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A NOVEL APPROACH FOR THE STUDY OF THROMBOXANE A_2 AND PROSTAGLANDIN H_2 RECEPTORS USING AN ^{125}I-LABELED LIGAND

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Abstract—Previous studies of eicosanoid receptors have utilized ^{3}H-labeled ligands. However, ^{125}I has a higher theoretical specific activity (~2000 Ci/mmole) than ^{3}H (29 Ci/mmole), which provides a potential advantage for ^{125}I ligand binding studies when the receptor density is low. Since eicosanoids do not possess an easily iodinatable structure (e.g. a phenol or imidazole ring), it is not feasible to directly incorporate ^{125}I into the molecule. The thromboxane A_2/prostaglandin H_2 receptor antagonist, *cis*-7-(2-*p*-hydroxyphenylethanolaminocyclopentyl)-heptanoic acid (*cis*-APO), was synthesized to test the concept that it could be labeled with ^{125}I and used as a ligand for binding studies. *cis*-APO is a structural analog of 13-azaprostanoic acid, a TXA_2/PGH_2 antagonist [G. C. Le Breton, D. L. Venton, S. E. Enke and P. V. Halushka, *Proc. natn. Acad. Sci. U.S.A.* **76**, 4097 (1979)], in which the ω aliphatic chain was substituted with 2-*p*-hydroxyphenylethanol, which contains a phenolic group. [^{127}I]*cis*-APO was synthesized by insertion of ^{127}I (stable isotope) into the phenolic portion of the molecule under alkaline conditions. [^{125}I]-*cis*-APO was synthesized via insertion of ^{125}I (unstable isotope) into the molecule in the presence of chloramine T. *cis*-APO inhibited human platelet aggregation induced by the thromboxane mimetic U46619 [C. Malmsten, *Life Sci.* **18**, 169 (1976)]. The IC_{50} for *cis*-APO was 6.4 ± 0.7 μM and for [^{127}I]-*cis*-APO was 9.8 ± 1.3 μM ($P < 0.001$). [^{125}I]-*cis*-APO binding to a human platelet membrane preparation at 4° was time and protein concentration dependent, saturable, and reduced or abolished by trypsin or boiling respectively. The K_d for iodo-*cis*-APO determined at equilibrium using a Scatchard analysis was 1.48 μM and the maximum binding capacity was 18.7 pmoles/mg protein. The forward rate constant (k_{+1}) was $2.3 \times 10^3\ M^{-1}\ s^{-1}$ and the dissociation constant (k_{-1}) was $2.12 \times 10^{-3}\ s^{-1}$. The K_d determined from k_{-1}/k_{+1} was 0.92 μM. These observations show that the ω side chain of eicosanoid analogs can be substituted with a phenolic group, iodinated, and retain biological activity. These molecules may then be utilized to study thromboxane A_2 or prostaglandin H_2 receptors.

^{3}H and ^{125}I are commonly used to rabiolabel molecules for radioligand binding studies. Incorporation of ^{125}I into the molecule has the advantage of yielding a theoretical specific activity of ~2000 Ci/mmole, compared to 29 Ci/mmole incorporated for ^{3}H. Iodination procedures are easily performed without the need for highly specialized equipment or specific radiochemical syntheses. In situations where the receptor density is low or the amount of biological material is limited, ^{125}I-labeled molecules provide a useful tool for identification of the receptors. However, iodination with ^{125}I has several potential disadvantages compared to ^{3}H. ^{125}I has a shorter $t_{1/2}$ (60 days) than ^{3}H (12 yr). Insertion of ^{125}I into the molecule, or the iodination procedure itself, may alter or destroy the biological activity of the molecule. In addition, the molecule must contain a phenol or imidazole moiety for it to be iodinated by the simple procedures described below.

