Biochemical Pharmacology, Vol. 40, No. 7, pp. 1457–1461, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

PHOTOAFFINITY RECEPTOR ANTAGONIST FOR HUMAN PLATELET THROMBOXANE A_2/PROSTAGLANDIN H_2 RECEPTORS

DALE E. MAIS,* NOAH LIEL and PERRY V. HALUSHKA†

Department of Cell and Molecular Pharmacology and Experimental Therapeutics, and Department of Medicine, Medical University of South Carolina, Charleston, SC, U.S.A.

(*Received* 27 *July* 1989; *accepted* 1 *March* 1990)

Abstract—9,11-Dimethylmethano -11,12-methano-16-(3-azido-4-iodophenoxy)-13,14-dihydro-13-aza-15$\alpha\beta$-ω-tetranor TXA_2 (I-PTA-PON_3) was synthesized and evaluated as a potential photoaffinity probe of the human platelet thromboxane A_2/prostaglandin H_2 (TXA_2/PGH_2) receptor. I-PTA-PON_3 inhibited the aggregation of washed human platelets induced by the TXA_2 mimetic U46619 [(15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid]. Schild analysis of the data revealed a K_d of 9.5 nM and a slope not significantly different from −1. Equilibrium binding studies using [^{125}I]PTA-OH, a TXA_2/PGH_2 receptor antagonist, showed that I-PTA-PON_3 plus photolysis resulted in a 52% reduction in the number of binding sites (1252 ± 202/platelet) compared to the nonphotolyzed group (2557 ± 293/platelet) ($N = 5$, $P < 0.05$) with no significant change in the K_d. Repetition of the incubation with I-PTA-PON_3 and photolysis a second time resulted in a further 77% (578 ± 163 binding sites/platelet) reduction in the number of binding sites. Incubation of washed human platelets with I-PTA-PON_3 (163 nM) followed by photolysis and removal of the non-covalently bound I-PTA-PON_3 resulted in no change in the EC_{50} value for the TXA_2 mimetic, U46619, when compared to controls that were either exposed to I-PTA-PON_3 and not photolyzed or exposed only to photolysis. The second photolysis of I-PTA-PON_3 resulted in a significant 42% increase in the EC_{50} value of U46619-induced aggregation compared to the non-photolyzed group ($N = 4$, $P < 0.05$). These results suggest that I-PTA-PON_3 is a useful probe for the study of TXA_2/PGH_2 receptors and that spare TXA_2/PGH_2 receptors may exist in the platelet.

The arachidonic acid derived metabolites, thromboxane A_2 (TXA_2‡) and prostaglandin H_2 (PGH_2), appear to share and act through specific membrane receptors on platelets [1–3]. Following the binding of TXA_2/PGH_2 or their mimetics to these receptors, a series of biochemical events including shape change, increase in intracellular Ca^{2+} concentrations, aggregation, secretion and protein phosphorylation is initiated [4].

The development of stable radioactive TXA_2/PGH_2 analogs has made it possible to measure the binding of ligands to platelet TXA_2/PGH_2 receptors [5–7]. Two radioactive ligands that have been useful in characterizing the receptors are [^{125}I]PTA-OH, a TXA_2/PGH_2 receptor antagonist [7–9], and [^{3}H]U46619, a TXA_2/PGH_2 mimetic [10–12].

In addition to radioligand binding studies to characterize receptors, photoaffinity probes have also been utilized. These probes have found wide utility in the study of receptors, especially in combination with SDS–PAGE which can be used to determine molecular weights. In addition, they have been used for the purification to homogeneity of receptors [13], have facilitated the study of the mechanisms of receptor desensitization [14], and have been utilized to demonstrate the existence of spare receptors [15, 16].

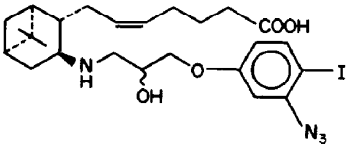

Fig. 1. Chemical structure of I-PTA-PON_3.

