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# Characterization of prostaglandin-$F_{2\alpha}$-binding sites on rat hepatocyte plasma membranes

Frank NEUSCHÄFER-RUBE, Gerhard P. PÜSCHEL and Kurt JUNGERMANN

Institut für Biochemie und Molekulare Zellbiologie, Fachbereich Medizin, Georg-August-Universität Göttingen, Federal Republic of Germany

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Prostaglandin (PG) $F_{2\alpha}$ has previously been shown to increase glucose output from perfused livers and isolated hepatocytes, where it stimulated glycogen phosphorylase via an inositol-trisphosphate-dependent signal pathway. In this study, $PGF_{2\alpha}$ binding sites on hepatocyte plasma membranes, that might represent the putative receptor, were characterized.

Binding studies could not be performed with intact hepatocytes, because $PGF_{2\alpha}$ accumulated within the cells even at 4 °C. The intracellular accumulation was an order of magnitude higher than binding to plasma membranes.

Purified hepatocyte plasma membranes had a high-affinity/low-capacity and a low-affinity/high-capacity binding site for $PGF_{2\alpha}$. The respective binding constants for the high-affinity site were $K_d$ = 3 nM and $B_{max}$ = 6 fmol/mg membrane protein, and for the low-affinity site $K_d$ = 426 nM and $B_{max}$ = 245 fmol/mg membrane protein.

Specific $PGF_{2\alpha}$ binding to the low-affinity site, but not to the high-affinity site, could be enhanced most potently by GTP[$\gamma$S] followed by GDP[$\beta$S] and GTP, but not by ATP[$\gamma$S] or GMP.

$PGF_{2\alpha}$ competed most potently with [$^3$H]$PGF_{2\alpha}$ for specific binding to hepatocyte plasma membranes, followed by $PGD_2$ and $PGE_2$.

Since the low-affinity $PGF_{2\alpha}$-binding site had a $K_d$ in the concentration range in which PG had previously been shown to be half-maximally active, and since this binding site showed a sensitivity to GTP, it is concluded that it might represent the receptor involved in the $PGF_{2\alpha}$ signal chain in hepatocytes. A biological function of the high-affinity site is currently not known.

Eicosanoids play an important role in the signal pathway between non-parenchymal and parenchymal liver cells [1–3]. They are involved in the regulation of liver metabolism and hemodynamics by sympathetic hepatic nerves [4, 5], cell-wall particles from yeast (zymosan) [6], endotoxins [7], heat-aggregated immunoglobulins [8], peptides of the activated complement system [9–11], platelet-activating factor [12] and extracellular nucleotides and nucleosides [13, 14]. Prostaglandins (PG) $F_{2\alpha}$, $E_2$ and $D_2$ stimulated glucose and lactate output and increased resistance in perfused rat liver [15–17]. $PGF_{2\alpha}$ and $PGE_2$ enhanced glucose output from isolated hepatocytes by an $\alpha_1$-adrenergic-like activation of glycogen phosphorylase via an inositol-trisphosphate-dependent signal pathway [18, 19]. Besides this glycogenolytic activity, PG have an antiglycogenolytic, $\alpha_2$-adrenergic-like effect, i.e. they attenuate the glucagon-stimulated elevation of cAMP and glycogen phosphorylase activity in hepatocytes [20–23].

In order to study hepatic PG-binding sites, plasma membranes have thus far been isolated from whole liver homogenates [24–27]. However, the liver is composed of different cell types, i.e. parenchymal cells (hepatocytes) and non-parenchymal cells comprising resident macrophages (Kupffer cells), endothelial cells and perisinusoidal cells (Ito or fat-storing cells). Hepatocytes represent about 90% of the liver-cell mass, but only about 60% of the total cell number and thus surface [28, 29]. Non-parenchymal liver cells also show biological responses to PG [30] and therefore must possess PG receptors that are not necessarily identical with PG receptors on hepatocytes. It was, therefore, the purpose of this study to characterize binding sites, which might represent the postulated $\alpha_1$-adrenergic-like $PGF_{2\alpha}$ receptor in hepatocyte plasma membranes not contaminated with non-parenchymal cell membranes.

It was found that hepatocyte plasma membranes contain a high-affinity/low-capacity binding site and a low-affinity/high-capacity binding site for $PGF_{2\alpha}$, the latter having the properties expected for an involvement in $\alpha_1$-adrenergic-like action.

*Correspondence to* G. P. Püschel, Institut für Biochemie und Molekulare Zellbiologie, Georg-August-Universität, Humboldtallee 23, W-3400 Göttingen, Federal Republic of Germany

*Abbreviations.* PG, prostaglandin(s); GTP[$\gamma$S], guanosine 5′-[$\gamma$-thio]triphosphate; GDP[$\beta$S], guanosine 5′-[$\beta$-thio]diphosphate; ATP[$\gamma$S], adenosine 5′-[$\gamma$-thio]triphosphate; G protein, guanine-nucleotide-binding regulatory protein.

