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Prostaglandin D_2 mediates the stimulation of glycogenolysis in the liver by phorbol ester

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The tumour-promoting phorbol ester, phorbol 12-myristate 13-acetate (PMA), when added to the perfused liver, stimulates glycogenolysis 2-fold. This stimulation is not seen when aspirin is present in the perfusion medium. In isolated parenchymal liver cells, PMA is not able to stimulate glycogenolysis, suggesting that its effect on glycogenolysis might be indirect and depends on the presence of the non-parenchymal liver cell types. To test the possible operation of an indirect mechanism, we measured the amount of prostaglandin (PG) D_2 in liver perfusates. After addition of PMA, the amount of PGD_2 is doubled, in parallel with the increase in glycogenolysis. Glycogenolysis in both isolated parenchymal liver cells and perfused liver could be stimulated by the addition of PGD_2. Our data indicate that stimulation of glycogenolysis in the liver by PMA may be mediated by non-parenchymal liver cells, which produce PGD_2 in response to PMA. Subsequently PGD_2 activates glycogenolysis in the parenchymal liver cells. The intercellular communication inside the liver in response to PMA adds a new mechanism to the complex regulation of glucose homoeostasis by the liver.

INTRODUCTION

Parenchymal liver cells are the site of hepatic glycogen storage. The synthesis and breakdown of glycogen in these cells is under strict hormonal regulation. Glycogenolysis is regulated at the site of phosphorylase. Glucagon activates phosphorylase through a cyclic-AMP-dependent mechanism [1,2]. Ca^{2+}-linked hormones such as angiotensin II, vasopressin and α_1-adrenergic agents also stimulate phosphorylase activity [1,2]. These hormones act via two different second messengers, i.e. inositol 1,4,5-trisphosphate, which triggers Ca^{2+} mobilization [3,4], and 1,2-diacylglycerol, which activates protein kinase C [5,6].

Raising the intracellular Ca^{2+} concentration by the addition of Ca^{2+} ionophore A23187 to isolated parenchymal liver cells results in increased phosphorylase activity [7]. Surprisingly, the phorbol ester PMA, a potent activator of protein kinase C, does not increase the phosphorylase activity in isolated parenchymal liver cells [8], or only very weakly [9] or at very high concentration [10]. In perfused liver [11–13], however, PMA does stimulate glycogenolysis, suggesting that in the intact liver the presence of liver cell types other than parenchymal cells may be involved in the stimulation of glycogenolysis by PMA. Platelet-activating factor has the same effect on glycogenolysis as PMA; it stimulates glycogenolysis in perfused liver, but fails to do so in isolated parenchymal liver cells [14–16]. Since the stimulation of glycogenolysis in perfused liver by PMA and platelet-activating factor is blocked by indomethacin, the involvement of prostaglandins has been suggested [12,17]. Patel [13] suggested that PMA causes vasoconstriction, which results in hypoxia, which in its turn triggers glycogenolysis in the parenchymal cells.

The liver contains, in addition to parenchymal cells, Kupffer cells, endothelial cells, fat-storing cells and pit cells [18]. Kupffer cells are known to produce several prostaglandins [19–21]. Recently it was shown [22] that endothelial liver cells also produce several prostaglandins. Evidence was obtained showing that the major prostaglandin produced by both Kupffer and endothelial liver cells is prostaglandin (PG) D_2 [22].

In the present work we investigated the possible role of PGD_2 as a mediator of the glycogenolytic effect of PMA in the intact liver.

MATERIALS AND METHODS

PMA, PGD_2 and collagenase type IV were from Sigma; *O*-acetylsalicylic acid (aspirin) was from BDH; PGD_2 radioimmunoassay kit was from Amersham; glucagon was from Novo.

Male Wistar rats, fed *ad libitum*, weighing 200–220 g were used. Nembutal (18 mg) was given intraperitoneally for anaesthesia, usually performed between 9:00 and 10:00 h.

Parenchymal liver cells were isolated after perfusion for 20 min with collagenase (type IV; 0.1%) by the method of Seglen [23], modified as previously described [24]. Parenchymal liver cells were incubated at 37 °C under constant shaking at 5 mg of protein/ml in Krebs–Ringer bicarbonate buffer (1.3 mM-$CaCl_2$), saturated with O_2/CO_2 (19:1), pH 7.4, which keeps the viability of the cells > 95%. At 10 min intervals, portions of cell suspension were withdrawn, rapidly cooled to 0 °C, centrifuged at 500 *g* for 5 min, and subsequently glucose was determined in the supernatant by the glucose oxidase–ABTS method [25].