Two prior reports have described affinity ligands for the human platelet TXA_2/PGH_2 receptor [17, 18]. Both of these ligands have limited utility, however, due to either the chemical nature of the ligand or modest affinity of the ligand for the receptor.

This study was conducted to determine the potential utility of a new photoaffinity probe for the study of TXA_2/PGH_2 receptors on human platelets.

METHODS

Materials. The structure of I-PTA-PON_3 is shown in Fig. 1, and its synthesis and characterization is

* Present address: Lilly Corporate Center, Indianapolis, IN 46185.

† Address correspondence to: Perry V. Halushka, M.D., Ph.D., Department of Cell and Molecular Pharmacology, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.

‡ Abbreviations: TXA_2, thromboxane A_2; PGH_2, prostaglandin H_2; I-PTA-PON_3, 9,11-dimethylmethano-11,12 - methano - 16 - (3 - azido - 4 - iodophenoxy) - 13,14-dihydro-13-aza-15$\alpha\beta$-ω-tetranor TXA_2; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PRP, platelet rich plasma; and U46619, (15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid.

described elsewhere [19]. [^{125}I]PTA-OH and 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] were synthesized as described previously [8, 20]. The reaction mixture generating [^{125}I]PTA-OH was injected onto a Whatman Partisil-5 ODS-3 reverse phase HPLC column (Whatman Inc., Clifton, NJ). The mobile phase consisted of 65% methanol:35% 0.1 M ammonium acetate at a flow rate of 1 mL/min. Fractions (1 mL) were collected into 10 mL of methanol (Burdick & Jackson, Muskegon, MI), and the fraction containing [^{125}I]PTA-OH eluted at 10 min under these conditions. U46619 [(15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid] was a gift from the Upjohn Co. (Kalamazoo, MI).

Preparation of washed platelets. Blood (20–50 mL) was drawn via venipuncture from normal human volunteers, who had not taken any medication for at least 10 days prior to study, into syringes containing indomethacin (10 μM) and EDTA (5 mM) (final concentrations). Informed consent was obtained from all subjects. This study was approved by the Medical University of South Carolina Institutional Review Board for Human Research. Blood was centrifuged at 100 *g* for 15 min at room temperature, and the platelet rich plasma (PRP) was pipetted off and placed into plastic centrifuge tubes. The PRP was then centrifuged at 1000 *g* for 15 min at room temperature, and the platelet pellet resuspended in buffer (50 mM Tris–HCl, 100 mM NaCl, 5 mM dextrose and 10 μM indomethacin at a final pH 7.4) to a concentration of 5×10^8 platelets/mL.

Photoaffinity labeling procedure. Platelets were incubated in a plastic conical tube at 37° in the presence or absence of I-PTA-PON_3 (163 nM) for 5 min followed by two 20-sec bursts of ultraviolet light from a hand-held ultraviolet lamp (Ultra-violet Products, Inc., San Gabriel, CA) at a distance of 5 cm. The lamp emits light at a wavelength of 254 nm with an intensity of 1250 $\mu W/cm^2$ at a distance of 15 cm. The platelets were then diluted 2-fold in modified Tyrode's buffer (NaCl, 70 mM; KCl, 2.7 mM; $MgCl_2$, 0.5 mM; Na_2HPO_4, 7.25 mM; dextrose, 5.5 mM; pH 7.4) and centrifuged for 10 min at 1000 *g*. The pellet was resuspended in modified Tyrode's buffer and centrifuged for 10 min at 1000 *g*. The platelet pellet was resuspended in Tris–NaCl buffer to a final concentration of 5×10^8 platelets/mL. In experiments using a second concentration of I-PTA-PON_3, following the first photolysis described above an additional 163 nM I-PTA-PON_3 was added (total cumulated concentration 326 nM) followed by another 5-min incubation at 37°. The platelets were then photolyzed with two further 20-sec bursts of ultraviolet light and washed of the I-PTA-PON_3 as described above.

