Enhancement of the antiaggregatory activity of prostacyclin by propranolol in human platelets

KARLEEN S. CALLAHAN, PH.D., ALICE R. JOHNSON, PH.D., AND WILLIAM B. CAMPBELL, PH.D.

ABSTRACT The β-adrenergic antagonist propranolol has been found to inhibit platelet aggregation. We investigated the possibility that propranolol exerts this action by stimulating the synthesis or enhancing the antiaggregatory activity of prostaglandin (PG) I₂. The media from cultures of human endothelial cells inhibited thrombin-induced platelet aggregation, an effect attributed to PGI₂ production by the cells. When endothelial cells were incubated with dl- or d-propranolol, the media had two to three times the inhibitory activity of control media. However, this increased activity was not due to increased synthesis of PGI₂ because control and propranolol-treated cultures synthesized similar amounts of the PGI₂ metabolite, 6-keto-PGF₁α. Instead, propranolol enhanced the antiaggregatory activity of PGI₂. Propranolol (1 µM) and PGI₂ (0.05 nM), when tested separately, inhibited aggregation by 19% and 13%, respectively, whereas the combination inhibited aggregation by 51%. PGI₂ inhibited platelet aggregation and thromboxane (Tx) B₂ production but stimulated cyclic AMP formation. The adenylyl cyclase inhibitor 2′,5′-dideoxycytidine (DDA) had no effect of its own on these parameters, but blocked the actions of PGI₂. Propranolol inhibited aggregation and TxB₂ synthesis without changing cyclic AMP levels. Unlike PGI₂, propranolol’s effects were not altered by DDA. While the combination of propranolol and PGI₂ inhibited aggregation to a greater extent than either agent alone, this enhanced effect with the combination did not extend to TxB₂ or cyclic AMP production. Propranolol, PGI₂, and the combination inhibited TxB₂ synthesis to a similar extent, and PGI₂ produced a similar increase in cyclic AMP in the presence and absence of propranolol. These findings indicate that propranolol and PGI₂ inhibit platelet aggregation through cyclic AMP-independent and dependent mechanisms, respectively. While propranolol does not alter the synthesis of PGI₂, it enhances the inhibition of aggregation by PGI₂, and this may contribute to its antiplatelet effect.


PROPRANOLOL is widely used in the treatment of hypertension and coronary artery disease. Various studies have indicated that propranolol inhibits platelet activation both in vitro and in vivo. We have found that propranolol inhibits platelet aggregation and decreases thromboxane (Tx) A₂ synthesis in platelet-rich plasma (PRP) obtained from normal subjects as well as in PRP prepared from hypertensive patients treated with this agent. Similar results have been reported with the 4-hydroxy metabolite of propranolol. Prostacyclin (prostaglandin [PG] I₂) is a potent vasodilator and inhibitor of platelet aggregation. The vessel wall appears to be a primary site of PGI₂ synthesis, with the intimal surface generating greater amounts than that formed by cells closer to the adventitia. Numerous investigators have demonstrated that the vascular endothelium synthesizes PGI₂. Since the vascular endothelium is in direct contact with platelets circulating in the blood, the stimulation of PGI₂ synthesis from this tissue by pharmacologic agents could produce an antiplatelet effect. The intent of the current investigation was to determine whether propranolol exerts a portion of its antiplatelet activity by increasing the synthesis or affecting the activity of PGI₂. Consequently, the effect of propranolol on PGI₂ synthesis was studied in cultured human endothelial cells. Additionally, the effects of propranolol on platelet aggregation, platelet cyclic AMP production, and
Tx synthesis were investigated in the presence and absence of PGI₂.

**Methods**

**Materials.** Drugs used and their sources were as follows: dl-propranolol (Ayerst), d-propranolol (Ayerst), thrombin (Parke-Davis), arachidonic acid (Sigma), adenosine-3,5-cyclic monophosphate (Sigma), monosuccinyl adenosine-3',5'-cyclic monophosphoric acid tyrosine methyl ester (Sigma), isobutylmethylxanthine (Aldrich), 2',5'-dideoxyadenosine (P.L. Biochemicals, Inc.)., TXB₂ (Upjohn), PGI₁ (Upjohn), PGE₂ (Upjohn), 3H-6-keto-PGFIₐ (Amersham), 3H-PGF₂α (Amersham), and 3H-TXB₂ (New England Nuclear).

