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[^{3}H]Iloprost and prostaglandin E_2 compete for the same receptor site on cardiac sarcolemmal membranes

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We have previously demonstrated that high-affinity PGE receptors are present on purified cardiac sarcolemmal (SL) membrane from bovine heart (Lopaschuk et al. (1989) Circ. Res. 65, 538–545). In this study we determined whether PGI_2 receptors are also present on the cardiac SL membrane. Due to the extreme lability of prostacyclin (PGI_2) under physiological conditions, the PGI_2 analogue, Iloprost was substituted for PGI_2. ^{3}H-Iloprost specifically bound to two sites on the SL membrane; one of high affinity (K_d = 0.3 nM, B_{max} = 97.0 fmol/mg SL), and one of lower affinity (K_d = 20.6 nM, B_{max} = 1589 fmol/mg SL). Competition studies demonstrated that the concentrations of PGE_2 and PGE_1 necessary to displace 50% of the specific binding of 20 nM [^{3}H]Iloprost on cardiac SL were 15-fold lower than the concentrations of unlabelled Iloprost necessary to displace 50% of binding. In contrast, a 15-fold higher concentration of unlabelled Iloprost was needed to displace 50% of specific binding of 2 nM [^{3}H]PGE_2 compared to the concentrations of PGE_1 or PGE_2 required to displace 50% of [^{3}H]PGE_2 binding. In summary, our results indicate that a prostacyclin receptor is present on the cardiac sarcolemmal membrane, and that PGI_2 competes for the same receptor site as PGE_2.

Introduction

PGE_2, prostacyclin (PGI_2) and $PGF_{2\alpha}$ are the major prostaglandins produced by the myocyte [1]. We have previously identified and characterized a PGE_2 receptor on cardiac sarcolemmal (SL) membrane [2–4]. The receptor is a 100 kDa protein and contains two high-affinity binding sites.

In platelets it has been demonstrated that PGE_2 and prostacyclin share common receptor sites [5–9]. In these membranes, two binding sites for the PGE/PGI_2 receptor(s) have been identified, one of high affinity and one of low affinity [7,8]. Studies on a purified human platelet PGE/PGI_2 receptor have suggested that the two binding affinities reside on the same protein molecule [9]. Ashby [7] has recently suggested that the different prostaglandin binding affinities for membrane binding may be a result of coupling of the prostaglandin receptor to both inhibitory and stimulatory G proteins.

Abbreviations: SL, sarcolemma; PMSF, phenylmethylsulfonyl fluoride; PG, prostaglandin.

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Although we have demonstrated that PGE_2 binds to the cardiac SL PGE_2 receptor with both a high and low affinity, it has not been determined if a specific prostacyclin binding site is present on the cardiac SL membrane. It has also not been determined if PGI_2 competes with PGE_2 for binding to the cardiac PGE_2 receptor. We therefore determined whether [^{3}H] Iloprost (the stable analogue of prostacyclin) specifically binds to cardiac SL and whether PGE_2 and Iloprost share the same binding site. Our results demonstrate that [^{3}H]Iloprost does specifically bind to cardiac SL membrane, and that both PGE_2 and prostacyclin share common receptor sites on these membranes.

Experimental procedures

Polymethylsulphonyl fluoride (PMSF), benzamidine, Hepes, PGE_2, PGE_1, PGD_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were purchased from Sigma. [5,6,8,11,12,14,15(n)-^{3}H]PGE_2 was obtained from New England Nuclear. Iloprost and [^{3}H]Iloprost were purchased from Schering (Germany). All other chemicals were of the highest commercial grade.

Purified cardiac SL vesicles were isolated from the left ventricle of fresh adult bovine hearts by a sucrose flotation method previously described [2,4]. This was

modified slightly by the addition to all buffers used in the preparation of the SL vesicles of the proteolytic enzyme inhibitors PMSF and benzamidine, both at the concentration of 0.1 mM. Vesicles were suspended at 3–4 mg protein/ml in 100 mM NaCl, 20 mM Hepes (pH 7.4), aliquoted, frozen in liquid nitrogen and stored at −80°C. Immediately before use frozen membranes were quickly thawed at 37°C. Excess thawed membrane was discarded. Protein concentration was determined by the method of Lowry et al. [10].