Aggregation studies. Washed platelets suspended in Tris–NaCl buffer containing $CaCl_2$ (250 μM) were incubated in aggregometer cuvettes (2.25×10^8 platelets/cuvette) (total volume 500 μL) with constant stirring at 37°. After 1 min of stirring, various concentrations of U46619 (final concentrations, 0.025 to 50 μM) were added and aggregation was recorded for up to 2 min. Aggregations were performed using a Chronolog aggregometer. Percent of the maximal aggregation at 1 min for each concentration of agonist was calculated by using the highest percent aggregation induced by U46619 in the control group.

In experiments designed to determine the pA_2 value of I-PTA-PON_3, the washed platelets were prepared by resuspending the platelet pellet obtained from centrifugation of PRP in buffer. The washed platelets (450 μL) were added to individual silanized glass cuvettes and preincubated with various concentrations of I-PTA-PON_3 or vehicle for 1 min at 37°. This was followed by the addition of the aggregating agent U46619 (final concentrations of 20 nM to 50 μM), and the aggregation response was observed for 2 min. Concentration–response curves were constructed for U46619, and the EC_{50} was determined from log-logit plots. The EC_{50} value was defined as the concentration required to produce 50% of the maximum aggregation occurring 1 min after the addition of U46619. The pA_2 value for I-PTA-PON_3 was determined from a Schild analysis of multiple concentration–response curves for U46619 in the presence or absence of varied concentrations of I-PTA-PON_3. Concentration ratios were calculated by comparing EC_{50} values in the presence of I-PTA-PON_3 to those in the presence of its vehicle [21, 22].

Radioligand binding studies. Incubations (200 μL) containing 5×10^7 platelets were performed in silanized (12 × 75 mm) glass tubes at 37° for 30 min. The incubation medium consisted of Tris–HCl buffer and [^{125}I]PTA-OH (~0.1 nM, ~5×10^4 cpm) per tube. The radiolabeled ligands were freshly prepared from the stock solution on the day of use. An aliquot was placed into a glass vial and dried under a stream of nitrogen at room temperature and then redissolved in the appropriate volume of Tris–NaCl buffer with vortex mixing for 1 min. For equilibrium or competition binding studies, concentrations of I-PTA-OH or I-PTA-PON_3 ranging from 10^{-9} to 10^{-5} M were also included. The reaction was terminated by the addition of 4 mL of ice-cold Tris–NaCl buffer (minus dextrose) followed by rapid filtration through Whatman GF/C glass fiber filters (Whatman Inc.). The filters were washed three more times with 4 mL of the ice-cold buffer. The filtration procedure was completed within 10 sec. Nonspecific binding was defined as the number of bound cpm remaining in the presence of 1 μM I-PTA-OH.

Data analysis. The EC_{50} values for aggregation were determined using log/logit transformations of the data. Equilibrium binding studies using [^{125}I]PTA-OH were analyzed using Scatchard plots and a Simplex computer program to fit the data [23, 24]. Competition binding assays using [^{125}I]PTA-OH and I-PTA-PON_3 were analyzed as log-logit plots, and the best fit regression line was determined. The IC_{50} value was determined from this line. All data are presented as mean ± SEM. A paired Student's *t*-test was used to determine if differences existed between the K_d, B_{max} and EC_{50} values between the control and experimental groups.

RESULTS

Determination of the K_d *value for* I-PTA-PON_3.

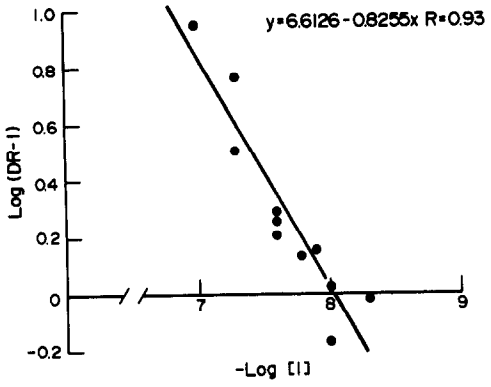

Fig. 2. Schild plot for I-PTA-PON_3 in washed human platelets. The x-axis is the -log of the concentration of I-PTA-PON_3. The y-axis is the ratio of the EC_{50} values obtained in the presence of antagonist to that obtained in the absence of antagonist. The graph presents the combined data from three individual experiments.