*Enzymes.* 5′-Nucleotidase (EC 3.1.3.5); glucose-6-phosphatase (EC 3.1.3.9).

## MATERIALS AND METHODS

### Chemicals

All chemicals were of reagent grade and from commercial sources. $PGF_{2\alpha}$, $PGE_2$ and $PGD_2$ were from Paesel (Frankfurt, FRG) and [$^3$H]PG from New England Nuclear

(Dreieich, FRG) or Amersham Buchler (Braunschweig, FRG). Glass-fiber filters GF52 were supplied by Schleicher und Schüll (Dassel, FRG). Sepac reverse-phase minicolumns were obtained from Waters (Milford, USA).

### Animals

Male Wistar rats (200–300 g, Winkelmann, Borchen, FRG) were kept on a 12-h day/night rhythm (light from 7 a.m. to 7 p.m.), with free access to water and food (standard rat diet, Ssniff, Soest, FRG). Rats were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body) for hepatocyte preparations (between 9 a.m. and 11 a.m.).

### Hepatocyte and plasma-membrane preparation

Hepatocytes were isolated according to Merredith without the use of collagenase [31] in order to prevent proteolysis of surface proteins during the isolation procedure. The method was slightly modified. The liver was perfused *in situ* without recirculation with a $Ca^{2+}$-free Krebs-Henseleit buffer containing 2 mM EDTA for 30–40 min. The liver capsule was opened and the hepatocytes were suspended in sodium phosphate, pH 7.4, containing 140 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$ and 1 mM $CaCl_2$. Hepatocytes were filtered through nylon gauze (60 μm pore size) then separated from detritus and non-parenchymal liver cells by centrifugation at 25 *g* for 2 min. The pellet was resuspended in the same buffer (about 200 mg/ml) and again centrifuged at 25 *g* for 2 min. This washing procedure was repeated three times. Thereafter, the hepatocytes were about 75% viable, as controlled by trypan-blue exclusion, and essentially free of contaminating non-parenchymal liver cells, as judged by light microscopy.

Hepatocytes (100 mg/ml) were homogenized in 250 mM sucrose in 10 mM Tris/HCl, pH 7.4, containing 0.5 mM $CaCl_2$, 0.5 mM phenylmethylsulphonyl fluoride and 0.5 mM benzamidine at 4 °C, in a glass homogenizer with a tight-fitting Teflon pestle, in a homogenization apparatus for 10 min with 40 strokes/min at 900 rpm. A plasma-membrane-enriched fraction was obtained by separation of membrane subfractions of the nuclear (1500 *g*) pellet and the postmitochondrial 100000 *g* pellet on sucrose gradients according to Pietras and Szego [32], except that the final purification by recentrifugation on a sucrose gradient was omitted. As marker enzyme for plasma membranes and endoplasmic reticulum, 5′-nucleotidase and glucose-6-phosphatase were determined by standard assays, respectively [33]. Protein content was determined by the method of Lowry [34]. The membrane fractions with the highest specific activity of 5′-nucleotidase and $PGF_{2\alpha}$ binding were pooled, aliquoted and frozen at −20 °C for later use.

### Determination of $PGF_{2\alpha}$ binding

Hepatocyte plasma membranes were incubated with [$^3$H]$PGF_{2\alpha}$ and varying amounts unlabelled $PGF_{2\alpha}$ at room temperature for 1 h if not stated otherwise. The assay buffer was a 10 mM Hepes, pH 7.4, containing 120 mM NaCl, 4.8 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$ and 1.2 mM $KH_2PO_4$ (buffer A). The assay contained 350–550 μg membrane protein/tube in a total volume of 0.1 ml. Bound label was separated from free ligand by vacuum filtration using GF52 glass-fiber filters. Specific binding was defined as the difference of the radioactivity bound to the plasma membranes in absence or presence of a 5000-fold excess of unlabelled $PGF_{2\alpha}$.

In saturation-binding assays hepatocyte plasma membranes were incubated with 0.5–25000 nM $PGF_{2\alpha}$ containing 7.66, 0.15 or 0.015 TBq/mmol [$^3$H]$PGF_{2\alpha}$. In competition binding assays, membranes were incubated with 5 nM [$^3$H]$PGF_{2\alpha}$ (7.66 TBq/mmol) and 0–10 μM unlabelled $PGF_{2\alpha}$, 0–32 μM $PGD_2$ or 0–32 μM $PGE_2$. An aliquot of each incubation was measured for total radioactivity. Non-specific binding and binding constants were calculated using the computer program LIGAND [35] provided by the NIH (Bethesda, MD, USA).

