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# Cellular communication inside the liver

## Binding, conversion and metabolic effect of prostaglandin $D_2$ on parenchymal liver cells

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The major eicosanoid produced within the rat liver, prostaglandin (PG) $D_2$, was studied for its ability to interact with the various liver cell types. It appeared that $PGD_2$ bound specifically to parenchymal liver cells, whereas the binding of $PGD_2$ to Kupffer and endothelial liver cells was quantitatively unimportant. Maximally 700 pg of $PGD_2$/mg of parenchymal-cell protein could be bound by a high-affinity site ($1 \times 10^6$ $PGD_2$-binding sites/cell). The recognition site for $PGD_2$ is probably a protein, because trypsin treatment of the cells virtually abolished the high-affinity binding. High-affinity binding of $PGD_2$ was a prerequisite for the induction of a metabolic effect in isolated parenchymal liver cells, i.e. the induction of glycogenolysis. High-affinity binding of $PGD_2$ by parenchymal cells was coupled to the conversion of $PGD_2$ into three metabolites, whereas no conversion of $PGD_2$ by Kupffer and endothelial liver cells was noticed. The temperature-sensitivity of the conversion of $PGD_2$ was consistent with a conversion of $PGD_2$ on or in the vicinity of the cell membrane. One of the $PGD_2$ metabolites could be identified as $9\alpha,11\beta$-$PGF_2$. It can be calculated that the conversion rate of $PGD_2$ by parenchymal liver cells exceeds the production rate of $PGD_2$ by Kupffer plus endothelial liver cells, indicating that $PGD_2$ is meant to exert its activity within the liver. The present finding that $PGD_2$ formed by the non-parenchymal liver cells is recognized by a specific receptor on parenchymal liver cells and that binding, conversion and metabolic effect of $PGD_2$ are interlinked by this receptor provides further support for the specific role of $PGD_2$ in the intercellular communication in the liver.

## INTRODUCTION

Recently the role of eicosanoids in several metabolic processes inside the liver was indicated. The induction of glycogenolysis after administration of phorbol ester [1,2], endotoxin [3] or platelet-activating factor [4] to the perfused liver was found to be meditated by eicosanoids produced in the liver. Eicosanoids were also reported to be involved in the induction of the enzyme ornithine decarboxylase in the liver after administration of phorbol ester *in vivo* [5–7]. Since phorbol ester and endotoxin were not able to induce these metabolic effects in isolated parenchymal liver cells, a new concept for the regulation of liver metabolism was presented (1–4,6,8). Within this concept eicosanoids, produced by non-parenchymal liver cells in response to phorbol ester or endotoxin, transduce the effect of these agents to the parenchymal liver cells [8]. In the rat liver, eicosanoids are almost exclusively produced by Kupffer and endothelial cells, and the major eicosanoid produced by both cell types is $PGD_2$, which represents about 50 % of the total amount of eicosanoids produced in the liver [9].

With isolated parenchymal liver cells it was found that prostaglandins were able to induce glycogenolysis [10], to change the degree of phosphorylation of three specific parenchymal-cell proteins [11] and to induce ornithine decarboxylase activity [6]. Among the various prostaglandins tested, $PGD_2$ was found to be the most potent inducer of these effects, and we therefore suggested that $PGD_2$ is the main eicosanoid involved in the intercellular communication between parenchymal and non-parenchymal liver cells [6,10,11].

In addition to the metabolic effects of eicosanoids in the liver, it appeared that eicosanoids are also rapidly converted within the liver. Prostacyclin [12], PGE [13–16] and several leukotrienes [17–19] are rapidly converted by the intact liver and by cultured parenchymal liver cells. In a perfused liver system it was shown that $PGD_2$ is rapidly transformed to $9\alpha,11\beta$-$PGF_2$ [20,21]. In cultured parenchymal cells a conversion into dinor-$PGD_1$ and tetranor-$PGD_1$ was observed [22,23], and with a hepatic microsomal enzyme preparation the formation of $PGF_{2\alpha}$ was found [24,25].

The aim of the present work was to characterize the binding of $PGD_2$ to parenchymal liver cells and to establish a relationship between binding, conversion and metabolic effect of $PGD_2$ in rat liver parenchymal cells.

## MATERIALS AND METHODS

### Materials

$PGD_2$, $PGE_2$ and $PGE_1$, collagenase types I and IV and bovine serum albumin (fraction V) were from Sigma, St. Louis, MO, U.S.A. [5,6,8,9,12,14,15(n)-$^3$H]$PGD_2$ (sp. radioactivity 158 Ci/mmol), radioimmunoassays specific

Abbreviation used: PG, prostaglandin.

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for $PGD_2$ and $9\alpha,11\beta$-$PGF_2$ were from Amersham International, Amersham, Bucks., U.K. Nycodenz was from Nycomed A/S, Oslo, Norway. Sep-Pak $C_{18}$, silica cartridges, h.p.l.c. filters HA (0.45 $\mu$m pore size) and Millex filters (0.45 $\mu$m pore size) were obtained from Waters/Millipore, Etten-Leur, The Netherlands. Prepacked h.p.l.c. columns (Zorbax $C_{18}$) were obtained from Chrompack, Middelburg, The Netherlands.