For liver-perfusion experiments, the portal vein was

Abbreviations used: PMA, phorbol 12-myristate 13-acetate; PG, prostaglandin.

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cannulated and the liver was perfused with non-recirculating Krebs–Ringer bicarbonate buffer [23]. The $CaCl_2$ concentration was 1.3 mM. The perfusion buffer was kept saturated with O_2/CO_2 (19:1) and the pH was 7.4. The perfusion flow was 34 ml/min, and the temperature was kept at 37 °C. Before additions were tested, the liver was pre-perfused for 40–50 min to obtain a constant glucose output. At 1 min intervals effluent was collected, in which glucose was determined by the glucose oxidase–ABTS method [25].

PGD_2 was determined in the effluent with a PGD_2 radioimmunoassay kit from Amersham. The zero value [26] was determined in the effluent of aspirin-treated (2 mM) livers and subtracted from other values to correct for non-specific binding.

Data were statistically analysed with a one-tailed paired Student's *t* test.

RESULTS

In the perfused liver the influence of PMA on the glucose output was studied. Fig. 1 shows that PMA stimulates glucose output; this stimulation has a lag phase of 3–6 min and consists in all experiments of two peaks. The stimulation by PMA (60 ng/ml) is almost 2-fold, and glucagon at 0.1 μM gives an almost 4-fold stimulation. When the perfusion is performed in the presence of 2 mM-aspirin, PMA stimulation is abolished, whereas the stimulation by glucagon is unaffected.

When PMA (60 ng/ml) was added to isolated parenchymal liver cells (Fig. 2), no stimulation of glucose production occurred, although 0.1 μM-glucagon gave a 4-fold stimulation.

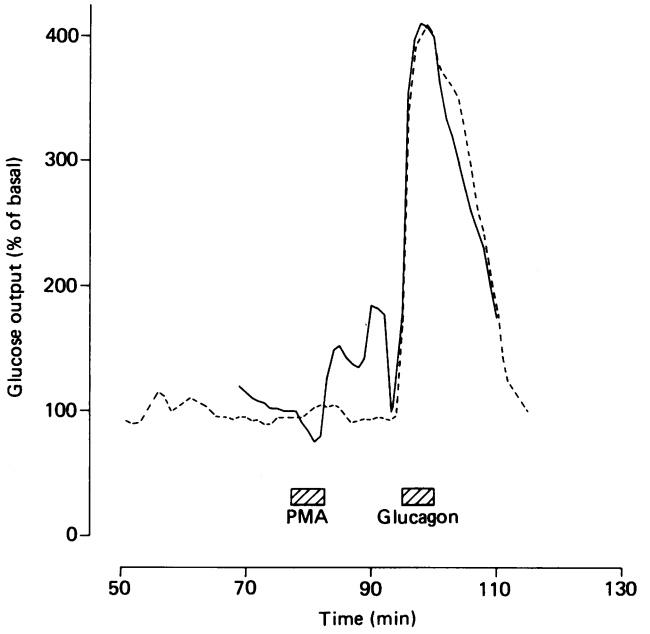

Fig. 1. Effect of PMA, glucagon and aspirin on the glucose output of perfused liver

Glucose was determined at 1 min intervals. PMA (60 ng/ml) and glucagon (0.1 μM) were given in 5 min pulses. In separate experiments livers were perfused with Krebs–Ringer buffer with (------) or without (——) aspirin present (2 mM). Data are from one typical experiment of six.

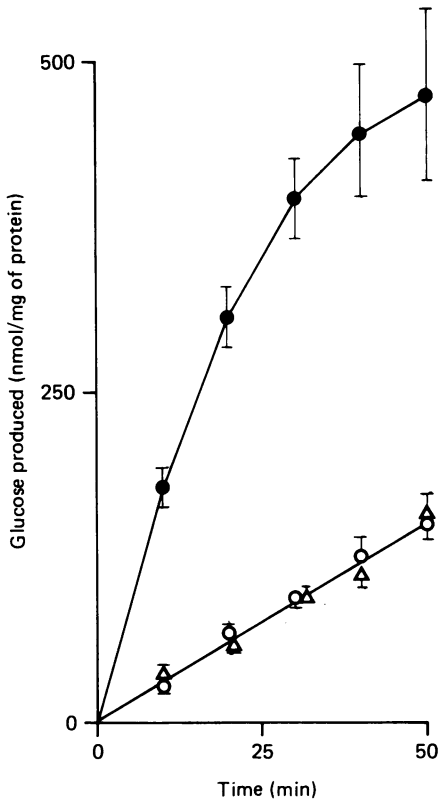

Fig. 2. Effect of PMA and glucagon on the glucose production by isolated parenchymal liver cells

○, Control; △, PMA (60 ng/ml); ●, glucagon (0.1 μM). Data are means ± S.E.M. for four experiments.