### Exclusion of PG degradation during the binding assays

[$^3$H]$PGE_2$, [$^3$H]$PGD_2$ or [$^3$H]$PGF_{2\alpha}$ (5 nM) were incubated with plasma membranes (400 μg membrane protein in 0.1 ml) for 1 h at room temperature. Samples were taken at times zero and at the end of the incubation time, then extracted with 5 ml 15% ethanol, applied to a Sepac column, which was then washed with 5 ml 15% ethanol, and subsequently eluted with 5 ml 100% ethanol. The eluate was concentrated then separated with an HPLC system (Kontron) equipped with an on-line radioactivity monitor (Berthold) on $C_{18}$ reverse-phase column (Spherisorb 50 DS2) by isocratic elution with 32.9% acetonitrile in 17 mM phosphoric acid, pH 3.5.

## RESULTS

### Binding studies with intact cells

Initially the binding studies for the characterization of the PG receptor were to be performed with freshly isolated intact hepatocytes. In order to prevent endocytosis, binding studies were carried out at 4 °C. Cells (30 mg/ml) were incubated for 2 h with 0.5 nM [$^3$H]$PGF_{2\alpha}$. Bound ligand was separated from free ligand by centrifugation (5 min, 300 *g*). The pellet was washed twice in assay buffer. After the last centrifugation 200 fmol $PGF_{2\alpha}$/g intact cells was retained, 95% of which could be ascribed to specific binding (Fig. 1). Bound ligand could not be released by incubation of the cells with trypsin for 1 h at 4 °C (not shown). Yet, if cells were lysed by freezing/thawing, prior to the last centrifugation, only 20% of the radioactivity bound to intact cells was retained in the pellet. These findings indicated that in intact cells the majority of $PGF_{2\alpha}$ was trapped inside the cells, possibly bound to an intracellular binding protein in a saturable manner, and did not represent ligand bound to an extracellular receptor on the plasma membrane.

Plasma membranes purified from hepatocytes were incubated at room temperature for 1 h with 5 nM [$^3$H]$PGF_{2\alpha}$, sedimented at 4000 *g* and washed twice in assay buffer. About 10 fmol $PGF_{2\alpha}$/mg membrane protein was bound, 45% of which could be ascribed to specific binding (Fig. 1). Freezing/thawing and subsequent washing of the membrane pellet with assay buffer did not change specific binding. Thus, trapping of label within membrane vesicles could be excluded. Therefore, the binding studies were not carried out with intact cells as initially intended, but with hepatocyte plasma membranes.

### Purification of plasma membranes

Since it was the aim of this study to characterize PG-binding sites on hepatocytes, plasma membranes were not isolated from whole livers but from purified hepatocytes in order to avoid contamination with plasma membranes from non-parenchymal liver cells.

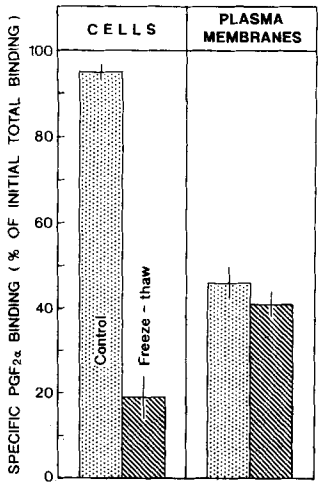


**Fig. 1. Trapping of $PGF_{2\alpha}$ in hepatocytes.** Hepatocytes (30 mg/ml) were incubated for 1 h at 4°C with 0.5 nM [$^3$H]$PGF_{2\alpha}$ with or without a 20000-fold excess of unlabelled $PGF_{2\alpha}$. Cells were sedimented by centrifugation at 300 *g* and either resuspended in assay buffer immediately or frozen/thawed prior to resuspension. Bound ligand was separated from free ligand by centrifugation at 4000 *g*. Hepatocyte plasma membranes (4 mg/ml), purified as described in Materials and Methods, were incubated at room temperature for 1 h with 5 nM [$^3$H]$PGF_{2\alpha}$, with or without a 5000-fold excess of unlabelled $PGF_{2\alpha}$, then treated as described for hepatocytes. Specific binding was defined as the difference of [$^3$H]$PGF_{2\alpha}$ bound in the presence or absence of excess unlabelled ligand. Maximal binding in absence of unlabelled ligand was set equal to 100%.

