grass polygraph (model RPS 7C8A).

In experiments using aortic rings from control rabbits to examine the contractile responses to [127I]BOP and ONO 11113, cumulative dose–response curves were performed, and EC50 values were calculated. In a separate set of experiments, dose–response curves to [125I]BOP were performed simultaneously in aortic rings from control and dexamethasone-treated rabbits. In some experiments, the potency of TXA2/PGH2 receptor antagonists [SQ29548, L657,925(–), or L657,926(+)] was determined by the method of Arunlakshana and Schild.10 Aortic rings were mounted as previously described, and cumulative dose–response curves to [127I]BOP and U46619 were generated. After maximal contractile responses were obtained, tissues were washed with Kreb's bicarbonate buffer until tension returned back to baseline values. Aortic rings were then incubated with a TXA2/PGH2 receptor antagonist (four different concentrations spanning a 20-fold range) for 15 minutes, and cumulative dose–response curves to [127I]BOP and U46619 were repeated. EC50 values in the absence and in the presence of the antagonists were calculated, and dose ratios (DRs) were obtained. From plots of the log(DR–1) versus the negative log molar concentration of the receptor antagonist added, pA2 values, slopes of the Schild plots, and drug-receptor dissociation constants (Kd) were calculated. In preliminary experiments, SQ29548 antagonized both [127I]BOP- and U46619-induced contractions to the same degree (pA2 = 7.78±0.09, slope = 0.82±0.06, and Kd = 23.6±0.83 nM for [127I]BOP [n=3] versus pA2 = 8.10±0.12, slope = 0.96±0.07, and Kd = 11.0±0.80 nM for U46619 [n=4]); therefore, due to limited quantities of [127I]BOP, U46619 was used as the agonist for Schild analysis comparing the potencies of L657,925(–) and L657,926(+).

Statistical Analysis

Results are expressed as mean±SEM. In most experiments, data were analyzed by unpaired Student’s t test. Pearson’s correlation coefficient was used to analyze the data in Tables 1 and 2. In contraction studies, the data were analyzed for significance by a two-way analysis of variance. If differences were noted, pairwise comparisons were made with a Duncan’s multiple-range test. The null hypothesis was rejected if p<0.05.

Results

Characterization of the Binding of [125I]BOP to Rabbit Aortic Membranes

The binding of [125I]BOP to rabbit aortic membranes was saturable, displaceable, and dependent on protein concentration (10–100 μg protein, data not shown). Figure 1 shows the Scatchard plot of the total displaceable binding of [125I]BOP with increasing concentrations of [127I]BOP in rabbit aortic membranes. After correction for nonspecific binding, Scatchard analysis (inset, Figure 1) of the equilibrium specific binding data (n=7) disclosed a single class of high affinity binding sites with a Kd of 0.44±0.13 nM and a receptor density (Bmax) of 114.4±5.2 fmol/mg protein. Removal of the vascular endothelium by mechanical denudation before aortic membrane preparation did not significantly alter the affinity or number of [125I]BOP binding sites (Kd=...
To further characterize the vascular [125I]BOP binding site, a kinetic analysis of association and dissociation of [125I]BOP was performed in aortic membranes. The time-dependent association (Figure 2) of [125I]BOP specifically bound to aortic membranes was rapid in onset and saturable after 15 minutes of incubation at 22°C. After linear transformation of the data, the pseudo-first-order rate constant, $K_{obs}$, was 0.185 min$^{-1}$. To determine the rate of [125I]BOP dissociation, membranes were incubated until equilibrium was attained. At equilibrium, the addition of excess

0.31±0.15 nM, $B_{max}=105.0±4.5$ fmol/mg protein, $n=3$ experiments in duplicate).