[^{3}H]PGE_2 displacement by PGE_2, PGE_1, PGD_2, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and Iloprost was performed as described previously [2]. [^{3}H]Iloprost displacement by unlabelled Iloprost, PGE_2, PGE_1, PGD_2, 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ was performed using the same procedure as described previously for [^{3}H]PGE_2 [2].

Saturation binding curves for [^{3}H]Iloprost were obtained using the same procedure as previously described for [^{3}H]PGE_2 [2,4]. Scatchard plot values were obtained using the Ligand program on an IBM personal computer with the validity of the model determined by both the 'runs test' (pass/fail $P \leqq 0.05$) and the partial F-test with $P \leqq 0.05$ considered significant [11].

Results and Discussion

The cardiac SL membrane preparation used in these studies consists of 70% tightly sealed right-side-out vesicles, 15% tightly sealed inside-out vesicles, and 15% 'leaky' vesicles [2,4]. This was determined by measuring alamethacin (a non-selective ionophore) sensitive adenylate cyclase activity. Therefore, the majority of extracellular receptor binding sites are on the external surface of these vesicles. The SL vesicles possess an electrogenic Na^+-Ca^{2+} exchanger, Ca^{2+}/calmodulin-dependent ATPase, ATP-dependent Ca^{2+} uptake activity, as well as high levels of both [^{3}H]nitrendipine and [^{3}H]quinuclinidyl binding; all indicating that the membranes isolated are of myocyte plasma membrane origin [2]. As well, this preparation was tested for endothelial membrane contamination, which was found to be less than 5% [2].

Fig. 1 shows that [^{3}H]Iloprost specifically binds to cardiac SL membrane vesicles. Binding was complete by 60 min and was linearly proportional to SL membrane concentration (data not shown). Scatchard analysis of this binding (Fig. 1B) shows that [^{3}H]Iloprost binds to two sites on the membrane, a high-affinity site with a K_d of 0.29 ± 0.37 nM, and B_{max} of 97.0 ± 59.0 fmol bound/mg protein, and a lower affinity site with an average K_d of 20.6 ± 5.0 mM and B_{max} of 1589 ± 294 fmol bound/mg protein. This compares with the values obtained previously with [^{3}H]PGE_2 using the same membrane preparation and kinetic analysis which gave a high-affinity site ($K_d = 0.018$ nM, $B_{max} = 77.1$ fmol bound/mg protein) and a low-affinity site ($K_d = 1.9$ nM, $B_{max} = 997.9$ fmol bound/mg protein) [4].

In competition experiments, 20 nM [^{3}H]Iloprost is displaced by unlabelled Iloprost concentrations in the range of 10^{-8} to 10^{-6} M. However, unlabelled PGE_2 and PGE_1 were approx. 15-fold more effective than unlabelled Iloprost in displacing 20 nM [^{3}H]Iloprost from the cardiac SL membrane (Fig. 2). PGD_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were found to be ineffective in displacing [^{3}H]Iloprost; concentrations of more than $3 \cdot 10^{-7}$ M of these ligands were needed before displacement of [^{3}H]Iloprost occurred (data not shown).

In competition experiments involving 2 nM [^{3}H]PGE_2, concentrations of unlabelled Iloprost in the range of 10^{-7} to 10^{-6} M were necessary to displace [^{3}H]PGE_2 from the cardiac SL (Fig. 3). In these experiments, unlabelled PGE_2 and PGE_1 were approx. 15-fold more effective than unlabelled Iloprost in displacing [^{3}H]PGE_2 from the cardiac SL membrane (Fig. 3). Again, as with the [^{3}H]Iloprost experiments, PGD_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were ineffective in displacing [^{3}H]PGE_2 from the membrane; concentrations higher than $2 \cdot 10^{-7}$ M of these ligands were needed before displacement of [^{3}H]PGE_2 occurred (data not shown).