**Table 1. Copurification of 5′-nucleotidase activity and specific $PGF_{2\alpha}$ binding during the preparation of hepatocyte plasma membranes.** Hepatocytes were homogenized in 10 mM Tris/HCl, pH 7.4, containing 250 mM sucrose, 0.5 mM $CaCl_2$, 0.5 mM phenylmethylsulfonyl fluoride and 0.5 mM benzamidine, with a Potter homogenizer. Nuclear (1500 *g*), mitochondrial (10000 *g*) and 100000 *g* fractions were prepared by differential centrifugation. The nuclear pellet and the 100000-*g* pellet were further separated by isopycnic centrifugation on sucrose gradients. 5′-Nucleotidase activity was determined in a standard assay; specific $PGF_{2\alpha}$ binding was determined as described in Materials and Methods. H, homogenate; N1, interface at between 31–37% (by mass) sucrose of the nuclear pellet; N2, interface at 37–41% (by mass) sucrose of the nuclear pellet; P1, interface at 31–37% (by mass) sucrose of the 100000-*g* pellet; P2, interface at 37–41% (by mass) of the 100000-*g* pellet; P3, 41–45% (by mass) sucrose of the 100000-*g* pellet; P4, 45–52% (by mass) of the 100000-*g* pellet.

| Sample | 5′-Nucleotide | Glucose 6-phosphatase | Specific $PGF_{2\alpha}$ binding |
|---|---|---|---|
| | nmol phosphate · $mg^{-1}$ · $min^{-1}$ | | fmol/mg |
| H | 22.2 ± 2.7 | 109.9 ± 9.5 | 3.2 ± 0.4 |
| N1 | 84.2 ± 17.9 | 40.9 ± 19.9 | 8.4 ± 1.5 |
| N2 | 132.1 ± 22.9 | 230.7 ± 49.4 | 15.6 ± 2.8 |
| P1 | 271.9 ± 46.2 | 191.1 ± 25.9 | 17.4 ± 1.8 |
| P2 | 194.7 ± 20.6 | 340.6 ± 22.1 | 14.4 ± 1.0 |
| P3 | 74.8 ± 19.1 | 424.3 ± 28.3 | 6.1 ± 0.3 |
| P4 | 43.1 ± 11.3 | 553.2 ± 46.6 | 4.2 ± 1.4 |

Hepatocyte plasma membranes were separated from other membrane fractions in the homogenate by isopycnic centrifugation on sucrose gradients. The highest specific activity for the plasma-membrane marker enzyme 5′-nucleotidase was found at the interfaces at 37%–41% (by mass)

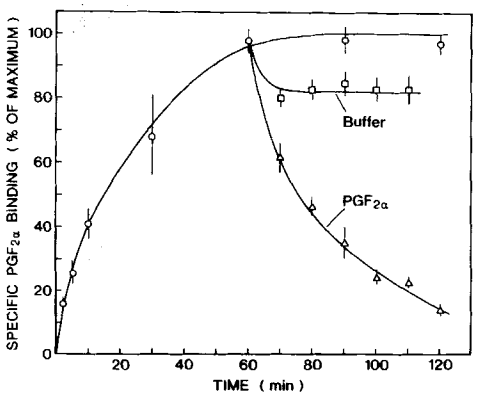


**Fig. 2. Time dependence and reversibility of $PGF_{2\alpha}$** binding to plasma membranes. Plasma membranes (400 µg protein in a volume of 0.1 ml buffer A) were incubated at room temperature with 5 nM [$^3$H]$PGF_{2\alpha}$ for the times indicated. To determine unspecific binding, a 5000-fold excess of unlabelled PG was included in parallel assays. After a 1-h incubation, membranes were sedimented by centrifugation at 4000 *g*, washed twice in assay buffer and resuspended in the original volume of assay buffer with or without 25 µM $PGF_{2\alpha}$. Values are means ± SEM of four experiments of two plasma-membrane preparations.

sucrose of the nuclear (1500 *g*) pellet and at 31%–37%, as well as 37%–41% (by mass) of the 100000 *g* pellet (Table 1). The fractions with the highest specific binding of $PGF_{2\alpha}$ copurified with 5′-nucleotidase (Table 1). The membrane fractions with highest specific binding were pooled for all subsequent assays. The pooled plasma-membrane fractions were enriched about 10-fold in 5′-nucleotidase activity and fivefold in specific $PGF_{2\alpha}$ binding compared to the homogenate. 5′-Nucleotidase is located only on the outer surface of the plasma membrane. Since its substrate cannot cross lipid membranes in a mixture of intact right-side-out and inside-out vesicles, the enzyme of the inside-out vesicles can only be measured after detergent treatment (latency). When used in binding assays, 5′-nucleotidase latency was less than 10% after Triton X-100 solubilization (data not shown). Hence, the plasma-membrane preparation consisted of either predominantly right-side-out or leaky vesicles.

