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PROSTAGLANDIN I_2 RECEPTORS IN A PARTICULATE FRACTION OF PLATELETS OF VARIOUS SPECIES

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Abstract—The binding of [^{3}H]PGI_2 to a particulate fraction of human, bovine, canine and rat platelets was measured. Saturation of the binding sites was reached within 10–15 min at 0°. The dissociation constant K_D was in the range of 3.5 to 6.3×10^{-8} moles/l with only little interspecies variation. A structure–binding relationship was obtained from the competition curves after a logit–log transformation. [^{3}H]PGI_2 was displaced by a five-fold higher concentration of PGE_1. PGE_2 and $PGF_{2\alpha}$ were much less effective. The high specificity of the platelet receptor should allow the characterization of stable PGI_2-derivatives.

The binding of PGI_2 to specific receptor sites at the cell membrane of thrombocytes can be considered as an essential step for its biological activity. Previous studies have demonstrated high specificity binding of [^{3}H]PGE_1 and [^{3}H]PGI_2 to intact platelets [1, 2]. Schafer *et al.* have recently shown binding of [^{3}H]PGE_1 to a particulate fraction of washed human platelets [3]. Due to close similarities in the biochemical profile between PGE_1 and PGI_2 it was inferred in these studies that the two prostaglandins may share the same receptor site.

We now wish to present direct evidence that [^{3}H]PGI_2 binds to a particulate fraction of platelets of various species and that PGE_1 competes for the same binding site.

MATERIAL AND METHODS

[9-^{3}H]PGI_2, tetramethylammonium salt (sp. act. 12.6 Ci/mmole), was purchased from New England Nuclear, Boston, MA, U.S.A. The radiochemical purity was greater than 90 per cent by H.P.L.C. (reversed phase chromatography on LiChrosorb RP-18, elution with a gradient of phosphate buffer/methanol) and t.l.c. (solvent system of ethyl acetate–acetic acid–iso-octane–water = 90:20:5:100, organic phase). Unlabeled prostaglandins were kindly provided by Dr. Skuballa and Dr. Radüchel from the Department of Synthetic Chemistry, Schering AG, F.R.G.

Blood from rats, dogs, bulls and human volunteers was collected using citric acid, citrate and glucose as anticoagulant according to the ACD USP XVII Formula B. The blood was centrifuged at 160 *g* for 20 min at room temperature to remove red and white blood cells. The following steps were performed in the cold. The platelet-rich plasma was further centrifuged for 10 min at 1600 *g* and the resulting pellet suspended in a medium containing 140 mmoles/l NaCl, 5 mmoles/l KCl, 5 mmoles/l glucose, 5.75 mmoles/l citric acid and 2 mmoles/l sodium citrate. The platelets were disrupted by freezing and thawing. The material was sonicated twice for 10 sec with a MSE sonifier. The sonicate was centrifuged at 105,000 *g* for 30 min. The precipitate was suspended in the glucose citrate medium which had been adjusted to pH 7.5 with 2 moles/l Tris base. The final protein concentration was 1.5–2.5 mg/ml determined according to the method of Lowry *et al.* [4].

Portions (10 pmoles) of [^{3}H]PGI_2 in 10 μl 0.02 moles/l Tris–HCl, pH 8, and 10 μl of unlabeled prostaglandins in appropriate concentrations, as shown in the figures, were added to 180 μl of the platelet particles in the suspension buffer of pH 7.5. The samples were incubated for 10 min in an ice bath and applied to a short Sephadex G-50 column to separate free from particle-bound PGI_2. To prepare the columns, Sephadex G-50 was suspended in 0.01 moles/l Tris–HCl, pH 7.5, containing 0.154 moles/l NaCl, and applied to Pasteur pipettes which had each been fitted with a small plug of quartz wool. The column bed volume was 2 ml. Prior experiments had shown that protein-bound PGI_2 was eluted with 1 ml of buffer. Unbound PGI_2 was quantitatively released from the column with 3.5 ml of Tris–NaCl buffer. The separation was performed at 4° in a cold room. The time required to elute bound PGI_2 was generally less than 2 min. After adjusting the volumes of the eluates with buffer, radioactivity was counted in both fractions with Instagel in a Packard Liquid Scintillation counter. Specific binding was defined as the amount of the total [^{3}H]PGI_2 bound that was displaced by 10^{-4} moles/l unlabeled prostaglandin.