To ascertain the specificity of [125I]BOP binding sites in rabbit aortic membranes, the displacement of [125I]BOP by agonists and antagonists that are known to interact with the vascular TXA$_2$/PGH$_2$ receptor was assessed. Figure 4 depicts the displacement of [125I]BOP by various TXA$_2$/PGH$_2$ analogues ([125I]BOP, ONO 11113, and U46619), PGF$_{2\alpha}$, TxB$_2$, and the thromboxane synthetase inhibitor CGS 13080. The specific binding of [125I]BOP was completely displaced in a concentration-dependent manner by [125I]BOP, ONO 11113, U46619, and PGF$_{2\alpha}$. In contrast with the effectiveness of the TXA$_2$/PGH$_2$ anal-

![Figure 1](image1.png)

**Figure 1.** Scatchard analysis of equilibrium binding data examining [125I]BOP binding to rabbit aortic membranes. B, [125I]BOP bound to membranes; B/F, ratio of bound to free [125I]BOP. Data are means from duplicate determinations in seven different membrane preparations. The standard error of the mean was less than 15% of mean values. The Scatchard plot of total binding data was not corrected for nonspecific binding. The inset shows the Scatchard plot of specific binding data (corrected for nonspecific binding); analysis using the computer program LIGAND revealed a single class of high affinity binding sites.

![Figure 2](image2.png)

**Figure 2.** Plot showing time course of [125I]BOP association to rabbit aortic membranes. B, specific binding of [125I]BOP; $B_{eq}$, specific binding of [125I]BOP at equilibrium. The data represent duplicate determinations. The inset shows the pseudo-first-order rate plot of the same data.

![Figure 3](image3.png)

**Figure 3.** Plot showing time course of [125I]BOP dissociation from rabbit aortic membranes. The data represent duplicate determinations. $B_t$, specific binding of [125I]BOP at time $t$; $B_0$, specific binding of [125I]BOP at time 0. At time 0, [125I]BOP (500 nM) was added to initiate dissociation of [125I]BOP from the membranes. The inset shows the linear transformation of the same data.

[125I]BOP (500 nM) initiated the rapid dissociation of specifically bound [125I]BOP from its membrane binding site, with a half-time of dissociation of 6.5 minutes (Figure 3). After linear transformation of the data, the first-order rate constant for dissociation, $K_{off}$, was 0.248 min$^{-1}$. By using the $k_{on}$ and $k_{off}$ values, the true first-order association constant, $K_a$, was calculated (see "Materials and Methods") to be 0.154 min$^{-1}$, and the kinetically determined $K_a$ was 0.620 nM.

To ascertain the specificity of [125I]BOP binding sites in rabbit aortic membranes, the displacement of [125I]BOP by agonists and antagonists that are known to interact with the vascular TXA$_2$/PGH$_2$ receptor was assessed. Figure 4 depicts the displacement of [125I]BOP by various TXA$_2$/PGH$_2$ analogues ([125I]BOP, ONO 11113, and U46619), PGF$_{2\alpha}$, TxB$_2$, and the thromboxane synthetase inhibitor CGS 13080. The specific binding of [125I]BOP was completely displaced in a concentration-dependent manner by [125I]BOP, ONO 11113, U46619, and PGF$_{2\alpha}$. In contrast with the effectiveness of the TXA$_2$/PGH$_2$ ana-
Table 1. Comparison of Competition Binding and Aortic Contractions to Various Agonists That Interact With the Vascular Thromboxane A2/Prostaglandin Endoperoxide H2 Receptor

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Binding IC50 (nM)</th>
<th>Contraction EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[125I]BOP</td>
<td>0.44±0.13 (7)</td>
<td>1.99±0.11 (17)</td>
</tr>
<tr>
<td>ONO 11113</td>
<td>0.74±0.04 (3)</td>
<td>2.97±0.27 (11)</td>
</tr>
<tr>
<td>U46619</td>
<td>5.88±1.51 (3)</td>
<td>10.30*</td>
</tr>
<tr>
<td>Prostaglandin F2a</td>
<td>2.990±350 (3)</td>
<td>2.120*</td>
</tr>
</tbody>
</table>

Values are mean±SEM; the number of experiments in aortic membranes or aortic rings is in parentheses. IC50 concentration of ligand yielding 50% reduction in displaceable binding of [125I]BOP in rabbit aortic membranes; EC50 concentration of agonist yielding 50% of the maximal response in rabbit aortic rings. IC50 and EC50 values were obtained from the experimental data as described in "Materials and Methods.