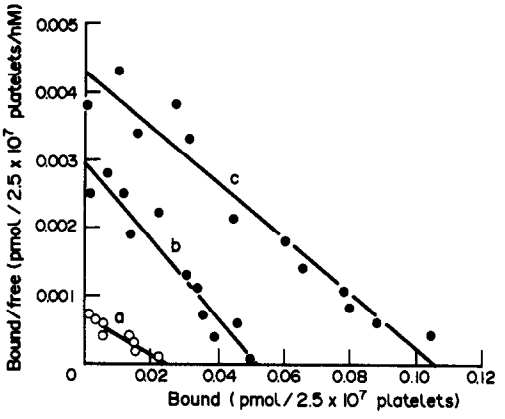

Fig. 3. Scatchard analysis for the equilibrium binding of [^{125}I]PTA-OH to washed human platelets that were incubated in the presence of I-PTA-PON_3 [326 nM (a) or 163 nM (b)] and photolyzed or not photolyzed (c). Each curve represents the pooled results of four individual experiments.

To determine if I-PTA-PON_3 was a competitive antagonist of the TXA_2/PGH_2 receptor, washed platelets were aggregated with various concentrations of the TXA_2PGH_2 mimetic U46619. The EC_{50} values for U46619-induced platelet aggregation were determined in the absence or presence of various concentrations of I-PTA-PON_3, and the results were analyzed by the method of Arunlakshana and Schild [21]. The pA_2 value obtained from the Schild plot was 8.02, which yielded a K_d of 9.5 nM (Fig. 2). The slope of the regression line (-0.93 ± 0.09, N = 3) was not significantly different from -1, indicating that I-PTA-PON_3 appears to be a competitive antagonist of U46619-induced aggregation of washed platelets.

In competition assays using [^{125}I]PTA-OH, I-PTA-PON_3 had an IC_{50} value of 36 ± 8 nM (N = 3) and the slope of the line determined by regression analysis of the log-logit transformation was -1.08 ± 0.09 (N = 3). I-PTA-PON_3 completely displaced the specifically bound radioligand.

Binding studies. To determine if I-PTA-PON_3 irreversibly reduced the number of putative TXA_2/PGH_2 receptor binding sites, equilibrium binding experiments using the radioiodinated TXA_2/PGH_2 antagonist [^{125}I]PTA-OH were performed. The results of these experiments are presented in Fig. 3. Scatchard analysis of the binding data of the control platelets from group C in Table 1 revealed a K_d for I-PTA-OH of 25 ± 7 nM and a B_{max} of 42.4 ± 4.9 fmol/10^7 platelets (N = 4) (2557 ± 293 sites per platelet). The control values were similar for the platelets incubated with either 163 or 326 nM I-PTA-PON_3; thus, the K_d and B_{max} values were pooled. The values for the K_d and B_{max} agree well with control values obtained previously [7, 9]. Equilibrium binding studies of platelets that were incubated with I-PTA-PON_3 at either 163 nM or 326 nM and photolyzed (group D in Table 1) showed no change in the K_d value for I-PTA-OH (18 ± 3 and 34 ± 12 nM respectively). However, the B_{max} values were significantly ($P < 0.05$) reduced by 52 and 77% for the platelets photolyzed in the presence of 163 or 326 nM I-PTA-PON_3 respectively. This represented a decrease in the number of [^{125}I]PTA-OH binding sites to 1252 ± 202 and 578 ± 163 sites per platelet respectively.