RESULTS

Binding of [^{3}H]PGI_2 to a particulate fraction of bovine platelets was readily saturable within 10–

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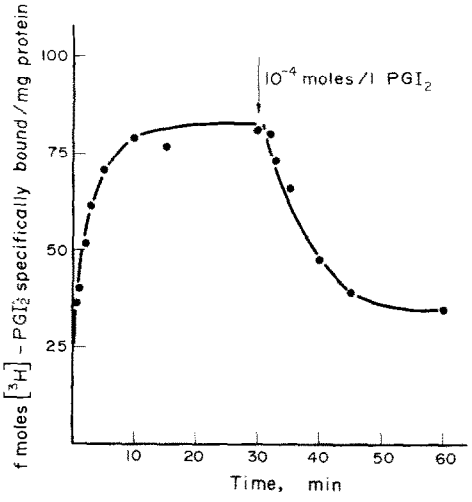

Fig. 1. Association and dissociation of $[^3H]PGI_2$ in a bovine platelet particulate fraction. After incubation of 5×10^{-8} moles/l $[^3H]PGI_2$ for 30 min, 10^{-4} moles/l unlabeled PGI_2 was added to measure dissociation. The points represent the mean of two experiments.

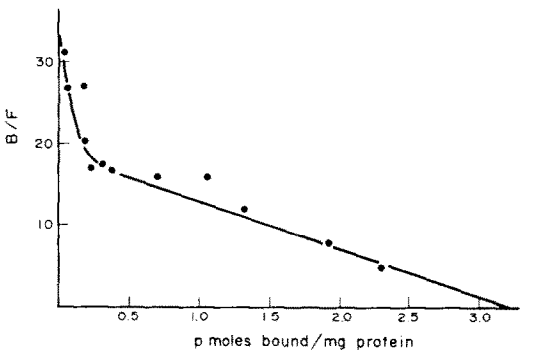

Fig. 3. Scatchard plot of $[^3H]PGI_2$ bound to a bovine platelet particulate fraction. The points represent the mean of three experiments.

15 min at 0°. Specifically bound $[^3H]PGI_2$ could be displaced to about 50 per cent by high concentrations of unlabeled PGI_2 (Fig. 1). Binding of PGI_2 was dependent on the protein concentration of the particles of the platelet preparation (Fig. 2). The transformation of saturation curves according to Scatchard [5] allowed the determination of binding parameters. Two binding sites were found in particles of bovine platelets with dissociation constants of $3.5 \pm 0.7 \times 10^{-8}$ moles/l and $3.2 \pm 1.9 \times 10^{-7}$ moles/l, respectively (Fig. 3). The high affinity site could bind 0.36 pmole/mg of protein and the low affinity site 2.82 pmoles/mg of protein. Dissociation constants were also determined in human, canine and rat platelets. The high affinity, low capacity sites from the three platelet types had dissociation constants of 6.3×10^{-8}, 4.2×10^{-8} and 5.5×10^{-8} moles/l, respectively. All experiments had been performed at least in duplicate. The interspecies differences were only small. Although there was little variation in the dissociation constants between each experiment, the number of binding sites varied considerably.

In order to determine the relative binding affinity of other prostaglandins, the displacement of $[^3H]PGI_2$ by unlabeled material was plotted as per cent binding vs the log molar concentration. Such displacement curves can be linearized by a logit–log transformation [6]. The results of parallel line assay analysis show that PGE_1 was 5.4 times less active than unlabeled PGI_2 in displacing radioactivity from the receptor (Fig. 4). PGE_2 was about 230 and $PGF_{2\alpha}$ more than 500 times less potent than PGI_2. 6-Keto-$PGF_{1\alpha}$, the stable isomerization product of PGI_2, had no affinity for the receptor. The fact that five times higher concentrations of PGE_1 were needed to displace PGI_2 from the binding site was in good agreement with the dissociation constant found from Scatchard plots with $[^3H]PGE_1$. The affinity was $1.2 \pm 0.1 \times 10^{-7}$ moles/l, which is about 3.5 times that of PGI_2. Because the displacement curves for PGI_2 after logit–log transformation were parallel and