All of the solvents used were of reagent grade and were purified by fractional distillation before use.

**Endothelial cell culture.** Endothelial cells were cultured from vessels of human lungs or umbilical cords as previously described. Briefly, the vessels were perfused with 0.2% collagenase in cell culture medium (medium 199, Gibco), then the ends of the vessels were clamped while the solution remained in the lumen for 15 min at 37°C. The detached cells were rinsed from the vessels with fresh culture medium, washed twice, and resuspended in culture medium supplemented with antibiotics, fetal calf serum, and human serum. The cells were plated in 25 cm² flasks and kept in an incubator at 37°C in an atmosphere of 5% CO₂ and 95% air. Umbilical cord cells were used without passage, but pulmonary cells had to be transferred at least once to obtain sufficient material for experiments.

**Prostacyclin bioassay experiments.** Supernatant media from the cultured endothelial cell monolayer were tested for PGI₂ production by bioassay on platelet aggregation in vitro. The cell monolayers were washed twice in protein-free culture medium and fresh medium with or without propranolol added. The cells were incubated for 15 min at 37°C, and the supernatant was collected into 10 mM Na₂CO₃ (pH 9.2), kept on ice, and tested within 1 hr for effects on platelet aggregation. These precautions were taken to minimize breakdown of PGI₂ into its inactive metabolite, 6-keto-PGF₁α.

**Prostaglandin synthesis from endothelial cultures.** For quantitative determination of 6-keto-PGF₁α and PGE₂, individual flasks of cells were incubated for 15 min at 37°C with medium alone, medium with thrombin (5.0 U/ml), or medium with varying amounts of dl-propranolol. The medium was removed from the cells, acidified with glacial acetic acid to pH 3.0, and extracted with 10× volume of ethyl acetate/cyclohexane. The organic phase was dried under nitrogen. This extract was reconstituted to the original volume in phosphate-buffered saline (pH 7.4) containing polyvinylpyrrolidone (0.1%). The PGs were measured by radioimmunoassay (see below) in duplicate aliquots from each sample.

**Platelet experiments in vitro.** Blood was obtained by venipuncture from normal subjects who had taken no drugs for at least 10 days before the study. The blood was diluted in one-tenth volume of 3.5% sodium citrate, pH 7.4. Platelet rich plasma (PRP) was prepared by centrifuging the blood at 150 g for 10 min. After removal of the PRP, the remaining red cell suspension was centrifuged at 1500 g for 10 min to obtain platelet-poor plasma (PPP). This PPP was used to standardize the aggregometer and to dilute the PRP. The platelet count in the PRP averaged 200 ± 10 x 10⁵ platelets/ml.

The PRP was kept in a tightly closed plastic container at room temperature, and experiments were completed within 3 hr of collection of blood. Platelet aggregation was monitored in vitro by the method of Born using a dual-channel Sieno aggregometer. One milliliter of PRP was incubated at 37°C with constant stirring at 1000 rpm. Propranolol or its vehicle (phosphate-buffered saline) was incubated with the PRP for 2 min before the addition of the aggregating agent. PGI₁ was added 30 sec before the addition of the aggregating agent. Aggregation was stimulated by either thrombin (0.5 U/ml) or arachidonic acid (3 x 10⁻⁸ M). PGI₁ was diluted in 50 mM Tris buffer, pH 9.4, kept on ice, and used within 2 hr of preparation. Arachidonic acid was dissolved in hexane and dried in the cuvette under nitrogen before addition of PRP.

For radioimmunoassay of TXB₂, PRP was sampled before the addition of the aggregating agent and at various times after addition of the aggregating substance. A 50 µl aliquot of PRP was removed from the cuvette, immediately added to 450 µl of ice-cold phosphate-buffered saline or distilled water containing indomethacin (1 µg/ml), and quick-frozen in a methanol–dry ice bath. These samples were kept frozen at −20°C until assay. For measurement of cyclic AMP (see below), samples were obtained by incubating 1.0 ml of PRP containing 100 µM isobutylmethylxanthine with the test drug(s) and the aggregating agent. The reaction was terminated after 1 min by transferring the platelets to a polypropylene tube containing 0.8 ml of 5% trichloracetic acid (TCA) and freezing the samples immediately in liquid nitrogen. The samples were kept frozen at −20°C until assay.