**Liver cell isolation and incubation**

Parenchymal liver cells were isolated from male Wistar rats (250–300 g) after perfusion of the liver for 20 min with collagenase (type IV; 0.05%) at 37 °C by the method of Seglen [26], modified as previously described [27].

Kupffer and endothelial rat liver cells were isolated from rat liver, which was perfused with collagenase (type I; 0.05%) at 37 °C for 20 min. After collagenase digestion the liver was excised, cut into pieces, and filtered through nylon gauze. Parenchymal and non-parenchymal cells were separated by differential centrifugation. In a subsequent centrifugation on a Nycodenz gradient, cell debris was removed from the non-parenchymal cell fraction. Finally, Kupffer and endothelial liver cells were purified by counterflow centrifugation in a Beckman elutriation rotor. The method has been described in detail elsewhere [28], and was used here except for the first elutriation step, which was replaced by a centrifugation step (2 min at 75 *g*). Kupffer cells were more than 90% pure, and endothelial liver cells were 95% pure. A differentiation between Kupffer and endothelial liver cells was made by peroxidase staining and Papanicolau counterstaining [29,30].

Freshly isolated Kupffer, parenchymal and endothelial liver cells were incubated in Krebs–Ringer bicarbonate buffer [3], saturated with $O_2/CO_2$ (19:1), pH 7.4. Cell suspensions in Kartell plastic tubes were placed on a circulating laboratory shaker (Adolf Kühner A.G., Basel, Switzerland). Viability, as checked by measuring ATP content and Trypan Blue exclusion, was above 95% for all liver cell types used throughout the incubations.

**Binding of $PGD_2$**

Binding of $PGD_2$ was determined by adding 0.25 $\mu$Ci of [$^3$H]$PGD_2$ and various amounts of unlabelled $PGD_2$ to freshly isolated parenchymal, endothelial and Kupffer cells, suspended in Krebs–Ringer bicarbonate buffer saturated with $O_2/CO_2$ (19:1) (pH 7.4). At the end of the incubation, liver cells were rapidly cooled and centrifuged (5 min, 500 *g*). The amount of unbound $PGD_2$ was determined by counting the radioactivity in the supernatant. Cells were washed three times with Krebs–Ringer bicarbonate buffer (4 °C), after which the amount of $PGD_2$ bound to various cell types was determined by liquid-scintillation counting of the $^3$H radioactivity in the cell pellet.

**Conversion of $PGD_2$**

[$^3$H]$PGD_2$ (0.25 $\mu$Ci; 50 ml) was perfused twice through the liver, or 0.25 $\mu$Ci of [$^3$H]prostaglandin $D_2$ was added to freshly isolated parenchymal, Kupffer and endothelial cells suspended in 1 ml of Krebs–Ringer bicarbonate buffer (pH 7.4). After incubation (15–120 min), the liver cell suspensions were centrifuged (500 *g*, 5 min). Supernatant and perfusate, containing [$^3$H]$PGD_2$ and $^3$H-labelled metabolic products of $PGD_2$, was applied to two Sep-Pak $C_{18}$ and silica cartridges (the cartridges were prewashed with 10 ml of ethanol and 10 ml of distilled water). The samples were eluted with 5 ml of ethanol, and the eluates were evaporated to dryness with a Speed-Vac concentrator (Savant) at 40 °C. The dried samples were dissolved in 0.3 ml of 0.1% solution of EDTA in tetrahydrofuran/methanol/water/acetic acid (250:300:450:1, by vol.) adjusted to pH 5.5 with conc. $NH_3$, centrifuged (2 min, 2800 *g*), purified by a Millex filter and kept in a h.p.l.c. microvial (Weidmann, Romanshorn, Switzerland).

Volumes of 100 $\mu$l were injected on to the columns and chromatographed on a 1082 B h.p.l.c. instrument (Hewlett Packard, Brussels, Belgium). Fractions were collected with a Superrac Fraction Collector (LKB–Pharmacia, Uppsala, Sweden) and counted for radioactivity in a liquid-scintillation counter (Packard). Reverse-phase h.p.l.c. of [$^3$H]$PGD_2$ and its metabolic products was performed on a Zorbax $C_{18}$ column. The solvent system was acetonitrile/benzene/water/acetic acid (240:2:760:1, by vol.) The flow rate was 2 ml/min, and the fractions were collected for 60 min for liquid-scintillation counting.

