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# DOWN-REGULATION OF PROSTAGLANDIN $E_2$ RECEPTORS IN REGENERATING RAT LIVER AND ITS PHYSIOLOGICAL SIGNIFICANCE

NAOAKI HASHIMOTO, TSUYOSHI WATANABE, YUSEI IKEDA, GOTARO TODA, HARUKI YAMADA, YUJI YOSHIKAWA, HIROSHI MITSUI, and KIYOSHI KUROKAWA

First Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, JAPAN

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SUMMARY The properties of prostaglandin(PG) $E_2$ receptors in regenerating liver were studied using rat hepatocytes in primary culture. The control cells possessed stereo-specific $PGE_2$ receptors with Bmax and Kd values, at $4^{o}C$, of 526 fmol/mg protein and 6.5 nM respectively. In cells from regenerating liver after 70% hepatectomy, Bmax was reduced to 42-43% that of the controls; Kd did not change. Administration of indomethacin before surgery prevented Bmax reduction. These results indicate that $PGE_2$, produced during the regeneration process, evoked cellular events and regulated the density of its receptors. 

Several authors (1-6) have suggested that prostaglandins (PGs) are involved in liver regeneration following partial hepatectomy. $PGE_2$ and $F_{2\alpha}$ production increased transiently in the regenerating liver (1), probably due to increased production by the non-parenchymal cells after surgery (2). Indomethacin, a potent inhibitor of PG synthesis, suppressed partial hepatectomy-induced DNA synthesis (3,4). Moreover, PGs stimulated DNA synthesis and mitosis in cultured neonatal rat hepatocytes (5) and played a role in inducing DNA synthesis in adult rat hepatocytes in primary culture (6). These reports suggest that PGs may be involved as growth factors in liver regeneration.

Abbreviations: PG, prostaglandin; Kd, dissociation constant; Bmax, maximum binding capacity; Hepes, 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid.

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However, whether PGs actually interact with the hepatocytes, *in vivo*, during the regeneration process remains to be elucidated.

In this study, we used primary cultures of regenerating hepatocytes and found that $PGE_2$ receptor density decreased in regenerating liver after partial hepatectomy compared with control. These data suggest that the cellular events in regenerating hepatocytes, including down-regulation of $PGE_2$ receptors, were evoked by the *in vivo* exposure to $PGE_2$ produced after the operation.

## MATERIALS AND METHODS

### Animals and Materials

Adult male Wistar rats (7-8 weeks old) were fed *ad libitum*. Partial (70%) hepatectomies were performed according to the method of Higgins and Anderson (7). In sham-treated group, only laparotomy was performed. All operations were performed between 8 am and noon. In fasted group, rats were starved for 24 h. Control rats received no treatment. In some experiments, indomethacin (7 mg/100g body weight) dissolved in polypropylene glycol (1) was administered intraperitoneally 4 h before partial hepatectomy. [5,6,8,11,12,14,15-$^3$H]$PGE_2$ was purchased from Amersham (UK) and [$^{125}$I]insulin was from New England Nuclear (Boston, MA, USA).

### Primary Cultures of Adult Rat Hepatocytes

Hepatocytes were isolated by the collagenase perfusion technique described by Tanaka *et al.* (8) and resuspended in Williams' medium E containing 5% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 ug/ml). Viability was determined by trypan blue exclusion and only preparations showing more than 90% viability were used. The cells were plated at a density of 5 x $10^5$ per 35-mm-diameter 6-well plate (Corning, NY, USA) and incubated under an atmosphere of 5% $CO_2$ in air at 37°C for 18-24 h.

### [$^3$H]Prostaglandin $E_2$ Binding Assay

The hepatocytes were washed with 1 ml/well of Williams' medium E, which contained 20 mM Hepes/NaOH (pH 7.2) (binding medium), then incubated in the binding medium, which contained 1 nM of [$^3$H] $PGE_2$, in the presence or absence of various concentrations of non-radioactive PGs $E_2$, $E_1$, $F_{2\alpha}$, or $D_2$ for 2 h. All procedures were performed at 4°C to prevent degradation of the ligand (9,10). The cells were solubilized with 0.5 N NaOH and the radioactivity was counted using a liquid scintillation system (Minaxi bete, Tri-carb C4430, Packard, USA). Specific binding of $PGE_2$ was calculated as the difference between total radioactivity measured and nonspecific radioactivity measured in the presence of 10 $\mu$M unlabelled $PGE_2$. Protein was measured by the methods of Bradford *et al.* (11) using bovine serum albumin as a standard.