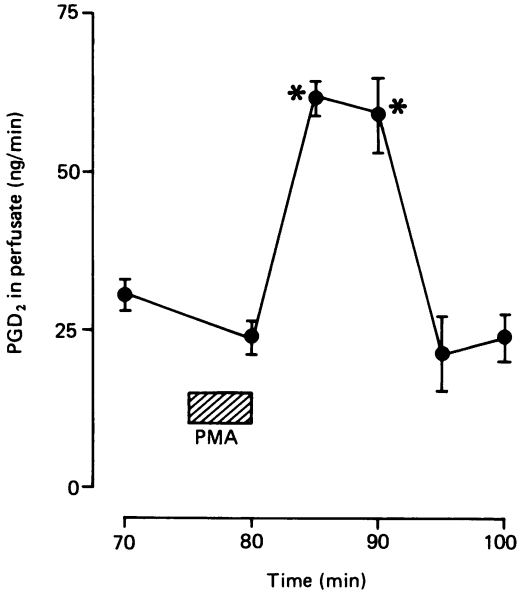

Fig. 3. Effect of PMA on PGD_2, recovered in liver perfusates

PMA (60 ng/ml) was given in a 5 min pulse. Data are means ± S.E.M. for four experiments; * indicates significant difference from control ($P < 0.05$).

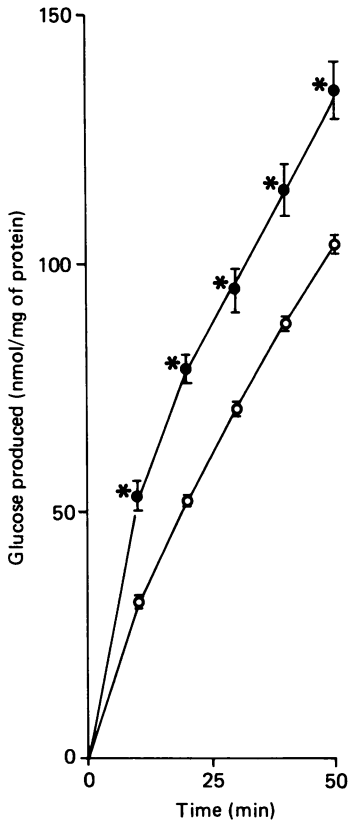

Fig. 4. Effect of PGD_2 on the glucose production by isolated parenchymal liver cells

○, Control; ●, PGD_2 (1 μM). Data are means ± S.E.M. for four experiments; * indicates significant difference from control ($P < 0.05$).

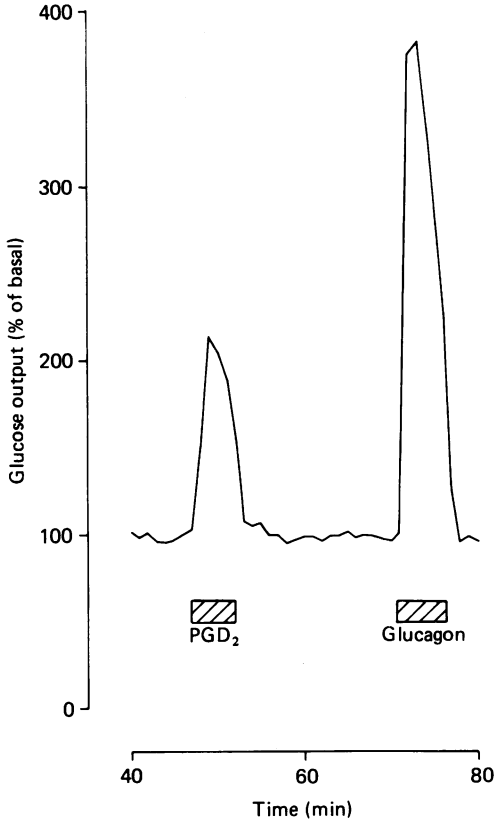

Fig. 5. Effect of PGD_2 and glucagon on the glucose output of perfused liver

Glucose was determined at 1 min intervals. PGD_2 (1 μM) and glucagon (0.1 μM) were given in 5 min pulses. Data are from one typical experiment of three.