Previous studies of prostaglandin receptors have only utilized ^{3}H-labeled prostanoids [1–4]. Because thromboxane A_2 ($t_{1/2}$ = 37 sec) [5] and prostaglandin H_2 ($t_{1/2}$ ~5 min) [6] are very labile, study of their putative receptors requires the synthesis of stable analogs which either mimic or antagonize their actions. These compounds may then be radiolabeled and used for study of their putative receptors. Eicosanoids do not contain phenol or imidazole; however, iodination of eicosanoid molecules has been carried out previously in a two-step reaction, namely formation of an amide at the C-1 carboxylic acid with tyramine, followed by iodination of the amide derivative with ^{125}I [7]. The resulting molecules have been used for radioimmunoassay [7] but are devoid of biological activity. Previous studies have shown that the lower (ω) side chain of eicosanoid molecules may be modified distal to the C-15 position without loss of biological activity [8]. Thus, a phenolic functional group might be introduced into the bottom side chain of an eicosanoid molecule beyond the C-15 position, without significant loss of its biological activity. The molecule could then be iodinated with ^{125}I yielding a high specific activity

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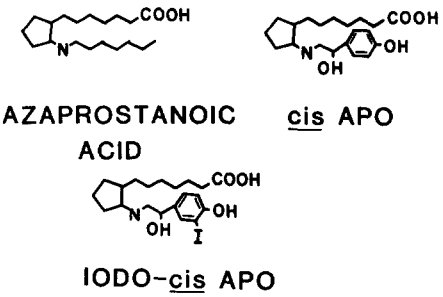

Fig. 1. Structures of 13-azaprostanoic acid, *cis*-APO and iodo-*cis*-APO.

radio-ligand for binding studies. We chose to test this concept by synthesizing an analog of 13-azaprostanoic acid, a thromboxane A_2/PGH_2 platelet receptor antagonist [9], in which the aliphatic lower side chain was replaced with 2-*p*-hydroxyphenylethanol (Fig. 1). The synthesis of this molecule is straightforward and provides a simple approach to test the concept that eicosanoid analogs may be iodinated without complete loss of biological activity.

This paper reports the synthesis of 7-(2-*p*-hydroxyphenethanolaminocyclopentyl)-heptanoic acid (*cis*-APO), [^{127}I]*cis*-APO and [^{125}I]*cis*-APO. The biological activities of these compounds have been compared, and preliminary binding data suggest that this is a promising strategy for examination of thromboxane A_2 or prostaglandin H_2 receptors.

MATERIALS AND METHODS

Synthesis of cis- *and* trans-7-(2-*p-hydroxyphenylethanolaminocyclopentyl)-heptanoic acid*

Preparation of methyl 7-[2-(p-*hydroxyphenylethanolamino)-cyclopentyl*]-*heptanoate*. A solution of 4.35 g of methyl 7-(2-oxocyclopentyl)-heptanoate [10] and 2.95 g of *d,l*-octopamine free base in 50 ml of anhydrous methanol was treated with 10 g of 3 Å molecular sieves and stirred under N_2 for 96 hr. The reaction was cooled in ice and treated with 800 mg of sodium borohydride in several portions to control foaming. The mixture was then stirred for 60 min in the cold and for an additional 30 min at room temperature. The sieves were filtered off, and the filtrate was evaporated to an amber oil which was partitioned between 150 ml of chloroform and 75 ml of distilled water containing 1 ml of glacial acetic acid. The organic layer was separated and dried over magnesium sulfate and evaporated to afford 6.7 g of oily product mixture which was dried *in vacuo*.

The *cis* and *trans* isomers were separated by high performance liquid chromatography on a Partisil M9 10/50 PAC column (a cyanopropyl-modified silica) with chloroform–methanol (97:3) at 5 ml/min while monitoring at 254 nm. The *cis* isomer eluted in about 15 min with the *trans* appearing at about 25 min. Run time was shortened by increasing the flow rate to 9.9 ml/min to collect the *trans* isomer. Yield of the *cis* isomer was 2.18 g and yield of *trans* was 2.4 g (total yield about 65%). Assignment of *cis* and *trans* isomers was based upon analogy to the retention times of the parent 13-azaprostanoic acid isomers [10]. The *cis* and *trans* isomers showed identical electron impact mass spectra as the di-trimethylsilyl derivatives (*m/z* 507[M^+], 386, 267, 254).