Our data suggest that Iloprost and PGE_2 bind to the same sites on cardiac SL. Evidence for this is provided by the following observations; (a) PGE_1 and PGE_2 will effectively displace [^{3}H]Iloprost from the membrane, at concentrations 15-fold lower then unlabelled Iloprost (Fig. 2), and (b) Iloprost will also displace [^{3}H]PGE_2, although 15-fold greater concentrations of unlabelled Iloprost were required than the concentration of unlabelled PGE_1 or PGE_2. The reason that higher concentrations of Iloprost are necessary probably reflects the observation that Iloprost has an approx. 20-fold lower affinity for binding on cardiac SL then does [^{3}H]PGE_2 (Fig. 1 and Refs. 2 and 4). Further evidence to support the concept of similar receptor sites for PGE_2 and PGI_2 will require the development of antagonists that bind to the PGE_2 and/or PGI_2 receptor site.

The primary evidence for a single receptor site shared by PGI_2 and PGE_1 originates from studies on platelet membranes [5–9]. Relatively few studies, however, have determined whether these two ligands share similar receptor sites in other tissues. Garrity et al. [13] studied this potential interrelationship in liver membranes, and concluded that PGE_1 and PGI_2 interact at distinct receptors. Even studies performed on platelet membranes are not in complete agreement that a common receptor site exists. Dutta-Roy and Sinha [9] have purified a protein from human blood platelets which binds [^{3}H]PGE_1 at both a high and low-affinity site. Unlabelled PGI_2 was shown to effectively compete with [^{3}H]PGE_1 for binding to the receptor protein. However, a recent study by Tsai et al. [12] which also