### Binding of [$^{125}$I]Insulin to Cultured Hepatocytes

The insulin binding assay was performed as described elsewhere (12).

### Statistical analysis

For Scatchard analysis, lines were drawn from least squares analysis of the data.

RESULTS

The [$^3$H] $PGE_2$ bound to the cultured control hepatocytes was displaced by unlabelled PGs in the order of PGs $E_1 = E_2 > F_{2\alpha} > D_2$ (Fig. 1). The cells had a high-affinity, single class of binding site with a dissociation constant (Kd) of 6.5 nM and a binding capacity (Bmax) of 526 fmol/mg protein, derived by Scatchard analysis (Fig. 2).

In the hepatocytes isolated 24 h after 70% hepatectomy, Bmax was decreased to 224 fmol/mg protein (43% of control) and Kd did not change (7.1 nM), whereas the Bmax of cells from sham-treated rats was virtually identical to that of controls (Fig. 2). In cells from rats fasted for 24 h, the Kd and Bmax values were almost the same as those of cells from control rats (Fig. 2). Reduced binding was observed in the cells isolated as early as 6 h after hepatectomy (220 fmol/mg protein, 42% of control). Although Bmax in cells from sham-treated rats showed a transient decrease (296 fmol/mg protein, 46% of control) at 6 h, it

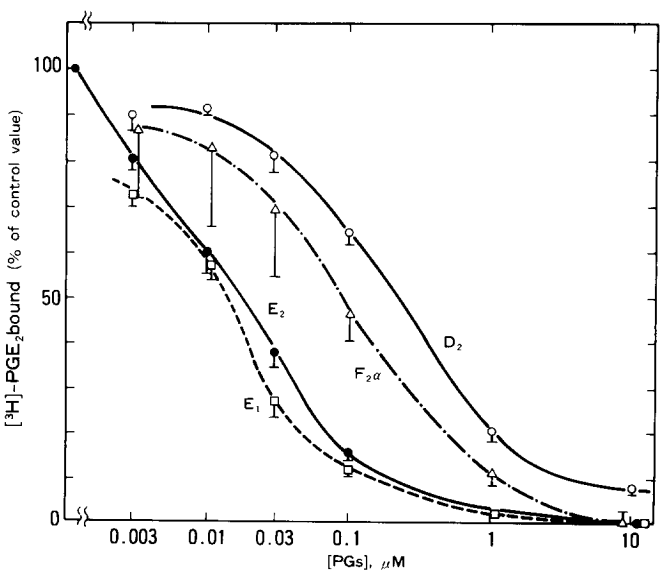


Fig. 1. Displacement curve for [$^3$H]$PGE_2$ by cold PGs $E_1$ (□), $E_2$ (●), $F_{2\alpha}$ (△) and $D_2$ (○). Each point is the mean+SEM of three independent experiments. Values are shown as percentages. The 100% control value was approximately 27,800 dpm/mg protein.

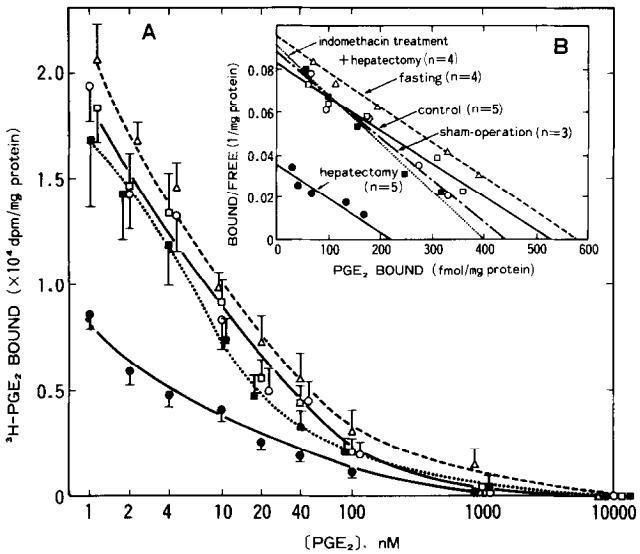


Fig. 2. Competition curves (A) and Scatchard analysis (B) of $PGE_2$ binding to cultured hepatocytes from rats under various conditions. Each point represents the mean of 3-5 independent experiments using different cell preparations. Cells were prepared from control rats (□), rats received 70% hepatectomy (●), sham-operation (○), indomethacin pretreatment before hepatectomy (■) or 24-fasting(△).

recovered to reach 438 fmol/mg protein (83% of control) at 24 h after the operation. The Kd values of $PGE_2$ receptors in the cells were essentially the same (5.2-7.1nM) in all treatment groups. The decrease of Bmax was prevented by treating the animal with indomethacin before partial hepatectomy (Fig. 2).