Preparation of cis-*APO*. A 700 mg portion of *cis*-APO methyl ester was dissolved in 20 ml of distilled water containing 200 mg of NaOH. The solution was refluxed for 4 hr and then cooled in ice. Addition of 400 μl of glacial acetic acid gave an immediate precipitate which was centrifuged. The supernatant fraction was concentrated on a rotary evaporator to about 10 ml volume, and the solid which separated was filtered off and washed with distilled water and dried *in vacuo*. TLC analysis showed that several impurities persisted and that considerable product was still in the filtrate. The dried solid portion was suspended in 8 ml of ethanol and heated to boiling, sonicated briefly, and allowed to cool. The insoluble solid was centrifuged, washed twice with 8-ml portions of fresh ethanol, and then recentrifuged and dried *in vacuo*. Yield of pure *cis*-APO was 208 mg (30%). The fast atom bombardment (FAB) mass spectrum showed a protonated molecular ion at *m/z* 350 with major fragments at *m/z* 332 ($MH^+ - H_2O$) and 226 ($MH^+ - HOC_6H_4CH_2OH$).

Iodination (^{127}*I*) *of* cis-*APO*. A 3 mg quantity of *cis*-APO was dissolved in 200 μl of distilled water containing 50 μl of concentrated ammonia. The solution was cooled to 0° and treated with 5.5 mg of iodine dissolved in 200 μl of 95% ethanol. The unreacted iodine was quenched with a drop of sodium metabisulfite solution. The reaction mixture was treated with small flakes of dry ice, and a precipitate which soon formed was centrifuged and washed with distilled water and ethanol and dried *in vacuo*. TLC analysis indicated this precipitate to be predominantly a single compound which migrated slightly faster than reference *cis*-13-APO, as would be anticipated for the iodinated analog. The compound was purified by preparative TLC and identified by FAB mass spectrometry as the monoiodinated compound (MH^+ = 476).

Iodination (^{125}*I*) *of* cis-*APO*. A stock solution of *cis*-APO (1 μg/μl) was prepared by dissolving it in 0.1 N NaOH. Under these conditions, the compound was stable at room temperature for several weeks. For the iodination procedure, the stock solution was diluted to a concentration of 0.05 μg/μl in 0.1 M phosphate buffer, pH 7.4. The iodination was carried out in a 1-ml reactivial. The following substances were added in the order given: 5 μl of 0.1 M phosphate buffer, pH 7.4; 10 μl *cis*-APO (0.05 μg/μl), 0.5 mCi [^{125}I]sodium (5 μl), and 10 μl Chloramine T (50 μg/ml). The vial was vortexed for several seconds and then allowed to stand at room temperature for 45 sec, after which 10 μl of sodium metabisulfite (30 mg/ml) was added and the vial was vortexed for an additional 5 sec. If incubation times of longer than 45 sec were used, di-iodo-*cis*-APO began to form.

The reaction mixture was applied to a silica gel thin-layer chromatography (250 μm thick) plate, except for approximately 1 μl which was applied to a second plate with 10 μg each of [^{127}I]*cis*-APO and *cis*-APO. The solvent system used was: *n*-hexane–glacial acetic acid–ethylacetate–ethylacetate/0.1 mM