To determine the rate of $PGD_2$ conversion, 200 ng of $PGD_2$ was added to parenchymal, Kupffer and endothelial liver cells suspended in 1 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) (0.1–0.2 mg of cell protein/ml). After an incubation period of maximally 5 min, cell suspensions were rapidly cooled and centrifuged (500 *g*, 5 min) and the amount of $PGD_2$ present in the resulting supernatant was determined by a radioimmunoassay specific for $PGD_2$. The control level of $PGD_2$ conversion was determined by performing the same incubations in the absence of liver cells. The amount of $PGD_2$ recovered in the control incubations was always more than 90%. The amount of $9\alpha,11\beta$-$PGF_2$ formed at the end of the incubation was determined by radioimmunoassay specific for $9\alpha,11\beta$-$PGF_2$. The effect of various agents on the conversion of $PGD_2$ by parenchymal liver cells was determined by incubating the parenchymal cells (0.1 mg of cell protein/ml) for 2 min in Krebs–Ringer bicarbonate buffer (pH 7.4) with 200 ng of $PGD_2$ and these agents at the indicated concentrations. Trypsin treatment of the parenchymal cells was performed by incubation of the cells for 45 min on an ice tray in a refrigerated room with 4 mg of trypsin/ml [31]. After trypsin treatment, cells were washed twice and incubated with 200 ng of $PGD_2$ for 2 min at 37 °C. Cell viability after trypsin treatment was more than 90%. Conversion of $PGD_2$ was measured as described above.

Temperature-sensitivity of $PGD_2$ conversion by parenchymal cells was determined by adding 200 ng of $PGD_2$ to parenchymal cells (0.1 mg in 1 ml of Krebs–Ringer bicarbonate buffer), which were preincubated for 20 min at the appropriate temperature (4–37 °C).

**Glucose production by isolated parenchymal cells**

Parenchymal liver cells were incubated at 37 °C with constant shaking at 5 mg of cell protein/ml in Krebs–Ringer bicarbonate buffer. At 10 min after the addition of various amounts of $PGD_2$, cell suspensions were rapidly cooled to 0 °C and centrifuged at 500 *g* for 5 min. Subsequently glucose was determined in the supernatant by the glucose oxidase–ABTS method [32] as previously described [10]. Glucose production by parenchymal cells

after stimulation with prostaglandins was compared with that after the addition of solvent. Data are expressed as stimulation (% over control); the control value of glucose output was $19.9 \pm 1.2$ nmol of glucose produced /10 min per mg of cell protein (mean $\pm$ S.D., $n = 3$).

### Biological effects of $PGD_2$ metabolites

Contraction of guinea-pig lung parenchymal strips and coronary-artery rings placed in Krebs–Ringer bicarbonate buffer (pH 7.4) at 37 °C was recorded as previously described [33].

## RESULTS

A comparison of the association of $PGD_2$ with parenchymal, Kupffer and endothelial liver cells is shown in Fig. 1. Initial association of $PGD_2$ with parenchymal cells (up to 60 min) was comparable at 4 °C and 37 °C, but for longer incubation times at 37 °C association of $PGD_2$ decreased, whereas for parenchymal cells incubated at 4 °C there was no such decrease. Kupffer and endothelial liver cells bound relatively small amounts of [$^3$H]$PGD_2$: less than 0.5 pmol of $PGD_2$ was bound per mg of cell protein, compared with 4 pmol/mg of cell protein by parenchymal cells.

Fig. 2 shows that [$^3$H]$PGD_2$ is converted into three metabolites by the perfused liver as identified after separation of the metabolites by reverse-phase h.p.l.c. After two circulations through the liver, 5% of the $PGD_2$ was recovered in an unchanged form (Table 1). Incubation of [$^3$H]$PGD_2$ with freshly isolated rat parenchymal cells resulted in the formation of the same three $^3$H-labelled metabolites. Both the retention times and the relative amounts of the $PGD_2$ metabolites formed by the parenchymal cells were identical with the $PGD_2$ metabolites formed in the perfused liver, and also here only 5% of the $PGD_2$ was found at a similar retention time as intact $PGD_2$ (Fig. 1, Table 1). Incubation of [$^3$H]$PGD_2$ with the Kupffer and endothelial liver cells did not result in any formation of metabolites, as is indicated by the h.p.l.c. profile (Fig. 2), and 95% of the $PGD_2$ was still recovered in an unchanged form (Table 1). The $PGD_2$ metabolites formed by the perfused liver and the isolated parenchymal liver cells were tested for their reactivity with immunoassays directed against the various types of prostaglandins. For peaks 1, 2 and 3 (Fig. 2) no cross-reactivity was observed with immunoassays directed