In perfusates obtained in experiments as described in Fig. 1, PGD_2 was determined in a radioimmunoassay. As shown in Fig. 3, PMA (60 ng/ml) temporarily stimulates PGD_2 production by the liver. As with the stimulation of glucose production, there is a lag phase of approx. 5 min. PGD_2 recovered in the perfusate is more than doubled after addition of PMA. At 15 min after PMA stimulation, PGD_2 returns at the starting value; subsequent glucagon infusion did not affect the PGD_2 production (results not shown).

The influence of PGD_2 on the glucose production by isolated parenchymal liver cells was investigated (Fig. 4). PGD_2 at 1 μM stimulates glucose production in isolated parenchymal liver cells; the percentage stimulation is largest (70%) at 10 min after addition.

In perfused liver, PGD_2 at 1 μM gives a more than 2-fold increase in glucose output (Fig. 5). The glucose output rises immediately after the addition of PGD_2 and begins to decline before the PGD_2 pulse has ended. The stimulation of glycogenolysis by PGD_2 was not affected by aspirin (cf. Fig. 1).

DISCUSSION

Our results confirm that PMA can stimulate glycogenolysis in perfused liver [11,12]. In agreement with reference [12], we observed a biphasic stimulatory response.

Since PMA does not stimulate glycogenolysis in isolated parenchymal liver cells (see also [8]), the intact liver or the presence of other liver cell types seems to be needed for a glycogenolytic response. The stimulation of glycogenolysis by PMA in the perfused liver was found to be abolished by including aspirin in the perfusion medium. Also, another inhibitor of prostanoid synthesis, indomethacin, abolishes the stimulation of PMA [12]. Therefore it seems likely that the stimulation of glycogenolysis by PMA is transduced by prostanoids. For Kupffer and endothelial liver cells, it is known that they can produce prostaglandins [19–22], and recently we obtained evidence that their major eicosanoid product is PGD_2 [22]. We therefore monitored the response of PGD_2 in the perfusate, as a consequence of PMA addition, and found that PGD_2 recovered in the perfusate is more than doubled on addition of PMA, indicating an increased production of PGD_2 by the liver. Although both Kupffer and endothelial liver cells can produce PGD_2, the capacity of Kupffer cells is much higher, so probably this cell type gives a major contribution.

The rise in PGD_2 occurs after a 5 min lag phase, which is also noticed in the glucose output. Because infusion of PGD_2 leads to an immediate increase in glucose output, it appears that the time-dependence of glucose output is consistent with an intermediate role of PGD_2. Thus our data suggest a causal relationship between increased PGD_2 production and stimulation of glucose production by PMA. Such a mechanism is consistent with recent observations in peritoneal macrophages that protein

kinase C is involved in the activation of eicosanoid synthesis [27]. The stimulation of glucose production in isolated parenchymal liver cells by PGD_2 shows that prostaglandins can act directly on these cells as glycogenolytic agents. For PMA and platelet-activity factor, and also for heat-aggregated IgG, stimulation of glycogenolysis in the liver via an indirect mechanism has been reported [28]. In that study, indomethacin was shown to block the stimulation of glycogenolysis in perfused liver by heat-aggregated IgG, and infusion of PGE_2 led to increased glycogenolysis. It was suggested that PGE_2 stimulated glycogenolysis via an induction of hepatic vasoconstriction, a mechanism that has also been suggested for platelet-activating-factor-stimulated glycogenolysis in perfused liver [29]. Here we show that PGD_2 has a stimulatory effect on glycogenolysis in both perfused liver and isolated parenchymal liver cells. Furthermore, we show here that PGD_2 production in the liver is stimulated by PMA, whereas production of PGE_2 and 6-oxo-$PGF_{1\alpha}$ has been reported not to be affected by PMA [13].

Considering the fact that PGD_2 is the most prominent prostaglandin produced by both Kupffer and endothelial liver cells [22], our data indicate that PGD_2 produced in non-parenchymal liver cells in response to PMA can directly stimulate glycogenolysis in parenchymal liver cells. Our data, however, do not exclude the possibility that, besides a direct effect of PGD_2 on glycogenolysis in parenchymal liver cells, an indirect effect of PGD_2 on glycogenolysis via vasoconstriction leading to hypoxia may occur.

The finding that products of non-parenchymal liver cell types may mediate the response to PMA adds a new type of mechanism to the complex regulation of glucose homoeostasis by the liver, and may also be relevant under pathophysiological conditions.

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