**Radioimmunoassay for prostanooids.** Synthesis of PGs by platelets and endothelial cells was measured by specific radioimmunoassays according to the method of Dray et al. as modified by Campbell et al. Specific antibodies were raised in rabbits against a PG-thyroglobulin complex. The TXB₂ antibody detected amounts as low as 1 pg/0.3 ml and cross-reacted less than 0.001% with PGE₂, PGD₂, PGF₂α, and 6-keto-PGF₁α, and less than 0.003% with PGD₂. The PGE₂ antibody detected 10 pg/0.3 ml and cross-reacted less than 0.7% with PGE₂, PGB₂, PGD₂, 15-keto-PGE₂, 13,14-dihydro-15-keto-PGE₂, PGF₂α, PGF₁α, and 6-keto-PGF₁α. It cross-reacted 14.0% with PGI₁. The 6-keto-PGF₁α antibody was able to detect 5 pg/0.3 ml and cross-reacted 14% with PGF₁α, 2% with PGE₁, and less than 0.6% with other known PGs and 6-keto-PGF₁α metabolites.

The radioimmunoassay consisted of combining 0.1 ml of the diluted sample with 0.1 ml of ³H-PG and 0.1 ml of specific antiserum in a 12 × 75 mm polypropylene culture tube. After overnight incubation at 4°C, the antibody-bound and free PGs were separated with dextran-coated charcoal. The bound radioactivity was counted in liquid scintillation spectrometer.

**Cyclic AMP radioimmunoassay.** Platelet samples were obtained as described above. The frozen samples were thawed on ice and homogenized for 30 sec with a Brinkman Polytron. The protein precipitate was removed by centrifugation at 5000 g for 30 min at 4°C. The supernatants were extracted four times with a 10-fold excess of water-saturated diethyl ether to remove the TCA. Residual ether was removed under a stream of nitrogen for 30 min. An aliquot of the sample was then assayed for cyclic AMP.

Platelet cyclic AMP levels were measured by radioimmunoassay according to the method of Steiner et al. with the acetylation modification of Harper and Brooker. The cyclic AMP antibody was a gift from Dr. Gary Brooker of Georgetown University. ¹²⁵I-cyclic AMP was prepared by the method of Brooker et al.

The cyclic AMP assay consisted of combining 50 µl of acetylated standard or sample, 50 µl of 50 mM sodium acetate buffer, 100 µl of the antibody solution, and 100 µl of ¹²⁵I-cyclic AMP (10,000 cpm) in a 12 × 75 mm glass tube. The antiserum for the cyclic AMP was used in a final dilution of 1:100,000. After an incubation at 4°C for 3 hr or overnight, the antibody-bound and free cyclic AMP were separated by the addition of charcoal. The bound radioactivity was counted with a Packard gamma counter.
scintillation spectrometer. The assay was sensitive to 50 fmol/0.3 ml.

**Statistical analysis.** Tx and cyclic AMP generation in the platelet experiments were evaluated by analysis of variance with the Neuman-Keuls multiple comparisons test. In aggregation experiments, when percent of maximal responses were compared, a Mann-Whitney U test was used since percentages do not follow a normal distribution.

**Results**

The release of PGI₂ from cultured endothelial cells could be shown by the ability of the incubation media to inhibit aggregation in PRP (figure 1). Thrombin was used to stimulate aggregation. The addition of a small volume (10 μl) of the media from vehicle-treated cells resulted in a slight diminution of the aggregatory response. The inhibition of aggregation was increased when a greater volume of media from the control flask was added to the PRP (data not shown). Media not exposed to cells had no inhibitory effect on platelet aggregation (data not shown). Ten microliters of media that contained dl-propranolol (10⁻⁶M), but was not exposed to the cultured cells, caused only a small decrease in the aggregatory response, as shown in the middle tracing in figure 1. In contrast, media from cells incubated with either dl- or d-propranolol (10⁻⁶M) caused marked attenuation of aggregation. When the results from several experiments were combined, propranolol-treated cultures were found to consistently have two to three times more inhibitory activity than medium from vehicle-treated cultures. Thus, propranolol treatment enhanced the antiaggregatory activity of media from endothelial cells, whereas the same concentration of propranolol added alone was not inhibitory. The results suggest that propranolol stimulated release of PGI₂ from endothelial cells.