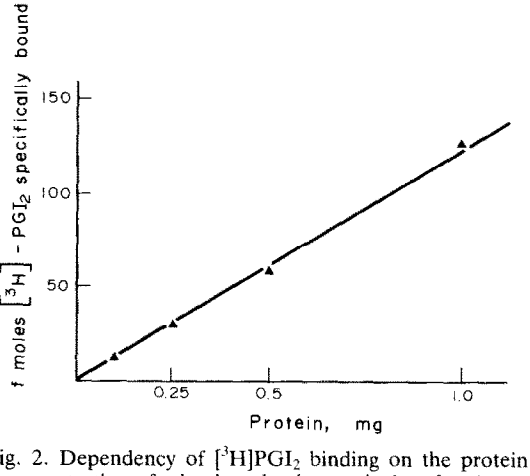

Fig. 2. Dependency of $[^3H]PGI_2$ binding on the protein concentration of a bovine platelet particulate fraction.

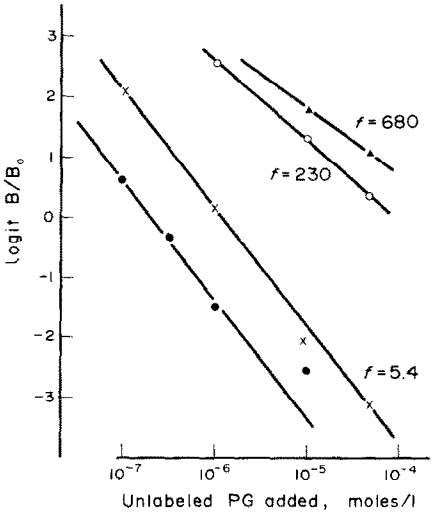

Fig. 4. Relative binding activity of PGI_2, (●), PGE_1 (×), PGE_2 (○) and $PGF_{2\alpha}$ (▲) after logit–log transformation.

because a double reciprocal plot of the data suggested competitive inhibition it is conceivable that PGI_2 and PGE_1 may share the same receptor molecule.

DISCUSSION

Saturation of the receptor molecules by prostaglandins is generally obtained within 30–60 min at room temperature [7, 8]. From the present experiments and also from those of Schafer *et al.* [3] it is apparent that the velocity of binding of PGI_2 is much faster than that seen with other prostaglandins. Even at 0° almost complete saturation of the binding sites is reached after only 10 min. The association of PGI_2 with the receptor *in vivo* is probably even more rapid. Similar fast association reactions are only seen with receptors of neurotransmitters [9].

The integrity of the PGI_2-receptor appears to be highly susceptible to inactivation. Already minute changes during the isolation procedure of the platelets prevented binding of [^{3}H]PGI_2 to the high affinity site.

The specificity of the PGI_2-binding site correlates well with the anti-aggregatory potency of these prostaglandins in platelet rich plasma. PGI_2 has so far been the most potent inhibitor of platelet aggregation, PGE_1 having about 6–12 per cent of the activity of PGI_2. PGE_2 prevents platelet aggregation only in very high concentrations whereas $PGF_{2\alpha}$ is practically inactive (W. F. Losert, personal communication). [^{3}H]PGI_2 is displaced from the receptor site by these prostaglandins in a corresponding fashion. We have previously shown that PGI_2 has no affinity for the PGE_2 receptor in human uterine particles, in contrast to PGE_1 which showed 100 per cent cross-reactivity [10]. It appears, therefore, that the anti-aggregatory PGE_1, unlike PGI_2, recognizes the geometry of both the PGI_2 and PGE_2 receptor molecule. Species variations in the characteristics of the PGI_2-binding site in platelet particulate fractions have not been observed.

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