*See Reference 11.

logues in displacing [125I]BOP, TXB2, and CGS 13080 did not significantly displace the radioligand. The rank order potency of agonist competition with [125I]BOP was [125I]BOP>ONO 11113>U46619>PGF2α. Comparison of IC50 values from competition studies to EC50 values generated in contraction studies (Table 1) reveals the same rank order potency for TXA2/PGH2 receptor agonists in both assays (r=0.75, p<0.0005).

Figure 5 demonstrates the ability of thromboxane receptor antagonists to compete with [125I]BOP for its membrane binding site. All three receptor antagonists displaced [125I]BOP to the level of nonspecific binding, with SQ29548 being the most potent of the antagonists tested (Table 2). L657,925(−) and L657,926(+) stereocemically antagonized [125I]BOP from interacting with its binding site; L657,925(−) was 37 times more potent than the dextrorotary isomer. Comparison of the IC50 values from displace ment studies to Kd values generated from Schild plots for antagonism of U46619-induced contractions of the rabbit aorta (Table 2) reveals the same rank order of potency (r=0.85, p<0.002). In addition, L657,925(−) and L657,926(+) stereoselectively displaced [125I]BOP binding and antagonized U46619-mediated contractions in a qualitatively similar fashion, with a 37-fold difference in binding studies versus a 20-fold difference in contraction studies.

![Figure 5](image-url) Figure 5. Plot showing thromboxane A2/prostaglandin H2 receptor antagonist displacement of [125I]BOP from rabbit aortic membranes. Results are expressed as means of three or four experiments, each performed in duplicate.

Table 2. Comparison of Displacement of [125I]BOP-Induced and Inhibition of U46619-Induced Aortic Contractions by Various Thromboxane A2/Prostaglandin H2 Receptor Antagonists

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Binding IC50 (nM)</th>
<th>Contraction Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQ29548</td>
<td>380±21.0 (4)</td>
<td>11±4.8 (4)</td>
</tr>
<tr>
<td>L657,925(−)</td>
<td>446±23.0 (3)</td>
<td>59±13.0 (4)</td>
</tr>
<tr>
<td>L657,926(+)</td>
<td>17,530±3,100.0 (3)</td>
<td>1,200±260.0 (4)</td>
</tr>
</tbody>
</table>

Values are mean±SEM; the number of experiments in aortic membranes or aortic rings is in parentheses. IC50 concentration of ligand yielding 50% reduction in the displaceable binding of [125I]BOP in rabbit aortic membranes; Kd equilibrium dissociation constant for the antagonists derived from pA2 values. IC50 and Kd values were obtained from the experimental data as described in "Materials and Methods."

[125I]BOP-Induced Contractions of Aortic Rings From Vehicle- and Dexamethasone-Treated Rabbits

Figure 6 depicts [125I]BOP-induced isometric tension development in aortic rings from rabbits treated with vehicle or dexamethasone for 6 days. [125I]BOP induced concentration-dependent contractions of the rabbit aorta, with EC50 values of 2.0±0.4 and 1.5±0.3 nM in vessels from vehicle-treated (n=8) and dexamethasone-treated (n=8) rabbits, respectively. Contrasting with the ability of [125I]BOP to evoke equipotent contractions in aortic rings from vehicle- and dexamethasone-treated rabbits, [125I]BOP was less efficacious in eliciting maximal contractile responses in aortic rings from dexamethasone-treated rabbits. [125I]BOP-induced tension development in aortic rings from dexamethasone-treated rabbits achieved 48% of the maximal response obtained with the contractile agonist in vessels from vehicle-treated rabbits, with maximal responses to [125I]BOP of 3.5±0.7 and 1.7±0.3 g of tension developed in aortic rings from vehicle- and dexamethasone-treated rabbits, respectively.

[125I]BOP Binding in Aortic Membranes Prepared From Vehicle and Dexamethasone-Treated Rabbits

The time- and protein-dependent bindings of [125I]BOP were qualitatively similar in aortic mem-
branes from control and dexamethasone-treated rabbits (data not shown). Scatchard analysis of equilibrium binding studies (Figure 7) in membranes from control and dexamethasone-treated rabbits revealed no significant change in the affinity of the receptor for the ligand \( K_a = 0.43 \pm 0.11 \) and \( 0.28 \pm 0.03 \) for control and dexamethasone-treated rabbits, respectively, \( n = 5 \) different membrane preparations) with a significant reduction in \( B_{\text{max}} \) from 114.0 \( \pm \) 7.1 fmol/mg protein in membranes from vehicle-treated rabbits to 84.8 \( \pm \) 6.7 fmol/mg protein in membranes from dexamethasone-treated rabbits \( p < 0.05 \), Figure 8).