### Time dependence and reversibility of $PGF_{2\alpha}$ binding to plasma membranes

At room temperature, specific binding increased with time and reached an equilibrium around 60 min (Fig. 2). If free ligand was removed after 1 h by centrifugation and the membranes were resuspended in the same volume of assay buffer, an initial drop in specific $PGF_{2\alpha}$ binding occurred, which was most likely due to a loss of material during the handling. The complex then remained stable for up to 1 h more (Fig. 2). If $^3$H-labelled ligand was removed after 1 h of incubation by centrifugation, and an excess of unlabelled ligand was added in the same volume of assay buffer, 85% of bound labelled [$^3$H]$PGF_{2\alpha}$ was replaced by unlabelled ligand during the next hour (Fig. 2). Bound ligand could be removed from the plasma membranes by trypsination or incubation of the plasma membranes in 10 mM $NaHCO_3$, pH 10, for 1 h at room temperature, but not by an acid wash with citrate buffer, pH 3.0 (not shown). The specific binding was linearly dependent on the concentration of plasma-membrane protein 100–800 µg/tube (not shown). All subsequent binding studies were carried out at room temperature with an incubation time of 1 h and 350

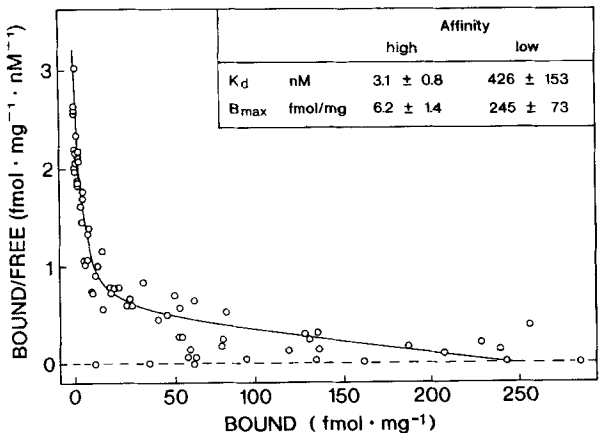


**Fig. 3. Scatchard analysis of a [$^3$H]PGF$_{2\alpha}$ saturation-binding assay with hepatocyte plasma membranes.** Hepatocyte plasma membranes (550 μg in 0.1 ml buffer A) were incubated for 1 h at room temperature with 0.5 – 25000 nM PGF$_{2\alpha}$ containing 7.66 – 0.015 TBq/mmol [$^3$H]-PGF$_{2\alpha}$. Bound and free ligand were separated by vacuum filtration. The binding data were analysed with the computer program LIGAND. The figure shows a conglomerate plot of the mean of duplicate points of five experiments of five plasma-membrane preparations.

– 550 μg membrane protein/tube. With these assay conditions, no appreciable degradation of PGF$_{2\alpha}$, PGD$_2$ or PGE$_2$ occurred, as determined by HPLC (not shown).

### Saturation-binding assays of PGF$_{2\alpha}$ to plasma membranes

In saturation-binding studies plasma-membrane-enriched fractions were incubated with 0.5 – 25000 nM [$^3$H]PGF$_{2\alpha}$ (see Materials and Methods). PGF$_{2\alpha}$ bound to the plasma membranes at saturable specific binding sites. The mathematical analysis of the binding data with the computer program LIGAND revealed the existence of a high-affinity and a low-affinity binding site (Fig. 3). The high-affinity binding site bound 6 fmol PGF$_{2\alpha}$/mg plasma-membrane protein with an apparent $K_d$ of 3 nM. The low-affinity binding site had an apparent $K_d$ of 426 nM and bound 245 fmol PGF$_{2\alpha}$/mg plasma-membrane protein. The binding constants of the low-affinity site could not be determined with the same accurancy as those of the high-affinity site, since the values for unspecific and specific binding were similar at PGF$_{2\alpha}$ concentrations close to the calculated $K_d$ of this site.

### Competition binding studies of PGF$_{2\alpha}$, PGE$_2$ and PGD$_2$ to plasma membranes

Membranes were incubated with 5 nM [$^3$H]PGF$_{2\alpha}$ and 0 – 10 μM PGF$_{2\alpha}$, 0 – 32 μM PGD$_2$ or 0 – 32 μM PGE$_2$. PGF$_{2\alpha}$ competed better than PGD$_2$ or PGE$_2$ for [$^3$H]PGF$_{2\alpha}$-binding sites (Fig. 4). The affinities and densities of the two binding sites for PGF$_{2\alpha}$ determined in the competition-binding studies were, as expected, not significantly different from those obtained in saturation-binding studies. A $K_d$ of 3.9 ± 1.6 nM and a $B_{max}$ of 10 ± 1.3 fmol/mg plasma-membrane protein for the high-affinity binding site, and a $K_d$ of 456 ± 333 nM and a $B_{max}$ of 163 ± 42 fmol/mg plasma-membrane protein for the low-affinity binding site, were determined by non-linear-regression analysis of the competition-binding data with LIGAND. Despite the large number of experiments performed ($n$ = 10), the binding constants for PGD$_2$ and