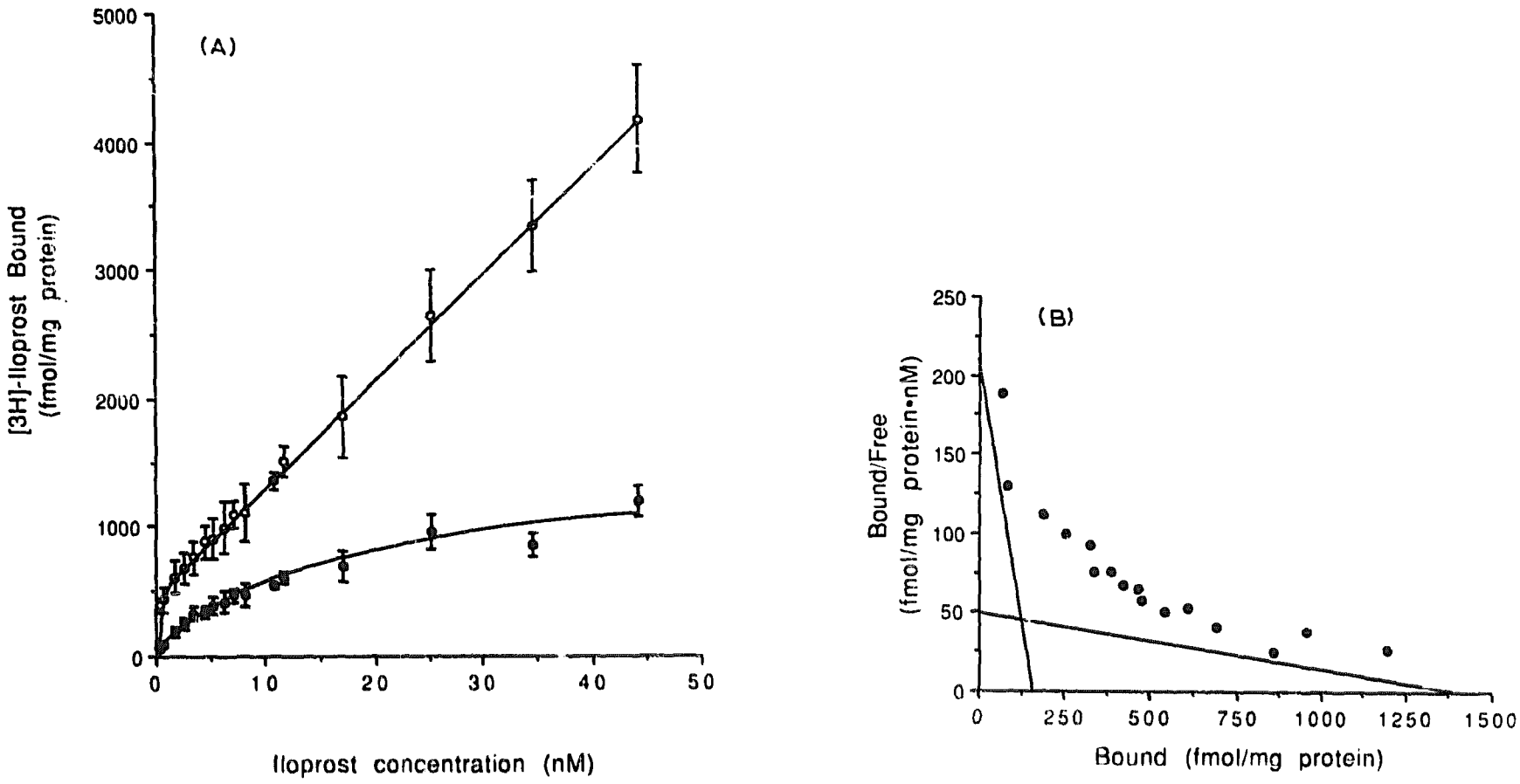

Fig. 1. Specific binding of [^{3}H]Iloprost to cardiac sarcolemmal vesicles. The [^{3}H]Iloprost binding curves (A) are the mean ± S.E. of four experiments performed in duplicate. (B) shows the Ligand generated Scatchard plot of the specific binding curve. In all cases, LIGAND analysis determined that two sites were preferred over one site with $P \leq 0.05$. Open circles, total [^{3}H]Iloprost bound; closed circles, specific [^{3}H]Iloprost bound.

used the solubilized platelet prostacyclin receptor, showed that binding of [^{3}H]Iloprost occurred at a single high-affinity site. In addition, these authors suggested that this site is distinct from the PGE_1 binding site. The reasons for the differences between these studies has yet to be established.

Although both cardiac SL and platelet membrane appear to contain a common PGE/PGI_2 receptor, a number of differences between these two membrane systems is evident. The affinity for PGE_1 or PGE_2 binding is considerably higher in cardiac SL than the affinity for PGE_1 binding to platelet membrane [2–9]. Furthermore, although [^{3}H]Iloprost binds to cardiac SL with a lower affinity than [^{3}H]PGE_2, the binding affinity is still considerably higher than the binding affinity of [^{3}H]Iloprost for the platelet receptor. The significance of these differences is unclear, but should be answered with further purification of the cardiac receptor. Another difference between the cardiac SL and platelet receptors is that both PGE1 and PGI2