phenol–H_2O (16:18:52:2;60). The organic layer of the two phases was used for developing the plates. After development to approximately 10 cm, the second plate was dried and scanned in a Packard radiochromatogram scanner. A representative tracing is shown in Fig. 2. The R_f (0.32) of the ^{125}I-compound was noted, and the plate was put in an iodine chamber to visualize the *cis*-APO and [^{127}I]-*cis*-APO. During this time the first plate was covered with aluminum foil until it was scraped. The zone corresponding to the [^{125}I]*cis*-APO on the radiochromatogram was marked on the first plate, and the silica was scraped into a glass conical test tube containing 1 ml of 0.1 N HCl and 2 ml ethylacetate/0.01 mM phenol. The mixture was vortexed for 1 min and then centrifuged to separate the layers. The ethylacetate/0.01 mM phenol layer was aspirated and retained. The procedure was repeated twice. The ethylacetate layer containing [^{125}I]*cis*-APO was stored for up to 2 months at −20°. Approximately 20% of the [^{125}I]sodium was recovered as [^{125}I]-*cis*-APO. The R_f values of the iodinated [^{125}I]*cis*-APO was identical to mono[^{127}I]*cis*-APO. Since [^{125}I]*cis*-APO was separated completely from *cis*-APO, the final specific activity was taken to be that of the sodium [^{125}I] used.

The radiolabeled ligand was prepared freshly on the day of use in the following manner. An aliquot of the ethylacetate solution was placed into a glass tube (12 × 75 mm) and dried under a stream of nitrogen at room temperature. The ligand was redissolved in the appropriate volume of assay buffer (25 mM Tris, pH 7.4), with vortex mixing for 1 min.

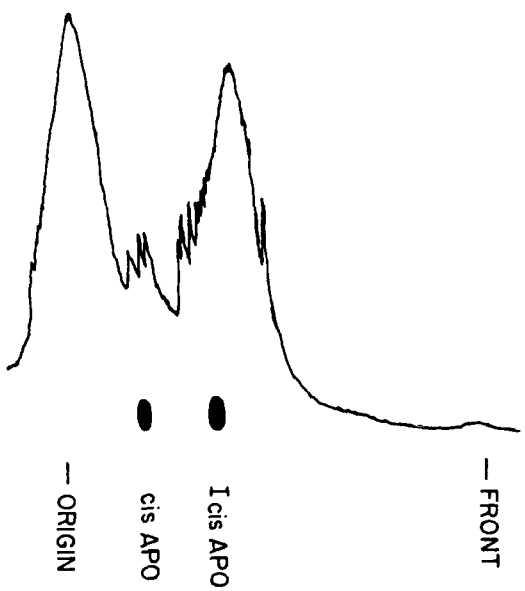

Fig. 2. Tracing of a representative thin-layer radiochromatogram scan of the iodination reaction mixture. The mobility of standard *cis*-APO and iodo-*cis*-APO are shown at the bottom of the scan. The radioactivity remaining at the origin corresponds to unreacted sodium [^{125}I].

Platelet aggregation

Platelet rich plasma (PRP) was prepared from blood of normal volunteers by a previously described procedure [11]. Platelet aggregation was carried out in a chronolog aggregometer using the method of Born [12]. PRP was incubated with indomethacin (10 μM) to prevent the metabolism of endogenous arachidonic acid to thromboxane A_2 and prostaglandins. Stock solutions of *cis*-APO and iodo-*cis*-APO were dissolved in 0.5 N NaOH and diluted with an equal volume of veronal-buffered saline (pH 7.4) (VBS) to a final concentration of 10 mM. Subsequent dilutions were made in VBS. PRP (0.5 ml) was preincubated at 37° with either vehicle, *cis*-APO or [^{127}I]*cis*-APO for 1 min prior to the addition of the stable prostaglandin endoperoxide analog U46619. A submaximal concentration of U46619 (0.09 to 0.2 μM) was added to the PRP, and the aggregation response was recorded for 2 min. Percent aggregation was calculated at 30, 60, and 120 sec after addition of U46619. Percent inhibition of aggregation was estimated 60 sec after the addition of U44619 using the following formula:

$$\% \text{ inhibition} = 100 \times \frac{\% \text{ control aggregation} - \text{aggregation in presence of antagonist}}{\% \text{ control aggregation}}$$