**Discussion**

This study demonstrates the presence of a single class of high affinity, low capacity binding sites in rabbit aortic membranes for \([^{125}\text{I}]\text{BOP} \), an agonist for the TXA\(_2\)/PGH\(_2\) receptor.\(^{12}\) Previous studies\(^{8-10}\) have demonstrated specific binding of \([^{3}\text{H}]\text{U46619} \) and \([^{3}\text{H}]\text{SQ29548} \) in porcine aortic membranes and of \([^{3}\text{H}]\text{SQ29548} \) in cultured rat aortic smooth muscle cells. In the present study, the density of specific binding sites for \([^{125}\text{I}]\text{BOP} \) in rabbit aortic membranes is comparable with the values reported for \([^{3}\text{H}]\text{U46619} \) and \([^{3}\text{H}]\text{SQ29548} \) in porcine aortic membranes. However, the \( K_a \) for \([^{125}\text{I}]\text{BOP} \) in rabbit aortic membranes is 50 to 100 times lower than the \( K_a \) values for the radiolabeled ligands in porcine membranes.\(^{8,9}\) The low \( K_a \) value for \([^{125}\text{I}]\text{BOP} \) in binding studies is in accord with pharmacological evidence that \([^{125}\text{I}]\text{BOP} \) is 10 times more potent than the TXA\(_2\)/PGH\(_2\) mimetic U46619 in contracting rabbit aortic smooth muscle (Table 1).

The binding of \([^{125}\text{I}]\text{BOP} \) to rabbit aortic membranes fulfills many of the criteria necessary for characterization of a functionally relevant TXA\(_2\)/PGH\(_2\) receptor. These criteria include time- and protein-dependent specific binding, comparable \( K_a \) values in equilibrium and kinetic binding studies, similar rank orders of potency for TXA\(_2\)/PGH\(_2\) receptor agonists and antagonists in both \([^{125}\text{I}]\text{BOP} \) displacement and vascular contraction studies, and, most importantly, stereoselective competition displacement of \([^{125}\text{I}]\text{BOP} \) for binding by the stereoisomeric thromboxane receptor antagonists L657,925(−) and L657,926(+).

In the present study, \( IC_{50} \) values for \([^{125}\text{I}]\text{BOP} \), ONO 11113, U46619, and PGF\(_{2\alpha} \) in displacement studies were well correlated with \( EC_{50} \) values generated with the agonists in contraction studies. Moreover, the selective interaction of \([^{125}\text{I}]\text{BOP} \) with vascular TXA\(_2\)/PGH\(_2\) binding sites is illustrated by the 1,000-fold molar excess of PGF\(_{2\alpha} \) necessary to displace membrane bound \([^{125}\text{I}]\text{BOP} \) and to elicit contractions of rabbit aortic rings compared with the other TXA\(_2\)/PGH\(_2\) mimetics tested. These data support the notion that PGF\(_{2\alpha} \) and U46619 cause contraction of vascular smooth muscle via interaction with a common receptor.\(^{5,17,18}\) However, as seen in Table 1, \( EC_{50} \) values for \([^{125}\text{I}]\text{BOP} \)-induced, ONO 11113−induced, and U46619-induced contractions were greater than the \( IC_{50} \) values generated in \([^{125}\text{I}]\text{BOP} \) competition studies.
with the agonists. Several plausible explanations could account for this discrepancy. First, it is possible that during preparation of aortic membranes we may have removed an inhibitory element that regulates the binding of [I]BOP; however, we have no evidence for such a factor. Second, higher amounts of agonist may be necessary to achieve an appropriate concentration in the organ bath due to the presence of physical sites for nonspecific binding, such as glass walls, platinum hooks, and wire. Third, in organ bath experiments, there is an unstirred water layer surrounding the tissue that may present a diffusional barrier for drug transport to the tissue receptor site. Thus, it is conceivable that higher concentrations of relatively hydrophobic compounds (i.e., [I]BOP, ONO 11113, and U46619) would be necessary to achieve adequate concentrations of the agonists at the receptor site. In support of this concept is the near unity correlation in ligand displacement and contraction studies with the relatively hydrophilic prostanoid PGF$_2\alpha$.

