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SPECIFIC BINDING SITES FOR PROSTAGLANDIN D_2 ON HUMAN PLATELETS

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SUMMARY

^{3}H-PGD_2 was biosynthesized from ^{3}H-arachidonate and used to study the binding of PGD_2 to intact human platelets. The binding of ^{3}H-PGD_2 to platelets was rapid, being essentially complete within two min. Bound ^{3}H-PGD_2 was rapidly displaced by the addition of a 300-fold excess of non-radioactive PGD_2. Scatchard analysis of concentration-dependent binding indicated a single class of binding sites with a dissociation constant (K_D) of 4.12×10^{-7}M and a capacity of 760 sites per platelet. The relative ability of PGD_2, PGE_2, PGE_1 and PGI_2 to displace ^{3}H-PGD_2 bound to these sites was 100:2:2<1. We conclude therefore, that these PGD_2 binding sites are specific for PGD_2 and independent of those previously demonstrated to recognize ^{3}H-PGI_2 and ^{3}H-PGE_1.

PGI_2, PGE_1, and PGD_2 are all potent inhibitors of platelet aggregation (1) and stimulators of platelet adenyl cyclase (2). PGE_1, although the most studied prostaglandin, is only formed in significant amounts by seminal vesicles (3) and is unlikely to play a major role in the circulatory system. On the other hand, PGI_2 is produced by vascular endothelium (4) and may function in the maintenance of normal blood flow. PGD_2, although not normally found in plasma, has been shown to be produced by platelets during aggregation (5,6) and hence may play a role in the thrombotic process. Preliminary investigations based on species specificity (7) and differences in the dose-response characteristics associated with the elevation of platelet cyclic AMP (8) led to the hypothesis that platelets possess at least two types of prostaglandin receptors; one which recognizes PGI_2 and PGE_1, and the other only PGD_2. Receptor binding studies with ^{3}H-PGI_2 (9) and ^{3}H-PGE_1 (10) have confirmed that PGD_2 does not appear to interact with these binding sites. In this paper we report the existence of specific binding sites for ^{3}H-PGD_2 on human platelets which do not interact with either PGI_2 or PGE_1.

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EXPERIMENTAL PROCEDURES

Materials

{5,6,8,9,11,12,15 ^{3}H (N)}-arachidonic acid (S.A. 61 Ci/mmol), {5,6,8,9,12,14,15 ^{3}H (N)}-PGD_2 (S.A. 100 Ci/mmol) and ^{14}C-sucrose (S.A. 45 Ci/mol) were obtained from New England Nuclear Corp., Boston, Mass. Non-radioactive prostaglandins were a gift from Dr. John Pike, The Upjohn Co., Kalamazoo, Mich. Fatty acid-poor bovine serum albumin was obtained from Sigma. St. Louis, Mo.

Methods

^{3}H-PGD_2 was biosynthesized from ^{3}H-arachidonic acid using washed rabbit platelets (preincubated with 2 mM imidazole to inhibit thromboxane synthetase (11)) as the enzyme source. Incubations (final vol 0.6 ml) were carried out at 37°C with stirring in an aggregometer cuvette. Incubation buffer (pH 8.2) was Tris-HCl (15 mM), saline (140 mM), glucose (1 mM), EDTA (1 mM). The synthesis was initiated by the addition of 100 uCi (1.4 nmol) ^{3}H-arachidonic acid in 10.0 ul ethanol. Fatty acid poor bovine serum albumin (4 mg/ml) was added after 3 min to accelerate the decomposition of prostaglandin endoperoxides and increase the yield of ^{3}H-PGD_2 (12). After a total of 15 min, the incubation was terminated by dilution to 3 ml with ice-cold saline and acidification to pH 3.0-3.5 with 1 M citric acid. The reaction mixture was extracted 3 times with 2 vol ethyl acetate. The ^{3}H-PGD_2 was purified by thin layer chromatography (TLC) using benzene:dioxane:acetic acid (40:20:2) as the solvent system. The ^{3}H-PGD_2 region, as determined by radiochromatography against authentic non-radioactive standards, was eluted with dry acetone and chromatographed using the organic phase of ethyl acetate:isooctane:acetic acid:water (55:25:10:50) as the solvent system. The ^{3}H-PGD_2 region was eluted and its purity was confirmed by measuring the percent conversion to ^{3}H-$PGF_{2\alpha}$ in the presence of excess $NaBH_4$. (The final purity of all preparations was between 90-95%). The yield of ^{3}H-PGD_2 by this method was 2-12%.