To further test this possibility, the release of PGI₂ from cultured endothelial cells was measured by radioimmunoassay as its stable metabolite, 6-keto-PGF₁α. Table 1 shows the results obtained in pulmonary endothelial cells. While both arterial and venous cells produced PGI₂, the venous cells synthesized more 6-keto-PGF₁α than did the arterial cells. Propranolol did not change basal synthesis at any dose tested. Umbilical venous endothelium was also tested. The basal and thrombin-stimulated production of 6-keto-PGF₁α and PGE₂ is shown in table 2. The basal levels of 6-
**TABLE 1**

Effect of *dl*-propranolol treatment on 6-keto-PGF$_{1\alpha}$ release from cultured human pulmonary endothelial cells

<table>
<thead>
<tr>
<th></th>
<th>Arterial</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.0 ± 0.4</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td><em>dl</em>-Propranolol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$1 \times 10^{-7}$M</td>
<td>1.8 ± 0.4</td>
<td>5.2 ± 1.1</td>
</tr>
<tr>
<td>$3 \times 10^{-7}$M</td>
<td>1.8 ± 0.3</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>$1 \times 10^{-6}$M</td>
<td>1.9 ± 0.4</td>
<td>5.1 ± 0.5</td>
</tr>
</tbody>
</table>

Data are ng of prostaglandin/10$^6$ cells as measured by radioimmunoassay. The values are mean ± SEM for three experiments.

6-keto-PGF$_{1\alpha}$ were considerably higher than those of PGE$_2$. The synthesis of both PGs was stimulated by thrombin treatment, but PGE$_2$ synthesis was enhanced to a greater extent than 6-keto-PGF$_{1\alpha}$ synthesis. Incubation with varying amounts of *dl*-propranolol for 30 min had no effect on either the basal or thrombin-stimulated generation of either PGE$_2$ or 6-keto-PGF$_{1\alpha}$. Identical results were obtained in endothelial cells incubated for 3 to 5 days with the drug (data not shown).

These results suggest that instead of increasing the production of PGI$_2$, propranolol instead may amplify the inhibitory activity of PGI$_2$. To test this hypothesis, the effects of *dl-* and *d*-propranolol on the antiaggregatory effects of various doses of PGI$_2$ were determined (figure 2). The left portion of the figure shows the dose-related decrement in aggregation caused by PGI$_2$. The threshold response for PGI$_2$ was 0.05 nM. The middle panel illustrates the effect of *dl*-propranolol on the antiaggregatory activity of PGI$_2$. Propranolol (10$^{-6}$M) alone caused a small (19%) inhibitory effect that was similar to that found with the 0.05 nM concentration of PGI$_2$ (13% inhibition). When propranolol was used in combination with the lowest concentration of PGI$_2$, there was a further inhibition of aggregation.

**FIGURE 2.** Inhibitory effect of various concentrations of PGI$_2$ either alone or in combination with *dl-* or *d*-propranolol (10$^{-6}$M) on thrombin-induced platelet aggregation. T = Thrombin.
The inclusion of dl-propranolol enhanced the antiaggregatory effect of PGI₂ at all concentrations tested. This enhanced effect was also observed with d-propranolol, as shown in the far right portion of figure 2. Thus, propranolol had a greater than additive effect in reducing platelet aggregability in vitro when used in combination with PGI₂, and this was most pronounced at low concentrations of PGI₂.

Figure 3 illustrates the effects of propranolol and PGI₂ on TxA₂ generation by platelets. Thrombin stimulated the production of TxB₂, which reached a maximum of 28.3 ng/ml of PRP. Platelets incubated with 1.25 nM PGI₂ produced a markedly decreased amount of TxB₂ (7.8 ng/ml), as did those incubated with 10⁻⁷M dl-propranolol (4.4 ng/ml). The TxB₂ production in the presence of both PGI₂ and propranolol (3.3 ng/ml) did not differ significantly from the levels measured in platelets incubated with either agent alone. However, in examining the aggregatory responses in these experiments, it was apparent that the effects of propranolol and PGI₂ were additive. Propranolol and PGI₂ alone attenuated aggregation by 29% and 56%, respectively, but the combination reduced aggregation by 73%.