Preparation of platelet membranes

Blood (80–100 ml) from normal volunteers was drawn into ice-cold syringes, containing indomethacin (50 μM) and EDTA (5 mM) (final concentrations). The blood was centrifuged at 100 *g* for 15 min at 4°, and the PRP was aspirated and placed into ice-cold plastic centrifuge tubes. The PRP was then centrifuged at 1000 *g* for 15 min at 4°, and the platelet pellet was resuspended in ice-cold 5 mM Tris–HCl buffer, pH 7.4. The platelet suspension was disrupted at 4° by twenty strokes in a tight-fitting Dounce homogenizer. The resulting platelet homogenate was centrifuged at 100,000 *g* for 20 min at 4°. The pellet contained the membranes and was washed once by resuspension in 5 mM Tris–HCl buffer, pH 7.4, and centrifuged at 100,000 *g* for 20 min. The pellet was resuspended in 25 mM Tris buffer at pH 7.4 at a protein concentration of 2–4 mg/ml. This suspension, referred to as membranes, was kept at 4° prior to assay or stored for several days at −80°. Protein concentrations were determined by a modification of the method of Lowry *et al.* [13].

Binding of [^{125}*I*]cis-*APO to platelet membranes*

Incubations (200 μl) containing 200–350 μg of platelet protein were performed in plastic (12 × 75 mm) tubes at either 4° or 30° for either 20 or 60 min. The incubation mixture contained 25 mM Tris–HCl buffer, pH 7.4, 20 mM $MgSO_4$, 10 mM $CaCl_2$ and [^{125}I]*cis*-APO at the concentrations shown. This combination of cations resulted in the highest total and specific binding of the ligand. The reaction was terminated by the addition of 4 ml of ice-cold 25 mM Tris buffer at pH 7.4, followed by rapid filtration through Whatman GF/C glass fiber filters. The filters were washed three times with 4 ml of the ice-cold buffer. The filtration procedure was completed in less than 20 sec. Nonspecific binding was determined in the presence of 30 or 100 μM [^{127}I]*cis*-

APO using the method of Chamness and McGuire [14]. Binding of ^{125}I to the filters was approximately 0.3% of the total added cpm.

In time–course experiments, single incubations of 3.0 to 3.5 ml were performed. Each contained the same molar concentrations of the reagents described above. At selected times after the commencement of the incubation, 200 μl volumes of this mixture were pipetted into plastic tubes containing 4 ml of ice-cold buffer, and filtration was performed as described above.

Statistics

The equilibrium binding data were corrected for nonspecific binding and fitted to a four parameter model identifying two independent binding sites using an iterative computer program for non-linear regression analysis based on the method of Koeppe and Hamann [15]. An analysis of variance of repeated measures was used to test for differences in the antagonist activity of [^{127}I]*cis*-APO and *cis*-APO as inhibitors of U46619-induced aggregation [16].

Materials

The following were purchased from commercial sources: sodium[^{125}I], Radiochemical Centre, Amersham, England; chloramine T, and sodium metabisulfite, Fisher Scientific; silica gel 60 thin-layer chromatography plates (250 μm thick) Brinkmann, West Germany; and Whatman GF/C glass fiber filters, Whatman, Maidstone, Kent, U.K. U46619 was a gift from Dr. John Pike, Upjohn Co. Kalamazoo, MI, U.S.A.

RESULTS

Inhibition of U46619-induced platelet aggregation

Since iodination of a molecule may alter or abolish its biological potency, it was important to determine if the iodination procedure resulted in loss of the biological activity of *cis*-APO. U46619 is a stable prostaglandin endoperoxide analog which aggregates platelets by interacting with either the platelet thromboxane A_2 and/or PGH_2 receptor [17]. Thus, it was used as the agonist for determining the antagonistic potencies of *cis*-APO and [^{127}I]*cis*-APO. The potencies of [^{127}I]*cis*-APO and *cis*-APO were compared in parallel in samples obtained from the same subjects over a concentration range of 5 to 25 μM. *Cis*-APO was significantly more potent ($P < 0.001$ by ANOVA) than [^{127}I]*cis*-APO. The IC_{50} of *cis*-APO was 6.4 ± 0.7 μM, and that of [^{127}I]*cis*-APO was 9.8 ± 1.3 μM (N = 4–7). The potency of *cis*-APO was similar to that of *cis*-13-azaprostanoic acid (data not shown).

