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Stimulation of hepatic glycogenolysis by phorbol 12-myristate 13-acetate

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In isolated perfused rat livers, infusion of phorbol 12-myristate 13-acetate (PMA) (150 nM) resulted in a 3-fold stimulation of the rate of glucose production. This response was maximal at a perfusate PMA concentration of 150 nM, and was significantly diminished at higher concentrations of PMA (e.g. 300 nM). Stimulation of glycogenolysis by PMA was greatly decreased in livers perfused with Ca^{2+}-free medium. PMA infusion into livers perfused in the absence of Ca^{2+} did not result in Ca^{2+} efflux from the livers. Additionally, in hepatocytes isolated from livers of fed rats, neither PMA nor 1-oleoyl-2-acetyl-*rac*-glycerol stimulated the rate of glucose production. Although indomethacin has been demonstrated to block PMA-stimulated hepatic glycogenolysis [Garcia-Sainz & Hernandez-Sotomayor (1985) Biochem. Biophys. Res. Commun. **132**, 204–209], infusion of PMA into perfused rat livers did not alter the rates of production of either prostaglandin E_2 or 6-oxo-prostaglandin $F_{1\alpha}$ in the livers. These data, along with the observed increases in the perfusion pressure and decrease in O_2 consumption in isolated perfused livers suggest that phorbol-ester-stimulated glycogenolysis is not a consequence of a direct effect of phorbol ester on liver parenchymal cells.

INTRODUCTION

The demonstration that phorbol 12-myristate 13-acetate (PMA) can directly stimulate protein kinase C activity (Castagna *et al.*, 1982; Kikkawa *et al.*, 1983; Parker *et al.*, 1984) has provided a convenient probe to investigate the role of diacylglycerol-mediated stimulation of protein kinase C in producing the complete physiological response to hormone (e.g. vasopressin) action (Kishimito *et al.*, 1980; Nishizuka, 1984). Using the isolated perfused rat liver preparation, Kimura *et al.* (1984) demonstrated that infusion of PMA into the livers transiently stimulated the rate of glucose production. Since this glycogenolytic response to PMA was blocked by verapamil and because efflux of $^{45}Ca^{2+}$ was decreased by PMA, Kimura *et. al.* (1984) concluded that PMA increases the Ca^{2+} influx into hepatocytes. Although such an increase would be expected to increase glycogen phosphorylase activity, in isolated hepatocyte preparations PMA has been shown neither to increase phosphorylase activity (Lynch *et al.*, 1985; Cooper *et al.*, 1985) nor to alter cytosolic free Ca^{2+} concentrations as measured by quin-2 fluorescence (Cooper *et al.*, 1985). Furthermore, Cooper *et al.* (1985) demonstrated that, in isolated hepatocyte preparations, PMA does not alter the rate of Ca^{2+} efflux.

More recently, Garcia-Sainz & Hernandez-Sotomayor (1985) reported that, in the isolated perfused rat liver, the effect of PMA on hepatic glycogenolysis was blocked by indomethacin. Therefore these authors suggested that PMA stimulated hepatic glycogenolysis via generation of cyclo-oxygenase products. However, it should be noted that indomethacin has been demonstrated to interfere with the participation of Ca^{2+} in biological processes (see, e.g., Northover, 1977), and the glycogenolytic effect of PMA is blocked by Ca^{2+} antagonists (Kimura *et al.*, 1984). Since the studies of Garcia-Sainz & Hernandez-Sotomayor (1985) did not differentiate between the Ca^{2+}-antagonistic effect of indomethacin and its ability to inhibit the cyclo-oxygenase system, the mechanism(s) by which PMA stimulates glycogenolysis in the perfused rat liver must be regarded as being unknown.

Therefore the studies described here were designed to elucidate the mechanism(s) by which PMA stimulates glycogenolysis in the perfused rat liver.

MATERIALS AND METHODS

PMA, phorbol 13-monoacetate and lactate dehydrogenase were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Glucose-6-phosphate dehydrogenase and hexokinase were purchased from Boehringer Mannheim Corp., Indianapolis, IN, U.S.A. 6-Oxo[5,8,9,11,12,14,15-^{3}H(n)]$PGF_{1\alpha}$, [5,6,8,11,12,14,15-^{3}H(n)]PGE_2, unlabelled prostaglandins and antibodies to prostaglandins were generously given by Dr. K. U. Malik, Department of Pharmacology, University of Tennessee, Memphis. All other chemicals were of the highest purity commercially available.