Platelet aggregation following photolysis. Table 1 shows the results from experiments with washed platelets that were incubated with I-PTA-PON_3 and photolyzed followed by washing and determination of the EC_{50} values for U46619-induced platelet aggregation. When platelets were incubated with 163 nM I-PTA-PON_3 and photolyzed, there was no significant change in the EC_{50} for the aggregation response to U46619 compared to the control groups. Group C which was incubated with I-PTA-PON_3 but not photolyzed served as the control for retained ligand not covalently bound to the platelets. Group B was the control for the effects of ultraviolet light in the absence of I-PTA-PON_3. All four groups were treated in an identical manner with respect to incubation at 37° and the washing procedure. In contrast, when platelets were incubated with an additional 163 nM I-PTA-PON_3 (326 nM cumulative concentration) followed by a further exposure to ultraviolet light, there was a significant increase in the EC_{50} value (423 ± 99 nM, group D) when compared with the control groups (270 ± 37 and 299 ± 68 nM for groups B and C respectively) ($P < 0.05$, N = 4). The maximum aggregation response was not significantly different among the four groups (data not shown).

DISCUSSION

This study describes a new affinity probe for the human platelet TXA_2/PGH_2 receptor and documents its irreversible incorporation into the receptor. I-PTA-PON_3 represents a significant improvement over previously described probes [17, 18]. Mais *et al.* [17] described an aryl diazonium salt that was photolyzed in the presence of human platelet membranes, and irreversible coupling of the ligand into

Table 1. EC_{50} Values for U46619-induced platelet aggregation following incubation of washed platelets with either vehicle or two concentrations of I-PTA-PON_3, photolysis and washing of the platelets

Group	EC_{50} Values for U46619 [I-PTA-PON_3] 163 nM (n = 5)	326 nM* (n = 4)
A Vehicle†	161 ± 27	249 ± 35
B Vehicle + photolysis	169 ± 31	270 ± 37
C I-PTA-PON_3	197 ± 37	299 ± 68
D I-PTA-PON_3 + photolysis	199 ± 23	423 ± 99‡

The EC_{50} value is the concentration of U46619 required to induce 50% of the maximal aggregation response at 1 min. The maximum attained aggregation response was the same for all four groups. Data are presented as means ± SE.

* Platelets were incubated with 163 nM I-PTA-PON_3, photolyzed, incubated with 163 nM again, and photolyzed.

† Vehicle for I-PTA-PON_3.

‡ $P < 0.05$ compared to groups B and C.

the TXA_2/PGH_2 receptor was shown. This molecule, however, could not be radioiodinated and its affinity for the receptor could not be determined. The second study described an iodinated azido ligand that apparently could be irreversibly incorporated into the TXA_2/PGH_2 receptor following incubation and photolysis [18]. The reported K_d value for the ligand in washed human platelets was 300 nM, an affinity which may be too low to allow for specific labeling of the receptor. The affinity of I-PTA-PON_3 was 9.5 nM based on Schild analysis of concentration–response curves and 36 nM based on competition binding assays.

In addition, this study shows that the number of TXA_2/PGH_2 receptor binding sites can be reduced irreversibly by the photoaffinity probe I-PTA-PON_3. This is indicated by the decrease in the number of binding sites for [^{125}I]PTA-OH following incubation of washed human platelets with I-PTA-PON_3 and photolysis. The data suggest that human platelets may possess a reserve of TXA_2/PGH_2 receptors since under conditions which decreased the number of binding sites by 52% as measured by equilibrium binding, there was no effect on U46619-induced platelet aggregation. Under conditions where 77% of the binding sites were blocked irreversibly, a modest and significant increase of 29% in the EC_{50} value for U46619-induced platelet aggregation was observed with no change in the maximum response. The notion that spare TXA_2/PGH_2 receptors exist was also supported by the report of an EC_{50}/K_d ratio of less than one for TXA_2/PGH_2 receptor agonists in human platelets [25]. The concept of spare receptors has been used as one explanation for EC_{50}/K_d ratios which differ from unity [26].

In its general form, receptor theory would dictate that a 50% loss in binding sites should shift the concentration–response curve approximately 0.3 log units to the right, whereas a 75% loss in binding sites would shift it 0.6 log units. That this was not observed for the TXA_2/PGH_2 receptor may reflect the method of determining the concentration–response curve. The measurement of aggregation is far downstream from the actual event of receptor occupancy, and in the platelet with its various amplification steps to promote aggregation, the measurement of this rightward shift could be lost.