The inhibition of platelet aggregation by PGI₂ is thought to be related to increases of cyclic AMP. We observed that the attenuation of TxB₂ formation by PGI₂ also correlated with increases in cyclic AMP (figure 4). There was a slight, but not statistically significant, increase in cyclic AMP levels with 1.25 nM PGI₂ from a basal value of 7.6 pmol/ml PRP to 8.5 pmol/ml PRP. However, at this concentration of PGI₂, there was significant inhibition of aggregation (60%) and TxB₂ synthesis (50%). With 2.5 nM PGI₂, cyclic AMP levels increased to 13.7 pmol/ml (p < .01), and aggregation and TxB₂ formation were reduced by 94% and 88%, respectively. At higher concentrations, PGI₂ stimulated further increments in cyclic AMP synthesis while both aggregation and TxB₂ production were completely inhibited.

2',5'-Dideoxyadenosine (DDA) is a potent inhibitor of platelet adenylate cyclase in both intact cells and membrane preparations. When DDA alone (100 µM) was incubated with PRP, there was no effect on the aggregatory response to either thrombin or arachidonic acid (figures 5 and 6). PGI₂ (2.5 nM) significantly inhibited the synthesis of TxB₂ and aggregation in platelets stimulated with thrombin (figure 5) or arachidonic acid (figure 6). When PRP was preincubated with DDA, the attenuating effects of PGI₂ on both responses were abolished.

To investigate whether cyclic AMP was involved in
FIGURE 4. The effect of various concentrations of PGI₂ on platelet aggregation (top), TxB₂ synthesis (middle), and cyclic AMP generation (bottom) in thrombin-treated platelets. *p < .01.

The inhibitory effect of propranolol, platelets were incubated with DDA and/or propranolol (figure 7). DDA alone had no effect on either response. Propranolol (10⁻⁴M) completely inhibited aggregation and attenuated the generation of TxB₂ by 55% (p < .05).

Contrary to the studies with PGI₂, pretreatment of the platelets with DDA did not alter the inhibitory actions of propranolol on either aggregation or TxB₂ synthesis.

When cyclic AMP levels were measured, PGI₁ (2.5 nM) significantly increased cyclic AMP and significantly inhibited aggregation (table 3). Pretreatment of PRP with DDA blocked the PGI₁-induced increase in cyclic AMP as well as its ability to inhibit platelet aggregation.

dl-Propranolol (10⁻⁴M) did not change the mean cyclic AMP level from the control value (8.6 pmol/ml for control vs 9.5 pmol/ml with propranolol). However, propranolol did significantly inhibit platelet aggregation (53%). When the effect of propranolol was tested over a wide range of concentrations (from 10⁻⁸ to 10⁻⁵M) there was no change from control in the amount of cyclic AMP produced even when aggregation was completely inhibited (data not shown). Finally, the cyclic AMP levels in platelets treated with both dl-propranolol and PGI₂ were significantly increased (16.9 pmol/ml) over control, but were not different from the values obtained with PGI₂ alone. Thus, propranolol alone did not increase the generation of cyclic AMP, nor did it affect PGI₂ stimulation of cyclic AMP synthesis.

Discussion

Propranolol has been found to inhibit platelet aggregation by some unidentified mechanism.¹⁻⁷ Since the vascular endothelium synthesizes PGI₂, a potent endogenous inhibitor of platelet aggregation,⁹ the current investigation was undertaken to examine if propranolol exerts its antiplatelet activity by stimulating the synthesis of PGI₂ by cultured human endothelial cells. Additionally, we investigated the effects of the combination of PGI₂ and propranolol on platelet aggregation and TxB₂ synthesis and the effects of these agents on platelet cyclic AMP accumulation.