Male rats of the Sprague–Dawley strain (160–180 g body wt.) were used in these studies. Animals were allowed free access to food and water. Experiments were performed between 08:30 and 11:00 h. After pentobarbital sodium anaesthesia, the rat livers were perfused by the non-recirculating perfusion technique described by Scholz *et al.* (1973). The haemoglobin-free and glucose-free perfusion medium, Krebs–Henseleit (1932) bicarbonate buffer, modified to contain 1.3 mM-$CaCl_2$, was saturated with O_2/CO_2 (19:1) and maintained at 37 °C. Phorbol ester was infused into the livers at a point proximal to the hepatic portal-vein cannula. The effluent

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

perfusate was collected at 30 s intervals and assayed for the various metabolites. Portal-vein pressure, which can be used as an index of intrahepatic pressure (Greenway, 1981, 1983), was monitored continuously by means of a pressure transducer connected to the portal cannula line.

Hepatocytes were isolated from livers of fed rats essentially as described by Seglen (1976). As assessed by their ability to exclude Trypan Blue, viability of hepatocytes was always found to be greater than 95%. The hepatocytes (25×10^6 cells) were incubated in 5 ml of the Krebs–Henseleit bicarbonate buffer described above. Samples (1 ml) of the incubation mixture were withdrawn after incubation for 0, 8, 16 or 24 min and acidified with 100 μl of 14% (w/v) $HClO_4$. After centrifugation and neutralization, the glucose content in the samples was determined as described below. Glucose production rates are expressed on the basis of tissue weight, by using the conversion 120×10^6 cells/g wet wt.

Glucose, pyruvate and lactate in the samples were determined by the enzymic methods described by Bergmeyer *et al.* (1974), Passonneau & Lowry (1974) and Gutmann & Wahlefeld (1974) respectively. For determination of prostaglandins, perfusate samples collected over 1 min periods were acidified to pH 2.7, passed through Sep-Pak columns containing ODS silica, and prostaglandins were extracted as described by Powell (1980). The methyl formate fractions containing prostaglandins were evaporated to dryness under nitrogen. The prostaglandin-containing extracts and standards of PGE_2 and 6-oxo-$PGF_{1\alpha}$ were then dissolved in Krebs–Henseleit bicarbonate buffer (0.9 ml). PGE_2 and 6-oxo-$PGF_{1\alpha}$ (the stable hydrolysis product of PGI_2) were measured by radioimmunoassay as described by Shaffer & Malik (1982).

RESULTS

Infusion of PMA into perfused rat livers resulted in a dose-dependent stimulation of glucose production (Fig. 1). As the concentration of PMA was elevated, the increase in the rate of glucose production from the liver was enhanced, and this response was maximal at perfusate PMA concentrations of 150 nM (Fig. 1). However, at higher PMA concentrations (e.g. 300 nM), the stimulation of glucose production was significantly lower than the maximal increase observed at 150 nM-PMA in the perfusion medium (Fig. 1).

Additionally, when Ca^{2+} was omitted from the perfusion medium, infusion of PMA into perfused rat livers resulted in stimulation of glucose production which was significantly lower than that observed in experiments with Ca^{2+}-replete medium (Fig. 1). However, the biphasic shape of the dose–response curve was maintained in both the presence and the absence of Ca^{2+} (Fig. 1).

Figs 2 and 3 show representative experiments which illustrate the temporal changes associated with PMA infusion into rat livers perfused in the presence (Fig. 2) and in the absence (Fig. 3) of Ca^{2+}. In rat livers perfused with Ca^{2+}-replete medium, infusion of PMA resulted in a simultaneous increase in the rate of glucose production, the rate of glycolysis (lactate + pyruvate production), and portal pressure (Fig. 2). Invariably, the increase in the rates of glucose production and glycolysis reached a maximum between 8 and 10 min after initiation of PMA infusion (Fig. 2). Thereafter, the rates of glucose

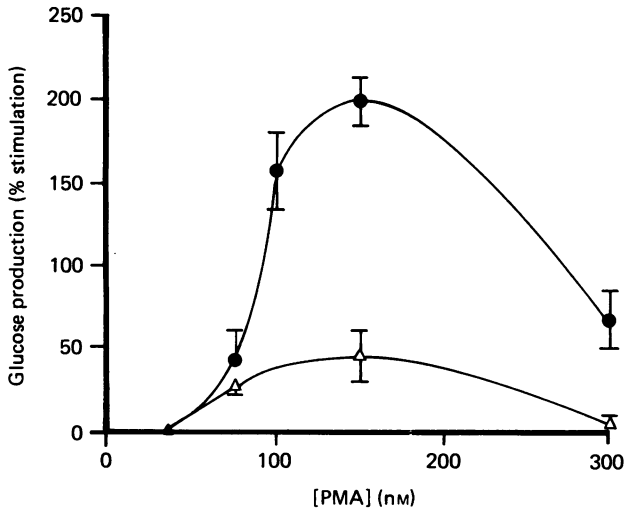

Fig. 1. Effect of perfusate PMA concentration on the rate of glucose production from rat livers perfused in the presence (●) and absence (△) of Ca^{2+}