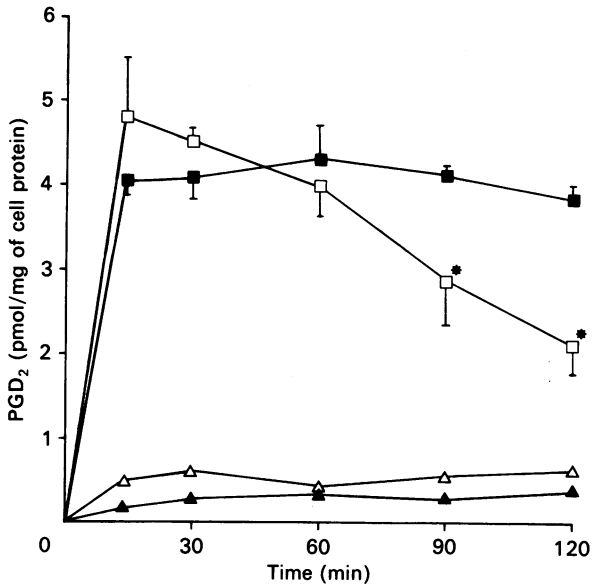


**Fig. 1. Association of [$^3$H]$PGD_2$ with Kupffer, endothelial and parenchymal liver cells**

[$^3$H]$PGD_2$ (100 pmol) was added to incubations of Kupffer (▲), endothelial (△) and parenchymal (■, □) liver cells (1 mg of cell protein). At the indicated time, the amount of $PGD_2$ associated with the various liver cell types was determined by counting the amount of $^3$H radioactivity bound to the cells. Association of $PGD_2$ with parenchymal cells at 4 °C (■) was compared with that at 37 °C (□). * represents significant difference from 4 °C value ($P < 0.01$).

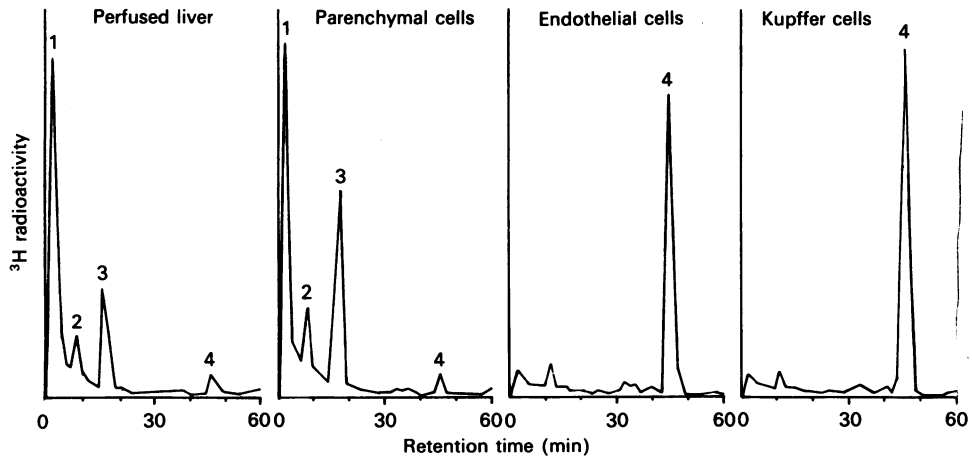


**Fig. 2. Profile of [$^3$H]$PGD_2$ metabolites formed by the perfused liver and by isolated Kupffer, endothelial and parenchymal liver cells**

[$^3$H]$PGD_2$ (0.25 $\mu$Ci) was incubated for 2 h with freshly isolated Kupffer, endothelial and parenchymal liver cells (1 mg of cell protein), or 0.25 $\mu$Ci of [$^3$H]$PGD_2$ was perfused twice through the liver. Supernatants of the cell incubations or the perfusate, containing $PGD_2$ and its metabolites, were concentrated and separated by reverse-phase h.p.l.c. (see the Materials and methods section).

**Table 1. Relative formation of $PGD_2$ metabolites by the perfused liver and by isolated Kupffer, endothelial and parenchymal cells**

[$^3$H]$PGD_2$ (0.25 $\mu$Ci) was either perfused through the rat liver (twice) or incubated for 2 h with isolated parenchymal, Kupffer and endothelial liver cells. The metabolites formed were concentrated and separated by reverse-phase h.p.l.c. The amount of radioactivity recovered in the various peaks (1–4, Fig. 2) was determined. Results are means ± S.D. of three experiments; n.d., not detectable.

| Peak no. | Retention time (min) | Relative amount (%) | | | |
|---|---|---|---|---|---|
| | | Perfused liver | Parenchymal cells | Endothelial cells | Kupffer cells |
| 1 | 2.25 | 48.1±5.1 | 44.9±4.9 | n.d. | n.d. |
| 2 | 8.25 | 12.1±4.7 | 15.1±4.0 | n.d. | n.d. |
| 3 | 17.25 | 28.1±6.1 | 33.0±4.8 | n.d. | n.d. |
| 4 | 45.75 | 5.1±2.1 | 5.7±0.9 | 95.3±3.4 | 96.1±2.8 |