To rule out the possibility that decreased cell viability was responsible for the reduction of $PGE_2$ receptors, we examined the binding properties of the insulin receptors, the Bmax of which has been demonstrated to be increased in regenerating liver (13). The binding capacity of the insulin receptors was increased by 67%, compared with controls, in cells isolated 24 h after 70% hepatectomy; Kd values did not change (Fig. 3).

## DISCUSSION

It has been suggested that PGs are involved in liver regeneration, as PG production increases after partial hepatectomy (1,2) and PGs stimulate DNA synthesis in cultured

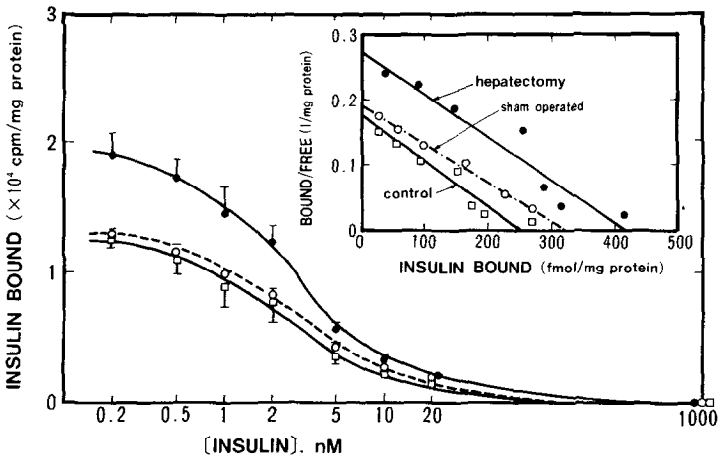


Fig. 3. Competition curves and Scatchard analysis of insulin binding to cultured hepatocytes from rats under various conditions. Each point represents the mean of 2-3 independent experiments, in each of which the binding assay was performed in duplicate. Symbols used were the same as shown in Fig. 2.

hepatocytes (5,6). However, the mechanism whereby PGs exert their effect on the regenerating liver and their physiological significance, in vivo, has not been elucidated.

PGs are believed to exert their effects via stereospecific receptors, which exist on the cell surface (14-16). The hepatocyte plasma membrane $PGE_2$ receptor has been reported to be stereospecific (14,15), which we have confirmed using cultured hepatocytes. The Kd and Bmax values of the cultured hepatocyte receptor were almost identical to those reported by Okumura, et al. (10).

We have shown that the density of $PGE_2$ receptors on the hepatocytes was reduced after partial hepatectomy. This reduction contrasts with the increased insulin receptor density we observed, which indicate that it was not due to decreased cell viability. Increased Bmax of insulin receptors in regenerating liver plasma membrane fraction has been reported, and is considered to represent up-regulation of the receptor, which reflects hypoinsulinemia following partial hepatectomy (13). Garrity et al.(17) reported that the density of $PGE_2$ receptors

was diminished in the hepatocytes from rats fasted for 24 h. In this study, however, hepatocytes showed no reduction of Bmax after 24-h fasting, which suggests that fasting was not responsible for the decreased density of the receptor after hepatectomy. The reason for the discrepancy between their report and ours is unclear.

Another possible explanation for reduction of the receptor density is that down-regulation is induced by exposure, *in vivo*, to $PGE_2$ produced after partial hepatectomy. Down-regulation of $PGE_2$ receptors has been reported in the plasma membrane preparation from rats treated with 16,16 dimethyl $PGE_2$ (18). Therefore, we investigated the effects of indomethacin treatment prior to partial hepatectomy on the density of the hepatocyte receptors. Indomethacin pretreatment prevented the Bmax reduction, which indicates that down-regulation played a role in the reduction of $PGE_2$ receptor density.

These data suggest that the rat hepatocytes were exposed to $PGE_2$ produced, *in vivo*, after the partial hepatectomy, which induced the down- regulation of $PGE_2$ receptors on the hepatocyte surface. Further study is needed in order to elucidate the mechanisms of action of PGs on hepatocytes during the regenerating process, *in vivo*.

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