Binding of [^{125}I]cis-*APO to platelet membranes*

Having established that [^{127}I]*cis*-APO was biologically active, binding of [^{125}I]- and [^{127}I]*cis*-APO to human platelet membranes was characterized. Binding was performed at 4° to minimize the rate of dissociation of the ligand from its binding site. Specific binding in this assay was linear over the range of 25 to 400 μg protein/tube (data not shown). In experiments designed to measure the rate constant

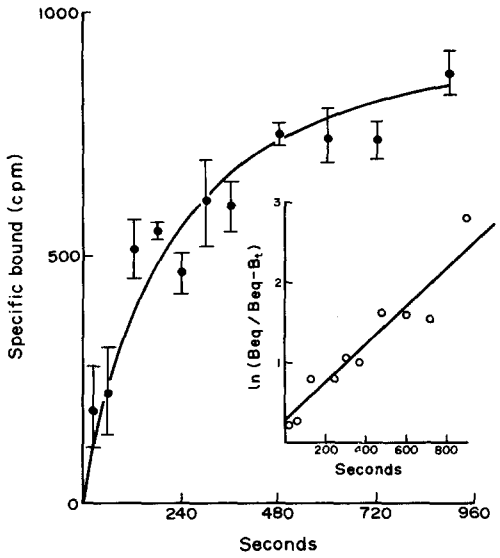

Fig. 3. Association of [^{125}I]*cis*-APO to human platelet membranes. Each data point represents the mean of triplicate determinations. The incubation was performed at 4°. The inset shows the pseudo-first-order rate plot of the same data, where B_{eq} is the specific binding at equilibrium and B_t is the specific binding at particular times (t).

(k_{+1}) for the association of the ligand–receptor complex, binding of [^{125}I]*cis*-APO + [^{127}I]*cis*-APO (100 nM) was shown to approach equilibrium within 15 min (Fig. 3). The data are presented as a pseudo first-order rate plot, from which the value of the pseudo first-order rate constant k_{obs} (2.35×10^{-3} sec^{-1}) was determined from the slope.

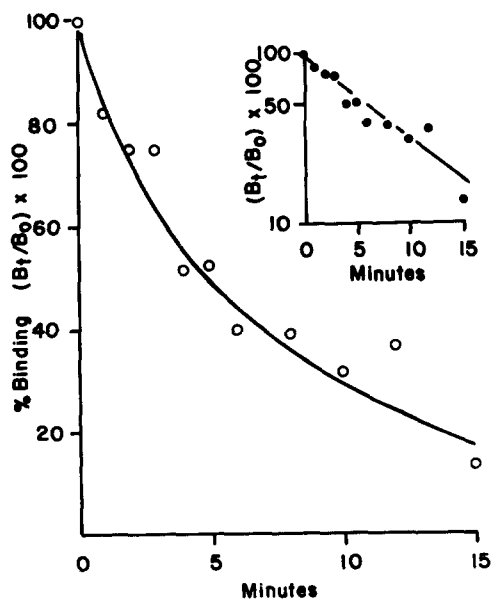

Fig. 4. Dissociation of [^{125}I]*cis*-APO from human platelet membranes. Each data point represents the mean of triplicate determinations. The inset shows a semi-logarithmic plot of the same data, where B_t is the amount of [^{125}I]*cis*-APO at each time and B_0 is the amount of [^{125}I]*cis*-APO bound at time zero.