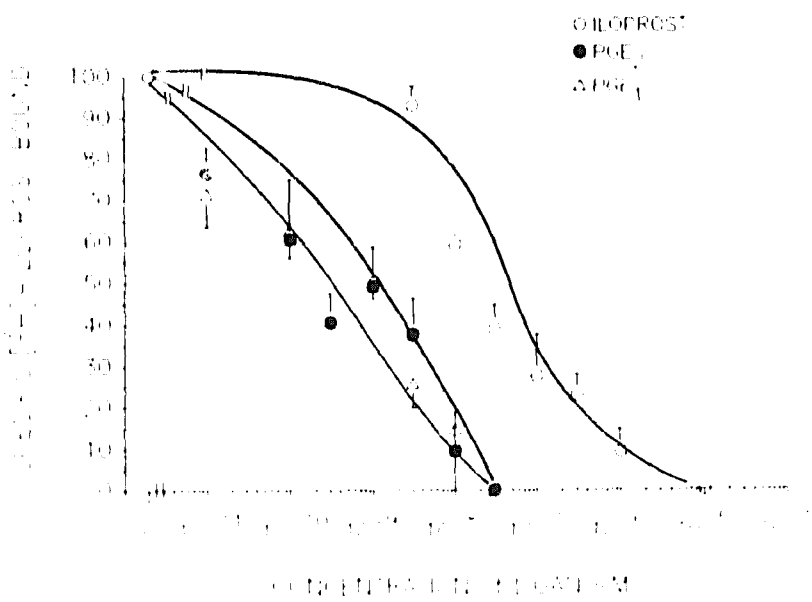

Fig. 2. Competition of [^{3}H]Iloprost binding to cardiac sarcolemmal vesicles by unlabelled PGE_1, PGE_2, and Iloprost. A concentration of 20 nM [^{3}H]Iloprost was used in these experiments. Each curve is mean ± S.E. of three experiments performed in duplicate. Open circles, unlabelled Iloprost; closed circles, unlabelled PGE_2; open triangles, PGE_1.

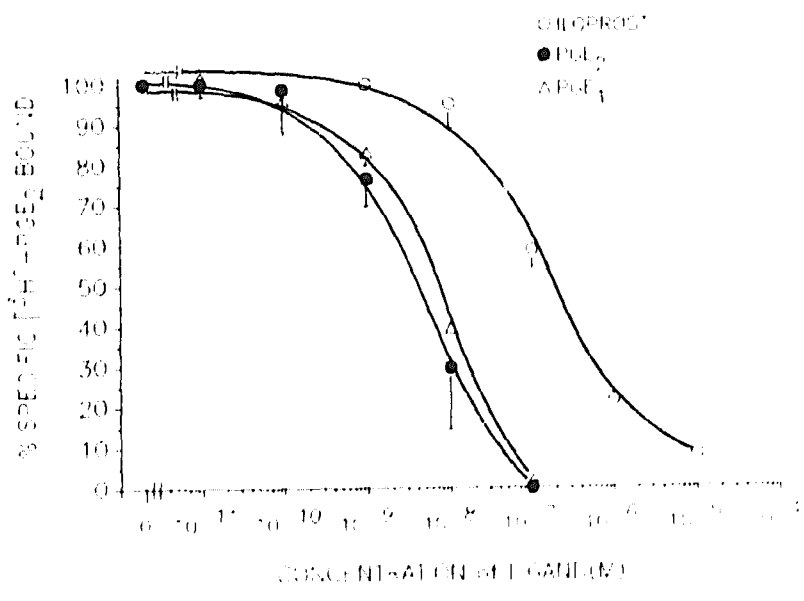

Fig. 3. Competition of [^{3}H]PGE_2 binding to cardiac sarcolemmal vesicles by unlabelled PGE_1, PGE_2 and Iloprost. A concentration of 2 nM [^{3}H]PGE_2 was used in these experiments. Each curve is the mean ± S.E. of three experiments performed in duplicate. Open circles, unlabelled Iloprost; closed circles, unlabelled PGE_2; open triangles, PGE_1.

stimulate adenylyl cyclase activity equally in platelet membrane [9]. In contrast, in the cardiac SL membrane PGE2 attenuates adenylyl cyclase activity at low concentrations [2,4], while prostacyclin has been shown to increase cAMP formation in myocytes [11]. The different binding affinities of PGE2 and prostacyclin/Iloprost may be due to coupling to inhibitory and stimulatory sites as has been proposed for platelets [7]. The effects of Iloprost on adenylyl cyclase activity in cardiac SL membranes has yet to be investigated.

In summary, we have identified a specific binding site for prostacyclin/Iloprost in bovine cardiac SL membrane which can be resolved into two sites (or state of one site); one of high affinity and low capacity, and one site of lower affinity and higher capacity. Iloprost binding is effectively competed for by PGE_2 and PGE_1 but not PGD_2, 6-keto-$PGF_{1\alpha}$ or $PGF_{2\alpha}$. The use of highly purified membranes and stable prostacyclin analogues should be useful in further characterizing the direct effects of prostacyclin and other eicosanoids on the heart.

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