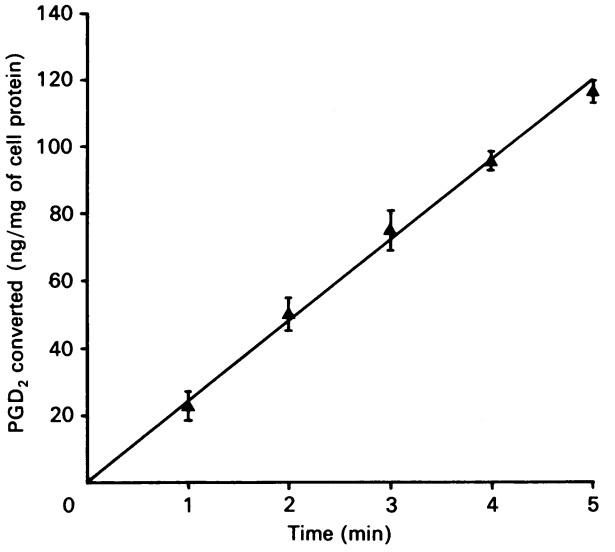


**Fig. 3. Rate of metabolism of $PGD_2$ by isolated parenchymal liver cells**

$PGD_2$ (200 ng) was incubated for the indicated times with parenchymal cells (0.1–0.2 mg of cell protein). At the end of the incubations the resulting amount of $PGD_2$ was detected by a radioimmunoassay and compared with the amount of $PGD_2$ recovered in incubations performed without parenchymal cells. Results are means ± S.D. for four experiments.

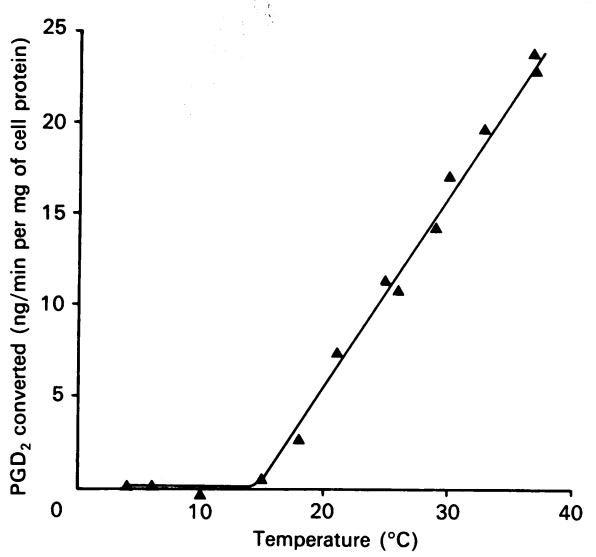


**Fig. 4. Effect of incubation temperature on metabolism of $PGD_2$ by parenchymal liver cells**

$PGD_2$ (200 ng) was added to an incubation of parenchymal liver cells (0.1 mg of cell protein). The cells were pre-incubated for 15 min at the indicated temperature. Metabolism of $PGD_2$ was determined by comparing the amounts of $PGD_2$ recovered in incubations with and without cells.

against $PGE_2$, $PGF_{2\alpha}$, 6-oxo-$PGF_{1\alpha}$, thromboxane $B_2$ and $PGD_2$. Peak 4 was identified as $PGD_2$ in an immunoassay.

Since the $PGD_2$ metabolites showed no cross-reactivity with an immunoassay specific for $PGD_2$, this assay was used to study the rate of $PGD_2$ conversion by parenchymal liver cells. The rate of $PGD_2$ conversion by parenchymal liver cells after addition of 200 ng of $PGD_2$ to the cells (0.1 mg) is shown in Fig. 3. From 0 to 5 min a constant rate of $PGD_2$ conversion was observed. At higher $PGD_2$ concentrations the rate of $PGD_2$ conversion by parenchymal cells did not increase, indicating that the values represent the maximal capacity of the cells to convert $PGD_2$.

Fig. 4 shows the temperature-sensitivity of the conversion of $PGD_2$ by parenchymal liver cells. Conversion of $PGD_2$ was negligible below 14 °C, whereas above 14 °C a linear relation was found between the temperature and the conversion rate, resulting in a conversion rate of 24 ng of $PGD_2$/min per mg of cell protein at 37 °C.

Under conditions in which $PGD_2$ conversion was negligible (4 °C) and $PGD_2$ binding to parenchymal cells reached the same level as at 37 °C, the saturation kinetics of the $PGD_2$ binding to rat parenchymal liver cells was studied. The Scatchard plot of this experiment is shown in Fig. 5. The saturation kinetics were indicative of the presence of a high- and a low-affinity component. About 700 pg of $PGD_2$/mg of cell protein was bound by a high-affinity process, indicative of the presence of $1 \times 10^6$ binding sites for $PGD_2$ per parenchymal liver cell. Part of the $PGD_2$ (42%) could not be displaced by an excess of $PGD_2$, indicative of a relatively high proportion of unspecific interaction. Both specific and non-specific