Blood was drawn from healthy, drug-free volunteers into 1/10 vol 3.8% citrate. Platelet rich plasma (PRP) was obtained by centrifugation at 180 g x 15 min at 20°C. Platelet counts were determined using a Coulter Counter, Coulter Electronics Corp., Hileah, Fla. Binding experiments were performed as described previously (9). ^{3}H-PGD_2, obtained as described above, was diluted to the appropriate specific activity with non-radioactive PGD_2. Similar experiments were also performed with commercially available ^{3}H-PGD_2 and identical results were obtained. All samples were oxidized in a Packard 306 oxidizer and the radioactivity determined in a Packard 3003 liquid scintillation counter. Bound ^{3}H-PGD_2 (fmoles/10^8 platelets) was determined after correction for background, plasma space and specific activity.

RESULTS

As can be seen in Figure 1, the binding of ^{3}H-PGD_2 is rapid, being essentially complete within 2 min and remaining at approximately this level for at least the next 15 min. When 10 uM non-radioactive PGD_2 was added, 4 min after the beginning of the incubation, there was an extremely rapid displacement of the bound ^{3}H-PGD_2. This indicated that the binding was reversible and not due to uptake by the platelet. A semi-quantitative kinetic analysis of the bind-

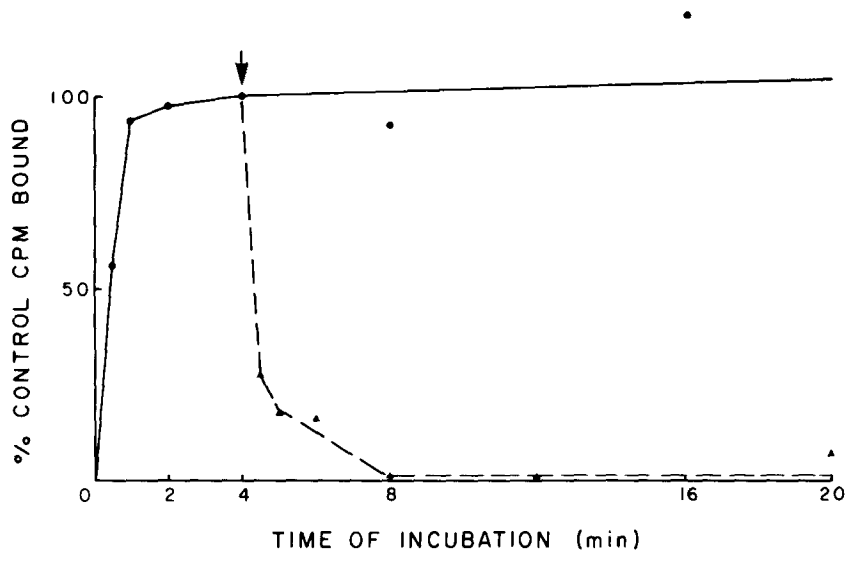

Figure 1 Time Course of Binding and Displacement of ^{3}H-PGD$_2$. Experiments were performed as described previously (9). Each point is the mean of 6 determinations on three subjects. 100% control is the amount of ^{3}H-PGD$_2$ bound to platelets 4 min after the addition of 30 nM ^{3}H-PGD$_2$ to platelet rich plasma (5.68 ± 0.23 fmoles/10^8 platelets). Displacement experiments were initiated by the addition of 10 uM non-radioactive PGD$_2$ at the 4 min (100% control) time point as indicated by the arrow. Platelet counts were 5.43 ± 0.15 x 10^8/ml. ●——● time course of binding; ▲----▲ time course of displacement.

ing and displacement data yielded an estimated dissociation constant (K_D) whose lower limit was 2 x 10^{-7}M (analysis not shown).

Concentration-dependent binding of ^{3}H-PGD$_2$ indicated a single class of binding sites upon Scatchard analysis (14) (Fig. 2). This line (r^2 = 0.987, $p<.001$) defines a set of binding sites with a dissociation constant of 4.12 (± 1.76) x 10^{-7}M and a capacity of 760 (± 120) sites per platelet.

The specificity of this ^{3}H-PGD$_2$ binding site was tested by measuring the ability of various non-radioactive prostaglandins to displace bound ^{3}H-PGD$_2$ as shown in Figure 3. The concentrations required for 50% displacement of 50 nM ^{3}H-PGD$_2$ were estimated as 2.5 x 10^{-6}M, 1.5 x 10^{-4}M and 5 x 10^{-4}M for PGD$_2$, PGE$_1$, and PGI$_2$ respectively. The concentrations of PGE$_2$, TxB$_2$ and PGF$_{2\alpha}$ required for 50% displacement were estimated as 1.25 x 10^{-4}M, 3 x 10^{-4}M and >5 x 10^{-4}M. The relative affinity of this ^{3}H-PGD$_2$ binding site for PGD$_2$, PGE$_2$, PGE$_1$, TxB$_2$, PGI$_2$ and PGF$_{2\alpha}$ was 100:2:2:<1:<1:<1 respectively.