The media from cultures of human endothelial cells have been found to inhibit platelet aggregation and to contain PGI₂.¹⁴ ¹⁶ This antiaggregatory activity has been attributed to PGI₂ since this activity is abolished by heating to 100°C, acidification to pH 4, cyclooxygenase inhibitors, or the prostacyclin-synthetase inhibitor tranylcypromine. When endothelial cells were incubated with propranolol, the inhibitory activity of the culture media was greater than that observed in media from untreated cultures. This result suggested that propranolol treatment inhibited aggregation by stimulating the synthesis of PGI₂ in the endothelial cells. However, when PGI₁ synthesis was determined by radioimmunoassay of 6-keto-PGF₁α, propranolol did not change either basal or thrombin-stimulated PGI₁ synthesis over a wide range of concentrations. There was also no effect of propranolol on PGE₂ generation. Thus, the antiaggregatory effects of propranolol did not appear to be due to increased prostanoid synthesis in these cultured cells.

Rigas and Levine²⁴ examined the effect of propranolol on arachidonic acid metabolism by studying various types of cultured cells, but endothelial cells were
FIGURE 5. Effect of PGI₂ and/or DDA on thrombin-induced TxB₂ synthesis (left) and platelet aggregation (right). The concentrations of PGI₂ and DDA were 2.5 nM and 100 μM, respectively. Each value is the mean ± SEM (left) or the mean (right) from three experiments. *p < .01.

FIGURE 6. Effect of PGI₂ and/or DDA on arachidonic acid–induced TxB₂ synthesis (left) and platelet aggregation (right). The concentrations of PGI₂ and DDA were 2.5 nM and 100 μM, respectively. Each value is the mean ± SEM (left) or the mean (right) from six experiments. *p < .01.
not studied. Their results showed that propranolol stimulated PG synthesis (i.e., PGE₂, PGF₂α, and 6-keto-PGF₁α) in cultured cells of dog kidney, rat basophil leukemia, mouse adrenal, and mouse neuroblastoma, but not in bovine aortic smooth muscle or rat glioma cells. Consequently, they suggested that the renin-independent antihypertensive action of propranolol might be due to increased synthesis of PGI₂. The difference in propranolol’s efficacy in stimulating PGI₂ generation in the present study compared with that of Rigas and Levine may be attributable to the different cell types that were examined. Additionally, the concentrations used by Rigas and Levine (2 × 10⁻⁴M) were considerably higher than those used in our study.

If propranolol does not stimulate PGI₂ synthesis, why did the media from propranolol-treated cultures have an enhanced antiaggregatory effect? This question was addressed by examining the interaction of PGI₁ with propranolol on platelet aggregation. When both agents were present, the inhibition of platelet aggregation was greater than that observed with either agent alone. The additivity observed with propranolol and PGI₁ suggests that these agents act by different mechanisms. Consistent with this suggestion were the results obtained in experiments directed toward changes in the platelet cyclic AMP levels. Various studies indicate that inhibition of platelet aggregation by PGI₁ is mediated by increases in intracellular cyclic AMP.²⁴, ²⁵, ²⁷ We have confirmed that PGI₁, in a concentration-related manner, increases cyclic AMP formation, reduces TxB₂ levels, and inhibits aggregation in platelets. However, both aggregation and Tx synthesis were significantly reduced at a concentration of PGI₁ that failed to significantly increase cyclic AMP levels. Similarly, Tateson et al.²⁴ reported that PGI₁ inhibited arachidonic acid–induced aggregation while causing only small increases in the platelet cyclic AMP formation. These findings suggest that the inhibition of platelet aggregation and TxB₂ formation is dependent on cyclic AMP at high concentrations of PGI₁ (>2.5 nM), but is independent of cyclic AMP at low concentrations (<2.5 nM). Alternatively, cyclic AMP may mediate the effects of PGI₁ at all concentrations, but total platelet cyclic AMP content may inadequately access the accumulation of the nucleotide at regulatory