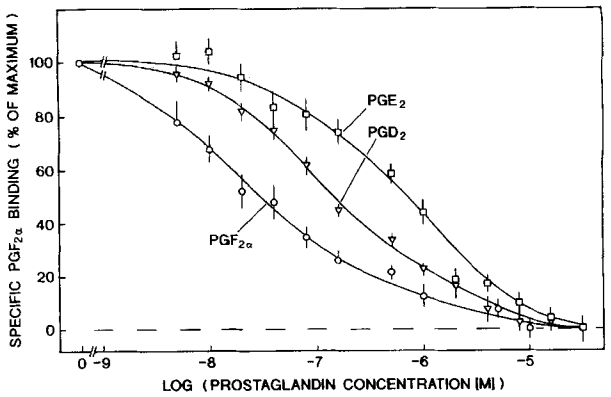


**Fig. 4. Competition of PGF$_{2\alpha}$, PGD$_2$ and PGE$_2$ for [$^3$H]PGF$_{2\alpha}$-binding sites on hepatocyte plasma membranes.** Hepatocyte plasma membranes (350 μg protein in 0.1 ml buffer A) were incubated for 1 h at room temperature with 5 nM [$^3$H]PGF$_{2\alpha}$ (7.66 TBq/mmol) and 0 – 10 μM unlabelled PGF$_{2\alpha}$, 0 – 32 μM PGD$_2$ or 0 – 32 μM PGE$_2$. Bound and free ligand were separated by vacuum filtration. The maximal specific binding of [$^3$H]PGF$_{2\alpha}$ was set at 100%. Values are means ± SEM of five (PGF$_{2\alpha}$), 10 (PGD$_2$) and 10 (PGE$_2$) experiments.

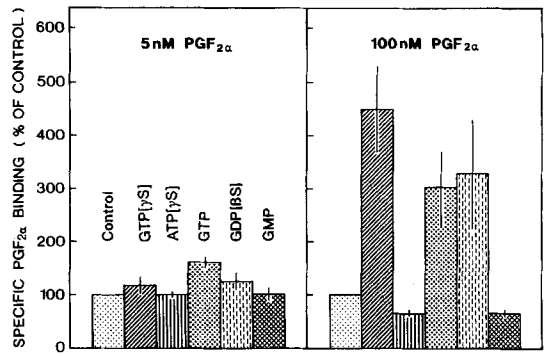


**Fig. 5. Increase by guanine nucleotides of specific PGF$_{2\alpha}$ binding to hepatocyte plasma membranes.** Hepatocyte plasma membranes (400 μg in 0.1 ml buffer A) were incubated for 1 h at room temperature with 5 nM [$^3$H]PGF$_{2\alpha}$ (7.66 TBq/mmol) or 100 nM [$^3$H]PGF$_{2\alpha}$ (0.38 TBq/mmol). Excess unlabelled PGF$_{2\alpha}$ (25 μM) was added in parallel assay points to determine unspecific binding. The different nucleotides were added to the reaction mixture at 100 μM. The data shown are means ± SEM of duplicate points of three experiments.

PGE$_2$ could not be determined with sufficient statistical reliability. However, regression analysis indicated that PGD$_2$ and PGE$_2$ bound to the high-affinity binding site with fourfold and sevenfold lower affinity, respectively, than PGF$_{2\alpha}$. PGD$_2$ bound to the low-affinity binding site with the same affinity as PGF$_{2\alpha}$, whereas PGE$_2$ bound with a 4.5-fold lower affinity than PGF$_{2\alpha}$.

### Increase in specific PGF$_{2\alpha}$ binding to hepatocyte plasma membranes by guanine nucleotides

Hepatocyte plasma membranes were incubated in presence or absence of nucleotides with 5 nM [$^3$H]PGF$_{2\alpha}$ (7.66 TBq/mmol) or 100 nM [$^3$H]PGF$_{2\alpha}$ (0.38 TBq/mmol). Guanosine 5′-[γ-thio]triphosphate (GTP[γS]), and to a lesser extent guanosine 5′-[β-thio]triphosphate GDP[βS] and GTP, increased specific PGF$_{2\alpha}$ binding, if the total PG concentration was close to the $K_d$ of the low-affinity binding site, i.e. 100 nM (Fig. 5). They had, however, no effect on PGF$_{2\alpha}$ binding, if