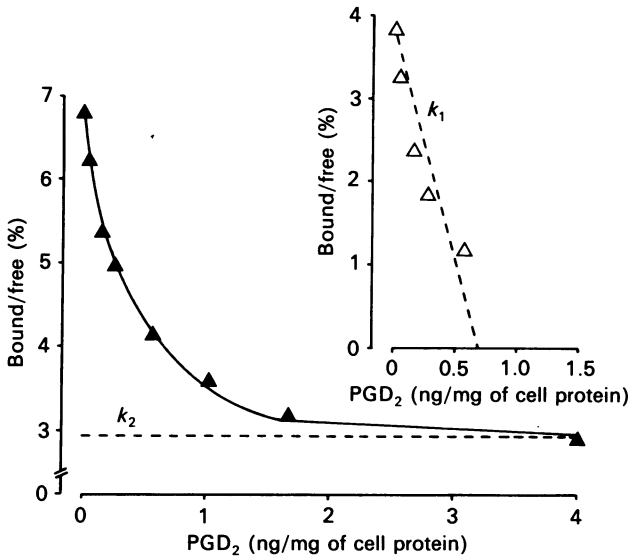


**Fig. 5. Scatchard plot of the binding of $PGD_2$ to parenchymal liver cells**

The binding of $PGD_2$ by parenchymal liver cells was studied by adding 0.25 $\mu$Ci of [$^3$H]$PGD_2$ together with increasing amounts of unlabelled $PGD_2$. After a 10 min incubation at 4 °C, the amount of $PGD_2$ bound to the parenchymal cells and the amount of unbound $PGD_2$ were determined by counting $^3$H radioactivity. From these data the bound/free $PGD_2$ (%) and the amount of $PGD_2$ per mg of parenchymal-cell protein could be calculated (▲). $k_1$ (inset, △) represents the high-affinity component of the $PGD_2$ binding, and $k_2$ represents the non-saturable low-affinity component.

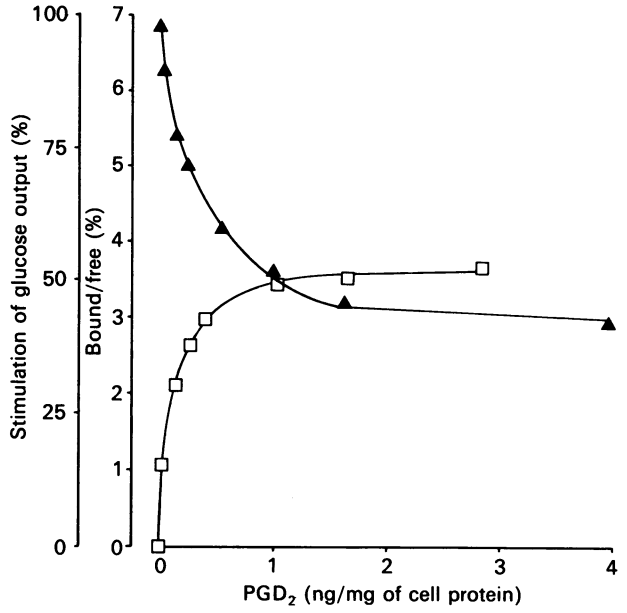


**Fig. 6. Comparison of specific binding of $PGD_2$ by parenchymal liver cells and the induction of glucose output in parenchymal cells by $PGD_2$**

Binding of $PGD_2$ [▲; bound/free (%)] was determined as described in the legend of Fig. 5. The induction of glucose output by $PGD_2$ (□; % increase over control) was determined by adding increasing amounts of $PGD_2$ to parenchymal liver cells, which were incubated for 10 min at 37 °C. The amount of glucose produced by the parenchymal cells in the presence of $PGD_2$ was compared with that produced in its absence as described in ref. [12].

**Table 2. Effect of various prostaglandins on the binding of $PGD_2$ to parenchymal liver cells**

The effect of $PGD_2$, $PGE_2$ and $PGE_1$ on the binding of 0.25 $\mu$Ci of [$^3$H]$PGD_2$ to parenchymal cells was studied. After incubation for 10 min at 4 °C the amount of [$^3$H]$PGD_2$ bound to the parenchymal cells was determined, as described in the legend of Fig. 5. Results (means ± S.D., $n = 3$) are expressed as inhibition of [$^3$H]$PGD_2$ binding to parenchymal cells.

| | Inhibition of $PGD_2$ binding (%) |
|---|---|
| $PGE_1$ | 4±2 |
| $PGE_2$ | 3±1 |
| $PGD_2$ | 58±6 |

binding to parenchymal cells was, in the cell-protein concentration range 1–4 mg/ml, linear with the amount of added protein. Two other prostanoids, $PGE_2$ and $PGE_1$, did not compete for $PGD_2$ binding (Table 2). To determine the relation between $PGD_2$ binding and metabolic effect, we compared the binding kinetics of $PGD_2$ with the concentration-dependency of the stimulation of glycogenolysis by $PGD_2$ in isolated parenchymal cells (Fig. 6). Maximal stimulation of the glucose output by parenchymal cells was found at a concentration of 0.5 $\mu$M-$PGD_2$, whereas a half-maximal effect was observed at 20 nM-$PGD_2$.