$[125$I]BOP displacement from aortic membranes by TXA$_2$/PGH$_2$ receptor antagonists also correlated well with the ability of the antagonists to interfere with U46619-induced contractions of rabbit aortic rings. Furthermore, L657,925(−) and L657,926(+) stereoselectively inhibited the binding of $[125$I]BOP in membranes and aortic contractions elicited by U46619. However, IC$_{50}$ values in displacement binding experiments were nine to 25 times greater than the $K_B$ values from pharmacological contraction studies. The high IC$_{50}$ values for TXA$_2$/PGH$_2$ receptor antagonist displacement of $[125$I]BOP may be related to the presence of high affinity binding sites for SO29548, which were not expressed under the experimental conditions implemented in our binding studies. Alternatively, it is possible that subtypes of vascular TXA$_2$/PGH$_2$ receptors exist, since the $K_B$ value for SO29548 antagonism of $[125$I]BOP-induced contractions of rabbit aortic rings was twofold greater than the $K_B$ value for SQ29548 antagonism of U46619-induced contractions. Future studies with $[125$I]BOP and TXA$_2$/PGH$_2$ receptor antagonists in vascular smooth muscle may provide explanations for these discrepancies.

Recently, we have reported that dexamethasone treatment of rabbits specifically attenuates aortic, carotid, and renal vascular responses to U46619. The present study demonstrates that dexamethasone also interferes with the expression of $[127$I]BOP-induced contractions of rabbit aortic smooth muscle. The reduced effectiveness of $[127$I]BOP to elicit a maximal contractile response in aortic rings from dexamethasone-treated rabbits was accompanied by a 25% reduction in the density of specific $[125$I]BOP binding sites in rabbit aortic membranes, suggestive of a regulatory influence of dexamethasone on TXA$_2$/PGH$_2$ receptor function. Relative to this point, there are reports that glucocorticoids increase β-adrenergic receptor density in cultured adipocytes and lung cells$^{20,21}$ and inhibit the expression of immunoglobulin E receptors in monocytes and of complement receptors in granulocytes.$^{22,23}$

That dexamethasone treatment of rabbits reduces the density of vascular $[125$I]BOP binding sites and interferes with the magnitude of $[127$I]BOP-induced aortic tension development may tentatively suggest a cause–effect relation between the events. However, since the precise nature of the second messenger system coupled to agonist occupancy of the vascular TXA$_2$/PGH$_2$ receptor is not well defined$^{19,24-26}$ and the “gain” of the pharmacomechanical coupling events of TXA$_2$/PGH$_2$ analogues to vascular smooth muscle contraction is unclear, we cannot rule out the possibility that dexamethasone may also be interfering with postreceptor coupling or signaling events. To this end, dexamethasone has been shown to modulate a diverse range of postreceptor events. This includes increasing mRNA levels for the β-subunit of the G protein, G$_s$, stimulating adenylate cyclase– and cyclic AMP–dependent protein kinase activities,$^{28,29}$ increasing the number of functional sodium–proton antiporters,$^{30}$ inducing or inhibiting the synthesis of specific vascular proteins,$^{31}$ and inhibiting hormone-activated phospholipases.$^{32-34}$

In summary, this study demonstrates the presence of a specific high affinity binding site for [I]BOP in rabbit aortic membranes; this finding fits the biochemical and pharmacological criteria necessary for characterization of the vascular TXA$_2$/PGH$_2$ receptor. Furthermore, the data suggest that the vascular TXA$_2$/PGH$_2$ receptor is subject to inhibitory regulation by the glucocorticoid dexamethasone, a regulatory influence that may contribute to the reduced vascular responsiveness to vasoconstrictor prostanoids in rabbits treated with dexamethasone. Future studies examining the first influence of dexamethasone on TXA$_2$/PGH$_2$ receptor number and postreceptor signaling events will reveal the importance of such a regulatory mechanism in vascular smooth muscle function.

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