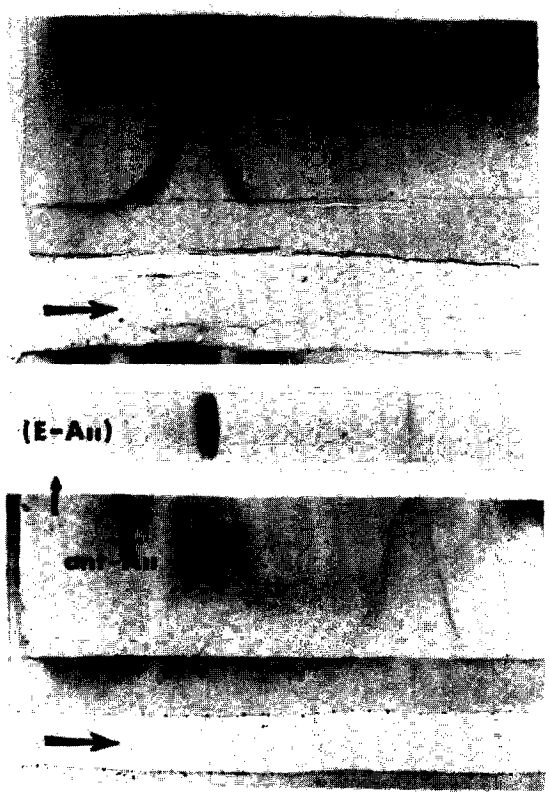

Fig. 3

Crossed immunoelectrophoresis of E-AII complex after reduction with mercaptoethanol. Horizontal arrows point to SDS gel slices imbedded in agarose containing 1% Triton X-100 and separately run in the second dimension containing either E antiserum (top) or AII antiserum (bottom). Precipitation rockets against AII are just visible near the AII and E regions respectively. A stained gel in the centre shows the position of E and AII proteins respectively.

be seen that I, II and E apoprotein reacted against E antisera, but not against AI or CII antisera. Only II (E-AII) reacted also against anti-AII. Neither reacted against anti-albumin. Other criteria for confirmation of immunochemical identity was shown by the complete fusion of the precipitin arcs of purified E and the E-AII and E trimer complexes (Fig. 2b). Additionally the identification of both E and AII

CONCLUSIONS AND DISCUSSION

To be relevant to the action of a hormone, data derived from hormone-receptor binding experiments should correlate with data on hormonal effects with regard to time and concentration. In addition, the hormone binding site should exhibit structural specificity and reversibility. The binding sites for ^{3}H-PGD_2 on human platelets appear to fulfill these criteria. The rapid binding of ^{3}H-PGD_2 agrees well with the rapid onset of inhibition of aggregation (unpublished data) and the rapid stimulation of adenyl cyclase (8). The rapid displacement of bound ^{3}H-PGD_2 by an excess of unlabeled PGD_2 indicates that this prostaglandin interacts reversibly with the platelet. The estimated dissociation constant obtained from kinetic analysis of the time course of binding and displacement (2×10^{-7}M) agrees well with the dissociation constant obtained from Scatchard analysis (4.12×10^{-7} M). This dissociation constant, in turn, agrees well with the ED_{50} (5×10^{-7}M) for the increase in platelet cAMP estimated from the results of Tateson et al (2) and confirmed by us (unpublished data). The finding that the ID_{50} for inhibition of platelet aggregation induced by 50 uM ADP is only 5×10^{-8}M (unpublished data) is not inconsistent with the above dissociation constants or with the concept that PGD_2 produces its effect directly through increases in platelet cAMP. Cuatrecasas and Hollenberg (14) have shown that, for many receptor systems, the further the effect is removed from the ligand-receptor interaction, the less likely there is to be a direct association between the ED_{50} for the biological response and the K_D determined by binding experiments. The specificity of the binding sites on platelets for PGD_2 as shown in Figure 3, indicates that they are indeed different from the PGI_2/PGE_1 binding sites.

We have demonstrated, therefore, binding sites for ^{3}H-PGD_2 on human platelets which appear to be the receptor sites involved in adenyl cyclase stimulation and inhibition of human platelet aggregation. Human platelets are the most sensitive to PGD_2 amongst several species examined (1,7). Further, Cooper et al(15) have reported that certain patients with myeloproliferative disorders exhibit

a decreased sensitivity to PGD_2 but not PGE_2 or PGI_2. These findings suggest that PGD_2 may play an important role in the human circulatory system.

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