Alternatively, if subtypes of TXA_2/PGH_2 receptors exist in platelets as has been suggested previously [27], I-PTA-PON_3 may be incorporating preferentially into only one of the subtypes in preference to the subtype actually coupled to the aggregation response.

These results of the present study are similar to those reported for the human platelet α_2-adrenergic receptor [16]. Over a 70% irreversible reduction in α_2-adrenergic receptors was required to produce only a 1.7-fold shift in the EC_{50} value for norepinephrine.

As can be seen in Table 1 group A, there was an increase in the EC_{50} value for U46619-induced platelet aggregation with an additional washing of the platelets. This probably reflects a decrease in platelet sensitivity due to the additional washings necessary to remove the higher concentrations of I-PTA-PON_3. This decreased sensitivity has been observed previously by Liel *et al.* [28]. That the shift in the EC_{50} value for U46619-induced platelet aggregation was not the result of retained photoaffinity ligand is indicated by the lack of a shift in group C of Table 1 when compared with groups A and B which were not incubated with I-PTA-PON_3. In addition, the binding studies were performed on groups C and D, with group C serving as the control. Since the number of [^{125}I]PTA-OH binding sites observed for group C in this study (2557 ± 293 binding sites/platelet) compares well with our previous studies (2530 ± 380 binding sites/platelet) [7] and those of Narumiya *et al.* (2360 ± 220 binding sites/platelet) [9], an effect of retained ligand at the concentrations used in this study can be excluded. However, at higher concentrations, it was not possible to remove all the ligand within a reasonable number of washes and maintain platelet viability. This negated the possibility of irreversibly blocking more of the TXA_2/PGH_2 receptors.

In conclusion, I-PTA-PON_3, a new affinity probe for the human platelet TXA_2/PGH_2 receptor, represents a significant improvement over previous

ligands and should be useful for further studies of TXA_2/PGH_2 receptors.

Acknowledgements—This work was supported in part by Grants HL36838 and HL29566. Dr. Liel was supported in part by an Upjohn Postdoctoral Clinical Pharmacology Fellowship and a Medical University of South Carolina Fellowship. Dr. Halushka is a Burroughs-Wellcome Scholar in Clinical Pharmacology.