To determine the rate constant (k_{-1}) for the dissociation of the ligand–receptor complex, platelet membranes were incubated with [^{125}I]*cis*-APO + [^{127}I]*cis*-APO (100 nM) for 30 min to ensure that equilibrium had been reached. At an arbitrary zero time point, [^{127}I]*cis*-APO was added to a final concentration of 100 μM, and the decrease in specific binding of the radioligand was measured for times up to 60 min. The results are shown in Fig. 4 which reveals an exponential decrease in specific binding. The time for 50% dissociation ($t_{1/2}$) of the high affinity site was found to be 328 sec. The value of k_{-1} was calculated to be 2.12×10^{-3} sec^{-1}. The value of k_{+1} for the association of the ligand–receptor complex is given by $\frac{k_{obs} - k_{-1}}{[L]}$, where $[L]$ = the ligand concentrations, from which k_{+1} was calculated to be 2.3×10^{3} M^{-1} sec^{-1}. The value of K_d is given by k_{-1}/k_{+1} and was 0.92 μM.

The competition for the binding of [^{125}I]*cis*-APO to platelet membranes at equilibrium was measured at a fixed concentration of [^{125}I]*cis*-APO (0.17 nM) in the presence of selected concentrations of [^{127}I]-*cis*-APO up to 30 μM. The results are shown in Fig. 5. Scatchard analysis of the data not corrected for nonspecific binding revealed a complex interaction, with a nonlinear relationship between total bound ligand and total bound/free ligand. The specific binding (Bsp) was determined from the value of total binding (B_t) and the free ligand concentration (F) [14] thus: $\text{Bsp} = B_t - F\left(\lim_{B\to\infty} \frac{B}{F}\right)$ where $\lim_{B\to\infty} \frac{B}{F}$, the value of B/F when $B = \infty$, was determined graphically (see Fig. 5). When the specific binding data were analyzed using a Scatchard analysis, a single class of specific binding sites was revealed, and the K_d (given by -1/slope) was 1.48 μM. The maximum binding capacity of the membranes was 18.7 pmoles/mg membrane protein.

Treatment of the membranes with trypsin (0.5 mg/ml) for 30 min at 37° reduced specific binding from 1325 ± 25 fmoles/mg protein to 322 ± 31 fmoles/mg protein (N = 3). Boiling the membranes for 10 min reduced specific binding to 140 ± 62 fmoles/mg protein.

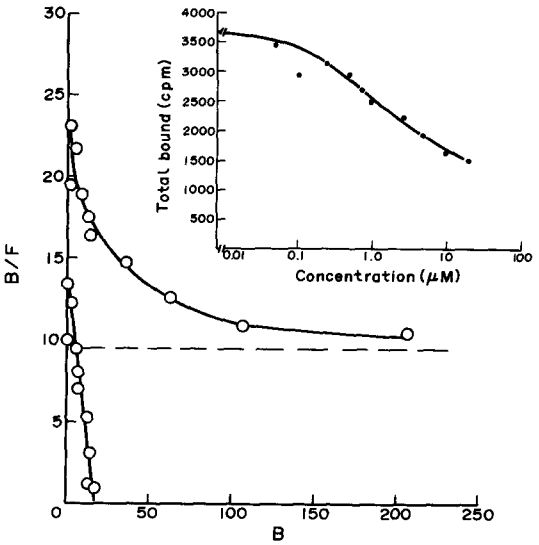

Fig. 5. Scatchard plot for the binding of [^{125}I]*cis*-APO to human platelet membranes. Each data point represents the mean of triplicate determinations. B = pmoles mg^{-1} protein and B/F = pmoles mg^{-1} protein/[ido-*cis*-APO, μM]. The line representing a single class of binding sites was estimated using the method of Chamness and McGuire [14] and fitted using a least square linear regression analysis ($r = 0.95$) [15]. The inset figure displays the actual displacement curve from which the Scatchard analysis was performed.

DISCUSSION

This study demonstrates that *cis*-APO, a platelet thromboxane A_2/prostaglandin H_2 receptor antagonist, can be labeled with ^{127}I or ^{125}I and retain biological activity and be used to study the putative platelet thromboxane A_2/prostaglandin H_2 receptor. Although iodination of the molecule did result in approximately a 50% loss of potency, sufficient biological activity remained to use this compound for radioligand binding studies. Thus, the stable isotope [^{127}I]*cis*-APO was used to compete with [^{125}I]*cis*-APO for binding to the platelet membrane homogenate.