**Table 3. Effect of various agents on the metabolism of $PGD_2$ by isolated parenchymal liver cells**

$PGD_2$ (200 ng) was incubated for 2 min with isolated parenchymal cells (0.1 mg of cell protein), in the presence of the various agents at the indicated concentrations, except for trpysin, which was used to pretreat the cells [33]. Metabolism of $PGD_2$ was determined by measuring the amount of $PGD_2$ before and after an incubation with parenchymal cells. Data are means ± S.D. for three experiments: * indicates significant difference from control ($P < 0.01$).

| Agent | Concn. | $PGD_2$ metabolized (ng/min per mg of cell protein) |
|---|---|---|
| None (control) | – | 20.3±0.7 |
| $NH_4Cl$ | 1 mM | 20.5±0.1 |
| KCN | 0.1 mM | 16.9±0.4* |
| Monensin | 0.2 nM | 21.0±2.1 |
| Cycloheximide | 5 $\mu$M | 20.2±1.8 |
| Colchicine | 10 $\mu$M | 19.9±1.4 |
| Leupeptin | 0.4 $\mu$M | 20.3±0.5 |
| Trypsin | 4 mg/ml | 2.3±2.1* |

Agents that influence receptor-mediated uptake and/or processing of ligands were tested for their effect on the conversion of $PGD_2$ (Table 3). Among the substances tested, cyanide inhibited the conversion of $PGD_2$ by parenchymal cells by only 20%. However,

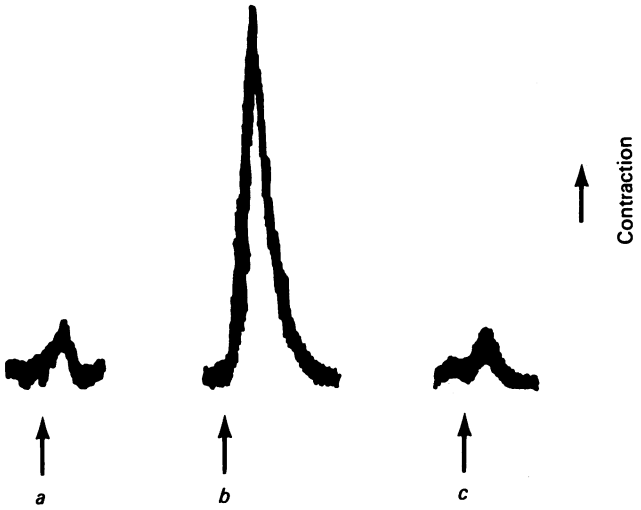


**Fig. 7. Comparison of the biological activity of $PGD_2$ and its metabolites**

$PGD_2$ (200 ng) was incubated for 30 min at 37 °C with parenchymal cells (2 mg of cell protein), and metabolites formed were tested for their ability to contract a guinea-pig lung parenchymal strip (*b*). The biological effect of the metabolites was compared with that of the initial amount of $PGD_2$ (*a*) and with that of $PGD_2$ that had been incubated for 30 min at 37 °C in the absence of parenchymal cells (*c*).

$PGD_2$ conversion could be completely blocked by pretreatment of the parenchymal cells with the protease trypsin (4 mg/ml) at 0 °C. Trypsin pretreatment led also to a 60% decrease in the $PGD_2$ binding to parenchymal cells (results not shown).

A comparison of the biological activity of $PGD_2$ and its metabolites on guinea-pig lung parenchymal strips and guinea-pig coronary-artery rings was performed (Fig. 7). The mixture of $PGD_2$ metabolites formed by parenchymal liver cells could elicit a contraction of the lung strips, whereas only a small contraction was observed after addition of the original amount of $PGD_2$. After separation of the $PGD_2$ metabolites by reverse-phase h.p.l.c., the metabolite recovered in peak 3 (Fig. 1) induced contraction of the lung parenchymal strips, whereas the metabolites 1 and 2 showed no biological activity. $PGD_2$ and its metabolites formed after incubation with parenchymal cells had the same slight dilatory effect on coronary-artery rings (results not shown). It was quantified that 22% of the amount of converted $PGD_2$ was $9\alpha,11\beta$-$PGF_2$, by using an immunoassay specific for $9\alpha,11\beta$-$PGD_2$. It also appeared that the retention time of [$^3$H]$9\alpha,11\beta$-$PGF_2$ on reverse-phase h.p.l.c. was identical with the retention time of peak 3, and that peak 3 (Fig. 1) showed cross-reactivity with the immunoassay for $9\alpha,11\beta$-$PGF_2$.

## DISCUSSION

Studies on the effects of phorbol ester [1,2], platelet-activating factor [4] and endotoxin [3] on glycogenolysis in the perfused liver indicated that prostaglandins, and in particular $PGD_2$, may play a mediating role in the induction of these processes. On the basis of studies with isolated cells, it became evident that the primary interaction of the aforementioned effectors occurred with the non-parenchymal liver cell types and that prostaglandins are utilized as messengers for the final expression of the signal in parenchymal liver cells.