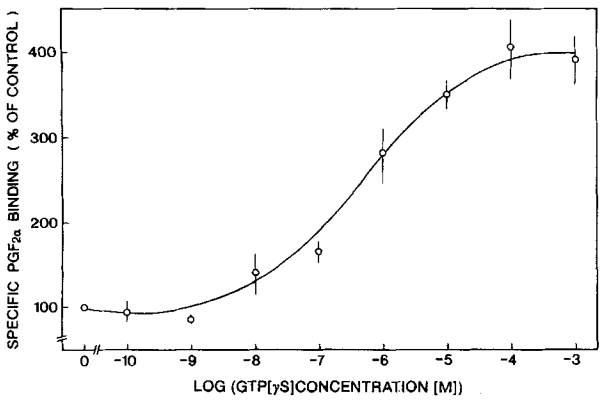


**Fig. 6. Dose dependence of the increase of specific $PGF_{2\alpha}$ binding to hepatocyte plasma membranes by GTP[γS].** Hepatocyte plasma membranes (400 μg in 0.1 ml buffer A) were incubated for 1 h at room temperature with 100 nM [$^3$H]$PGF_{2\alpha}$ (0.38 TBq/mmol). Excess unlabelled $PGF_{2\alpha}$ (25 μM) was included in parallel assays to determine unspecific binding. GTP[γS] was added to the reaction mixture at the concentration indicated. Values are means ± SEM of duplicate points of four experiments.

the $PGF_{2\alpha}$ concentration was in the range of the $K_d$ of the high-affinity binding site, i.e. 5 nM. Adenosine 5′-[γ-thio] triphosphate (ATP[γS]) and GMP had no influence on $PGF_{2\alpha}$ binding at either PG concentration (Fig. 5) GTP[γS] increased specific binding to hepatocyte plasma membranes of 100 nM $PGF_{2\alpha}$ dose-dependently in the range 0.01 – 100 μM (Fig. 6). The ranking order in which the different nucleotides increased $PGF_{2\alpha}$ binding to hepatocyte plasma membranes and the dose/response curve for GTP[γS] are similar to those reported for other guanine-nucleotide-binding-regulatory-protein(G protein)-associated receptors [36, 37]. Thus, these data indicate, that the low-affinity binding site, but not the high-affinity binding site, might be linked to a G protein and therefore represent a $PGF_{2\alpha}$ receptor.

## DISCUSSION

Studies to characterize PG-binding sites in liver have been performed previously either with intact cells or with plasma membranes purified from whole liver homogenate. The present study is the first investigation with plasma membranes purified from isolated hepatocytes free of non-parenchymal cells. The $K_d$ and $B_{max}$ values differ considerably between different publications, most likely because of the differences in the experimental protocol.

### PG receptors on intact hepatocytes

In hepatocyte suspensions, a binding site for $PGD_2$ with a $K_d$ of 20 nM and a lower affinity for $PGE_2$ and $PGF_{2\alpha}$ has been reported. The calculated receptor number of 1000000 copies/cell [3] was unexpectedly high in comparison with other receptors on hepatocytes [38 – 43]. On hepatocytes in primary culture, $PGE_1$-binding sites were demonstrated with an apparent $K_d$ of 12 nM and about $3.5 \times 10^{11}$-binding sites/mg hepatocyte protein [44], which corresponds to about 500000 sites/cell. However, as demonstrated in the current investigation, studies with whole cells have to be regarded with caution, since a considerable accumulation of PG within the cells may occur, even at 4 °C. Binding data obtained with whole cells might thus not necessarily represent binding to receptors on the cell surface.

### PG receptors on plasma membranes of whole liver

Early studies described PG-binding sites in whole liver plasma membranes with different affinities for $PGF_{2\alpha}$ and other PG. There was only one binding site for $PGF_{2\alpha}$ with a $K_d$ of 100 nM and a $B_{max}$ of 1 pmol/mg membrane protein [25], but three binding sites for $PGE_1$ with high, (1 nM), medium (25 nM) and low affinity with uncertain $K_d$. A later study confirmed the existence of the medium-affinity and low-affinity binding site for $PGE_1$, and also showed the existence of only one $PGF_{2\alpha}$-binding site with an apparent $K_d$ of 23 nM [24]. Evidence for a medium-affinity/high-capacity $PGF_{2\alpha}$-binding site, as described in these studies, was not obtained in the current investigation. This discrepancy might represent differences in the binding-site content of membranes obtained from whole liver plasma membranes, being a mixture of plasma membranes of different cell types and plasma membranes prepared from pure hepatocytes. It is, however, also possible that in the present study improved mathematical analysis of the binding data [35] revealed the existence of two $PGF_{2\alpha}$-binding sites that might have appeared as one binding site of medium affinity in previous studies.

A high-affinity/low-capacity binding site and a low-affinity/high-capacity binding site for $PGF_{2\alpha}$ were described in liver plasma membranes with a $K_d$ of about 40 nM and 1 μM, and a $B_{max}$ of 1 fmol/mg membrane protein and 600 fmol/mg membrane protein, respectively [45]. The binding data of the low-affinity binding site were close to those observed in the present investigation. The density of the low-affinity binding site was decreased in chronically endotoxin-treated animals [45]. Endotoxins were shown to increase hepatic glycogenolysis via PG release from non-parenchymal liver cells [7]. In line with this finding, chronically endotoxin-treated animals had elevated hepatic PG levels [46], that might cause a down-regulation of their functional receptor. This finding further supports the view that the low-affinity binding site described here might be responsible for the glycogenolytic effects of $PGF_{2\alpha}$.