REFERENCES

1. Hamberg M, Svensson J, Wakabayashi T and Samuelsson B, Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc Natl Acad Sci USA* **71**: 345–349, 1974.
2. Hamberg M, Svensson J and Samuelsson B, Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci USA* **72**: 2994–2998, 1975.
3. Saussy DL Jr, Mais DE, Knapp DR and Halushka PV, Thromboxane A_2 and prostaglandin endoperoxide receptors in platelets and vascular smooth muscle. *Circulation* **72**: 1202–1207, 1985.
4. Halushka PV and Knapp DR and Grimm L, Prostaglandins, thromboxanes and platelet function. *Curr Top Hematol* **2**: 75–143, 1979.
5. Hung SC, Ghali NI, Venton DL and LeBreton GC, Specific binding of the thromboxane A_2 antagonist 13-azaprostanoic acid to human platelet membranes. *Biochim Biophys Acta* **72**: 171–178, 1983.
6. Armstrong RA, Jones RL and Wilson NH, Ligand binding to thromboxane receptors on human platelets: Correlation with biological activity. *Br J Pharmacol* **79**: 953–964, 1983.
7. Mais DE, Burch RM, Saussy DL Jr, Kochel PJ and Halushka PV, Binding of a thromboxane A_2/prostaglandin H_2 receptor antagonist to washed human platelets. *J Pharmacol Exp Ther* **235**: 729–736, 1985.
8. Mais DE, Saussy D, Kochel P and Halushka PV, Binding of an ^{125}I-labelled thromboxane A_2/prostaglandin H_2 receptor antagonist to washed canine platelets. *Mol Pharmacol* **28**: 163–169, 1985.
9. Narumiya S, Okuma M and Ushikubi F, Binding of a radioiodinated 13-azapinane thromboxane antagonist to platelets: Correlation with antiaggregatory activity in different species. *Br J Pharmacol* **88**: 323–331, 1986.
10. Coleman RA, Humphrey PPA, Kennedy I, Levy GP and Lumley P, Comparison of the actions of U-46619, a prostaglandin H_2 analogue with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br J Pharmacol* **73**: 773–778, 1981.
11. diMinno G, Bertelé V, Bianchi L, Barbieri B, Cerletti C, Dejana E, deGaetanno G and Silver MJ, Effects of an epoxymethano stable analogue of prostaglandin endoperoxide (U-46619) on human platelets. *Thromb Haemost* **45**: 103–106, 1981.
12. Liel N, Mais DE and Halushka PV, Binding of a thromboxane A_2/prostaglandin H_2 agonist [^{3}H]U46619 to washed human platelets. *Prostaglandins* **33**: 789–798, 1987.
13. Simonds W, Burke T, Rice K, Jacobson A and Klee W, Purification of the opiate receptor of NG108-15 neuroblastoma-glioma hybrid cells. *Proc Natl Acad Sci* **82**: 4974–4978, 1985.
14. Rothman R, Danks J, Jacobson A, Burke T, Rice K, Tortella F and Holaday W, Morphine tolerance increases μ-noncompetitive δ binding sites. *Eur J Pharmacol* **124**: 113–120, 1986.
15. Atlas D and Steer ML, Clonidine *p*-isothiocyanate, an affinity label for α_2-adrenergic receptors on human platelets. *Proc Natl Acad Sci USA* **79**: 1378–1382, 1982.
16. Lenox RH, Ellis J, Van Riper D and Ehrlich YH, Alpha$_2$-adrenergic receptor-mediated regulation of adenylate cyclase in the intact human platelet. *Mol Pharmacol* **27**: 1–9, 1985.
17. Mais DE, Burch R, Oatis J, Knapp D and Halushka PV, Photoaffinity labelling of a thromboxane A_2/prostaglandin H_2 antagonist binding site in human platelets. *Biochem Biophys Res Commun* **140**: 128–133, 1986.
18. Arora S, Kattleman E, Lim C, LeBreton G and Venton P, Preparation and biological evaluation of a potential photoaffinity label for the prostaglandin H_2/thromboxane A_2 receptor. *J Med Chem* **30**: 918–924, 1987.
19. Mais DE and Halushka PV, Synthesis of an [^{125}I]-azido photoaffinity probe for the human platelet thromboxane A_2/prostaglandin H_2 receptor. *J Labelled Compd Radiopharm* **25**: 731–737, 1988.
20. Mais DE, Knapp D, Ballard K, Halushka PV and Hamanaka N, Synthesis of thromboxane receptor antagonists with the potential to radiolabel with ^{125}I. *Tetrahedron Lett* **25**: 4207–4210, 1984.
21. Arunlakshana O and Schild H, Some quantitative uses of drug antagonists. *Br J Pharmacol Chemother* **14**: 48–54, 1959.
22. Tallarida R, Cowan A and Adler M, pA_2 and receptor differentiation: A statistical analysis of competitive antagonism. *Life Sci* **25**: 637–643, 1979.
23. Nelder JA and Mead R, A simplex method for function minimization. *Comput J* **2**: 308–313, 1965.
24. Lam CF, Computer analysis and optimization of a class of nonlinear systems. *Ph.D. Dissertation*. Clemson University, Clemson, SC, 1980.
25. Halushka PV, Kochel PJ and Mais DE, Binding of thromboxane A_2/prostaglandin H_2 agonists to human platelets. *Br J Pharmacol* **91**: 223–227, 1987.
26. Stephenson RP, A modification of receptor theory. *Br J Pharmacol* **11**: 379, 1956.
27. Morinelli T, Niewiarowski S, Daniel J and Smith J, Receptor-mediated effects of a PGH_2 analogue (U46619) on human platelets. *Am J Physiol* **253**: H1035–H1043, 1987.
28. Liel N, Mais D and Halushka P, Desensitization of platelet thromboxane A_2/prostaglandin H_2 receptors by the mimetic U46619. *J Pharmacol Exp Ther* **247**: 1133–1138, 1988.