$PGD_2$ is the major eicosanoid formed in the liver [9,34,35], and inside the liver Kupffer and endothelial cells are responsible for 95% of the total $PGD_2$ production by the liver [9]. Our present studies on the binding and conversion of $PGD_2$ by the various liver cells are consistent with the concept that $PGD_2$ may play an important role in the intercellular communication between parenchymal and non-parenchymal liver cells, and that parenchymal liver cells may be considered as the metabolic target for $PGD_2$.

$PGD_2$ binds only to a significant extent to parenchymal cells, which were found to contain a specific binding site for $PGD_2$, with a maximal specific binding capacity of 700 pg of $PGD_2$/mg of cell protein. The specific binding of $PGD_2$ correlates well with the stimulation of glycogenolysis in parenchymal cells. These results may indicate that $PGD_2$ can only induce metabolic effects after binding to a specific binding site. The binding site is most probably a protein, since a trypsin pretreatment of the parenchymal cells at low temperature, which removes all the proteins present on the surface of the parenchymal cells, but leaves the intracellular sites unchanged [31], diminished both the binding and the transformation of $PGD_2$ by the parenchymal cells. Other prostaglandins ($PGE_2$ and $PGE_1$) did not influence the binding of $PGD_2$ to the parenchymal cells. These results suggest that the recently described binding site on parenchymal cells for $PGE_1$ [36] is different from the binding site for $PGD_2$ on the parenchymal cells. The observation that both $PGD_2$ and $PGE_1$ may possess a specific binding site on parenchymal cells is in agreement with our finding that $PGD_2$ and $PGE_1$, when added together to parenchymal cells, have an additional effect on the glucose output by these cells [10].

The association of $PGD_2$ with parenchymal cells at higher temperatures was coupled specifically to a rapid conversion, and maximally 24 ng of $PGD_2$/min per mg of cell protein could be converted by these cells. The rapid conversion of $PGD_2$ most probably explains the decrease in $PGD_2$ association with parenchymal cells at 37 °C at longer time intervals (see Fig. 1). No lag phase in the conversion of $PGD_2$ was observed, indicating either that internalization of $PGD_2$ by the parenchymal cells is a very rapid process or that the conversion of $PGD_2$ occurs in the vicinity of or on the cell membrane. The trypsin-sensitivity of the $PGD_2$ conversion indicates that specific binding of $PGD_2$ to its receptor on the surface of parenchymal cells is a prerequisite for the conversion of $PGD_2$ by the cells. Conversion of $PGD_2$ was not influenced by substances that affect endosomal and lysosomal processes ($NH_4Cl$, leupeptin, chloroquine, monensin), indicating that the pathway for the conversion of $PGD_2$ is different from the pathway which is common for receptor-mediated processes. $PGD_2$ conversion was only to a limited extent energy-dependent (20% inhibition by cyanide). Since between 14 and 37 °C a linear increase in $PGD_2$ conversion was observed, these data once more may indicate that the conversion of $PGD_2$ after binding of $PGD_2$ to the parenchymal cells does not require an endocytotic process, because in these processes a sharp increase in processing takes place at temperatures round 18 °C [37].

$PGD_2$ was converted by isolated parenchymal cells into three metabolites which have exactly the same

retention times on reverse-phase h.p.l.c. and are formed in the same relative amounts as the metabolites formed in the perfused liver. These metabolites induced a contraction of lung parenchymal strips, whereas $PGD_2$ had almost no effect on the strips. Further analysis indicated that the biological activity of the metabolites might be due to the formation of $9\alpha,11\beta$-$PGF_2$ [38], as based on cross-reactivity of the metabolites in an immunoasssay specific for $9\alpha,11\beta$-$PGF_2$. Formation of $9\alpha,11\beta$-$PGF_2$ as a metabolite of $PGD_2$ has also been observed in rat lung [39], in humans [20,40] and in rabbit liver [21]. Formation of $9\alpha,11\beta$-$PGF_2$ from $PGD_2$ by isolated rat parenchymal cells was not found previously [22,23], probably because the conversion of $PGD_2$ is trypsin-sensitive, and therefore a specifically modified isolation procedure [26,27] is necessary to obtain cells which are metabolically active and convert $PGD_2$ into the products described.

It can be calculated, from the relative contribution of the various liver cells to total liver protein [41], that the capacity to convert $PGD_2$ (22 ng/mg of liver protein) exceeds the capacity to produce $PGD_2$ (maximally 9.4 ng/mg of liver protein [9]). It is therefore most likely that the $PGD_2$ produced in the liver is only meant to exert its metabolic effect within the liver. The present finding that $PGD_2$ formed by the non-parenchymal liver cells is recognized by a specific protein receptor on parenchymal liver cells, of which binding, conversion and metabolic effect are interlinked, provides further support for our previous proposal concept [8] that $PGD_2$ plays a major role in intercellular communication inside the liver.

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