### PG receptor on plasma membranes of purified hepatocytes

Hepatocyte plasma membranes had a high-affinity/low-capacity $PGF_{2\alpha}$-binding site and a low-affinity/high-capacity $PGF_{2\alpha}$-binding site, the second of which might represent a receptor. The plasma membrane preparations were enriched 5 – 10-fold over the crude homogenate. Assuming that 150 mg protein in homogenate corresponds to 1 g wet hepatocytes, 15 mg of the membrane protein of the plasma-membrane-enriched fraction would correspond to 1 g hepatocytes. Assuming further that 1 g hepatocytes is about $10^8$ cells, the $B_{max}$ of 6 fmol/mg membrane protein for the high-affinity and 250 fmol/mg membrane protein for the low-affinity binding site (Fig. 3) correspond to about 500 copies/cell and 25000 copies/cell, respectively. These values can only be regarded as a rough estimates and could possibly be higher, since receptor degradation during the purification procedure may not be excluded. The calculated density of the low-affinity binding site is in the same range as the number of copies in each hepatocyte found for other receptors involved in metabolic regulation, e.g. the $\alpha_1$-receptor with about 500 fmol/mg membrane protein [47, 43], or the insulin receptor for which receptor numbers of 10000 copies/cell in binding studies [39, 40] or

100000 copies/cell in morphometric studies [38] have been reported.

The apparent $K_d$ of the low-affinity binding site was about 400 nM (Fig. 3) and was thus similar to concentrations of around 1 μM $PGF_{2\alpha}$, $PGD_2$ and $PGE_2$ that evoked a half-maximal stimulation of glucose output in perfused rat liver [15], and of glycogen phosphorylase activity in isolated rat hepatocytes (G. P. Püschel, A. Schröder and K. Jungermann, unpublished results) via the $\alpha_1$-adrenergic-like receptor. Ten-fold-higher concentrations of $PGF_{2\alpha}$ and $PGE_2$ increased inositol trisphosphate in hepatocytes [18, 19]. 0.1–1 μM PG also elicited the antiglycogenolytic effects mediated via the $\alpha_2$-receptor-like PG receptor [22, 23]. $PGF_{2\alpha}$ binding to hepatocyte plasma membranes was enhanced by GTP[γS], when the PG concentration was in the range of the $K_d$ of the low-affinity binding site, but not when the concentration was in the range of the $K_d$ of the high-affinity binding site (Fig. 5). Since the low-affinity/high-capacity binding site had a $K_d$ in the concentration range in which physiological responses to $PGF_{2\alpha}$ were observed and since GTP[γS] enhanced $PGF_{2\alpha}$ binding to this site specifically, it might represent a G-protein-linked $PGF_{2\alpha}$ receptor. It is not possible to judge from the present data, whether the binding site might correspond to the $\alpha_1$-adrenergic-like PG receptor mediating the glycogenolytic effects or the $\alpha_2$-adrenergic-like PG receptor, mediating the antiglycogenolytic effect, or whether this one receptor may be linked to two G proteins. There is, as yet, no functional correlate to the high-affinity/low-capacity binding site.

The concentrations of PG in the portal or hepatic vein are in the nanomolar range. This could imply that the high-affinity rather than the low-affinity site might be the one mediating biological responses. Yet, PG are rapidly metabolized by hepatocytes [3, 48, 49]. During one liver passage, more than 90% of exogenously added PG is degraded [50] (Püschel, G. P., Hülsmann, M. and Jungermann, K., unpublished results). Thus, the PG concentrations in the hepatovenous blood represent the small part of PG formed in the liver that escape degradation. The local PG concentrations at the sites of close proximity of non-parenchymal cells and hepatocytes in the space of Disse must be much higher. If a non-parenchymal liver cell with a diameter of 8 μm releases 1 amol $PGF_{2\alpha}$/min (corresponding to 2 pmol · $min^{-1}$ · ($10^6$ Kupffer cells)$^{-1}$) [51] into a surrounding space of 1 μm width for 1 min, then the local concentration can be expected to be about 4 μM, which would suffice to saturate the low-affinity binding site.

## Conclusion

The present study of PG-binding sites, apparently the first in plasma membranes of purified hepatocytes, has provided evidence for a G-protein-linked $PGF_{2\alpha}$ receptor, with low affinity and high capacity, which could be involved in the $\alpha_1$-adrenergic like stimulation of glycogenolysis by $PGF_{2\alpha}$ [18].

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