The probability that [^{125}I]*cis*-APO binds to a membrane protein is supported in part by the observation that trypsin and boiling both reduced significantly the specific binding. The K_d determined by equilibrium binding was 1.48 μM, and the kinetically derived K_d calculated from k_{-1}/k_{+1} was 0.92 μM. The K_d values estimated by the two different techniques agree well with each other and are similar to the IC_{50} for [^{127}I]-*cis*-APO (9.8 μM). The higher value for the IC_{50} than the K_d may be due in part to the fact that the IC_{50} was determined in the presence of a high affinity agonist (U46619). Since receptors have been identified on platelets for PGE_1 [1], PGD_2 [2, 18] and PGI_2 [3, 19], all of which inhibit platelet aggregation, it is possible that [^{125}I]*cis*-APO could be interacting with one of these receptors. However, in preliminary studies, PGE_1, PGD_2 and carbacyclin, a stable PGI_2 analog, in concentrations as high as 10 μM failed to displace [^{125}I]*cis*-APO (data not shown). These concentrations are 100- to 1000-fold greater than either their IC_{50} or K_d values [1–3]. [^{125}I]*cis*-APO in a preliminary study was displaced by ONO11113, a thromboxane mimetic [20], at concentrations in the range of its EC_{50}.

Armstrong *et al.* [21] also recently reported the binding of [^{3}H]-15S-9,11-epoxymethano-prostaglandin H_2 to intact washed human platelets. A comparison of their results with the current study is difficult since their study utilized washed platelets rather than platelet membranes. They described three compartments for binding, of which two were competed for by the cold ligand. K_d values for any of these binding sites were not presented.

Hung *et al.* [22] have reported on the binding of [^{3}H]-13-azaprostanoic acid to human platelet membranes. They observed two apparent binding sites of high and low affinities respectively. Scatchard analysis revealed that the high affinity site had a K_d of 100 nM and that the low affinity site was 3.5 μM. However, it should be noted that the determination of the high affinity site appeared to rely on only

two data points, considerably less than would be necessary to determine the K_d with a high degree of reliability. We were unable to identify a high affinity binding site for *cis*-APO after submitting the data to computer analysis [15]. However, the present data are in agreement with the observation of a binding site with a K_d in the low μM range and a B_{max} of 10 pmoles/mg protein [22]. The K_d for the binding site in this study is in agreement with the potency of iodo-*cis*-APO. The B_{max} found for iodo-*cis*-APO is approximately 100-fold higher than that reported for α-adrenergic receptors in human platelets [23]. Comparison of the B_{max} for iodo-*cis*-APO to that for PGI_2 in human platelets reveals that it is approximately 10-fold greater [3] but appears to be in the same range as that for platelet fibrinogen receptors [24].

The development of ^{125}I-labeled ligands which employ modification of the ω side chain of eicosanoid analogs may prove useful in the study of receptors not only for the unstable natural compounds such as thromboxane A_2 and prostaglandin H_2 but might also be useful for the study of receptors for the stable classic prostaglandins. The bottom side chain may be modified with a phenol substitution, shown in the present study, or may potentially be substituted with imidazole which can also be iodinated. The initial rationale for the synthesis of *cis*-APO was based on the observations that 13-azaprostanoic acid was a thromboxane A_2/prostaglandin H_2 receptor antagonist in human platelets and that the synthesis of this congener was straightforward and could be accomplished quickly. However, it was recognized at the time that it was only a weak antagonist (IC_{50} in the μM range) and that it might not be an ideal ligand to characterize the platelet thromboxane A_2/prostaglandin H_2 receptor. However, it could be used to test the notion that ^{125}I-labeled molecules could be synthesized to serve as useful radiolabeled ligands for characterization of thromboxane A_2 or prostaglandin H_2 receptors. Thus, this molecule has provided evidence that the chemical modification of eicosanoid analogs with subsequent iodination is feasible. Clearly more potent ligands will be required to study more extensively the putative thromboxane A_2 or prostaglandin H_2 receptors.

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