### TABLE 3

**Effect of PGI₁ and/or propranolol on cyclic AMP levels and aggregation in thrombin-treated platelets**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Cyclic AMP (pmol/ml PRP)</th>
<th>Aggregation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>8.6 ± 0.8</td>
<td>100</td>
</tr>
<tr>
<td>DDA (100 µM)</td>
<td>7</td>
<td>8.8 ± 1.2</td>
<td>93.5 ± 2.8</td>
</tr>
<tr>
<td>PGI₁ (2.5 nM)</td>
<td>9</td>
<td>16.6 ± 1.9ₐ</td>
<td>14.5 ± 4.4ₐ</td>
</tr>
<tr>
<td>DDA + PGI₁</td>
<td>8</td>
<td>9.5 ± 1.4</td>
<td>84.6 ± 3.2</td>
</tr>
<tr>
<td>dl-Propranolol (1 µM)</td>
<td>9</td>
<td>9.5 ± 1.3</td>
<td>46.9 ± 9.4ₐ</td>
</tr>
<tr>
<td>DDA + dl-propranolol</td>
<td>7</td>
<td>8.8 ± 1.9</td>
<td>52.0 ± 15.2ₐ</td>
</tr>
<tr>
<td>dl-Propranolol + PGI₁</td>
<td>7</td>
<td>16.9 ± 2.0ₐ</td>
<td>0ₐ</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM.

ₐp < .05, ₐₐp < .01 compared with control.
sites in the cell. Results of studies with DDA, a potent inhibitor of adenylate cyclase, also support a role for cyclic AMP in the antiaggregatory activity of PGI2 at higher concentrations. When given alone, DDA did not alter basal cyclic AMP levels, Tx synthesis, or platelet aggregation. However, DDA blocked the ability of PGI2 to inhibit arachidonic acid or thrombin stimulation of aggregation. Similar findings were reported by Haslam et al. Additionally, DDA blocked the increase in cyclic AMP and decrease in TxB2 caused by PGI2 treatment. These results support the hypothesis that the inhibitory effect of PGI2 on platelet aggregation is cyclic AMP dependent.

The role of cyclic AMP in the antiaggregatory activity of propranolol was investigated in a manner similar to that in the PGI2 studies. Propranolol inhibited TxB2 synthesis and platelet aggregation, but DDA did not reverse these effects of propranolol as it did with PGI2. In agreement with this finding, propranolol in concentrations that attenuate aggregation and Tx formation failed to increase cyclic AMP levels from control values. Furthermore, the ability of PGI2 to increase the production of cyclic AMP was not enhanced by propranolol even though the aggregatory response was enhanced. These observations support the conclusion that the antiaggregatory action of propranolol is independent of cyclic AMP synthesis. The exact mechanism by which propranolol inhibits platelet aggregation and thereby enhances the effect of PGI2 remains unclear. Previous studies have revealed that the drug inhibits the synthesis of TxB2 in platelets, an effect confirmed in the present study. Since PGI2, propranolol, and the combination of PGI2 and propranolol inhibited Tx synthesis to the same extent, it is unlikely that such a mechanism can explain the enhancement by propranolol of PGI2 inhibition of aggregation. The antiplatelet activity of propranolol has also been attributed to inhibition of calcium mobilization or utilization. While the present study does not address this mechanism, it may possibly explain the observed results.

The data indicate that PGI2 inhibits the synthesis of Tx, and this inhibition appears to be mediated by cyclic AMP at higher concentrations. Other investigators have also found that cyclic AMP and agents that stimulate its synthesis will inhibit the synthesis of Tx by platelets. However, it is unclear whether the inhibition occurs at the level of phospholipase or cyclooxygenase. We found that PGI2 inhibited both thrombin- and arachidonic acid–stimulated Tx synthesis. This would suggest a site of inhibition distal to arachidonic acid or at cyclooxygenase.

In summary, propranolol treatment did not increase PGI2 synthesis by vascular endothelium. However, the combination of PGI2 and propranolol resulted in a greater than additive effect on inhibition of platelet aggregation in vitro. The inhibitory action of PGI2 on platelets appears to be mediated by cyclic AMP, whereas the attenuation of aggregation by propranolol is independent of cyclic AMP. Instead, propranolol appears to inhibit the synthesis and the actions of TxA2. The additive effect of these agents on aggregation is probably the result of their different mechanisms of action. Propranolol is a drug that is widely used in the treatment of angina pectoris. Recent evidence suggests that propranolol treatment may reduce mortality after a myocardial infarction. It is possible that some of the benefit of the drug under these conditions may be related to its direct inhibitory effects on platelet aggregation or its indirect enhancement of the antiplatelet activity of endogenously synthesized PGI2.

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