Percentage increases in glucose production over control rates of 69.7 ± 2.2 μmol/h per g ($n = 44$) are represented as means ± S.E.M. ($n > 4$). After a 30 min equilibration period, perfusate samples were collected for a 15 min period to determine the control rate of glucose production. Infusion of PMA was then initiated for a 15 min period and the samples were analysed for glucose. The maximal increase in glucose production was used to determine the percentage increase.

production and glycolysis declined slightly, to attain new steady-state values which were significantly higher than control values (i.e. before PMA infusion) (Fig. 2). On the other hand, the increase in portal pressure during PMA infusion into the livers was gradual, and devoid of any transient changes (Fig. 2). In addition, infusion of PMA caused a decrease in O_2 consumption by the liver (Fig. 2). Withdrawal of PMA from the perfusion medium did not reverse either the increase in portal pressure or the stimulated rates of glucose production and glycolysis (Fig. 2). It should be noted that in control experiments in which livers were perfused either with the inactive phorbol ester phorbol 13-monoacetate or without any addition, the portal pressure and the rates of glucose production, glycolysis and O_2 consumption were maintained constant at values similar to those shown in Fig. 2 before PMA infusion (results not shown).

When livers were perfused with Ca^{2+}-free medium (Fig. 3), infusion of PMA resulted in alterations in the rates of glucose production, glycolysis and O_2 consumption and in portal pressure which, apart from the exceptions noted below, were very similar to those described above for livers perfused with Ca^{2+}-replete medium. First, omitting Ca^{2+} from the perfusion medium decreased the extent to which glucose production, glycolysis and portal pressure were elevated on infusion of PMA into the rat livers (cf. Figs. 2 and 3; see also Fig. 1). Similarly the decrease in O_2 consumption during PMA infusion into livers perfused with Ca^{2+}-depleted medium was also quantitatively less than that observed in livers perfused with medium containing Ca^{2+} (cf. Figs. 2 and 3). Secondly, in rat livers perfused with Ca^{2+}-free medium, the transient phase in the stimulated

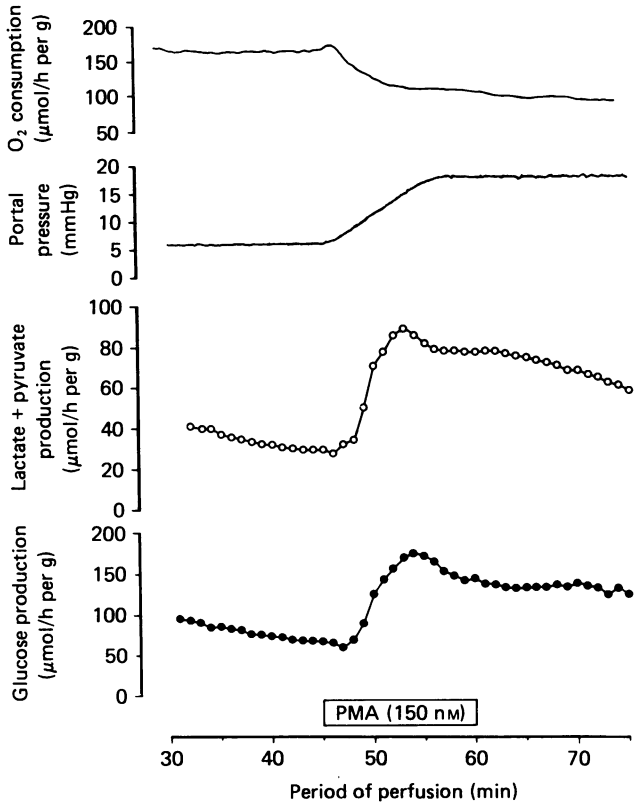

Fig. 2. Temporal changes in the rate of glucose production (●), glycolysis (○), portal pressure, and O_2 consumption in the effluent perfusate during PMA (150 nM) infusion into a rat liver perfused with Ca^{2+}-replete (1.3 mM-$CaCl_2$) medium

Experimental details are described in the Materials and methods section. PMA was present for the period indicated by the bar.

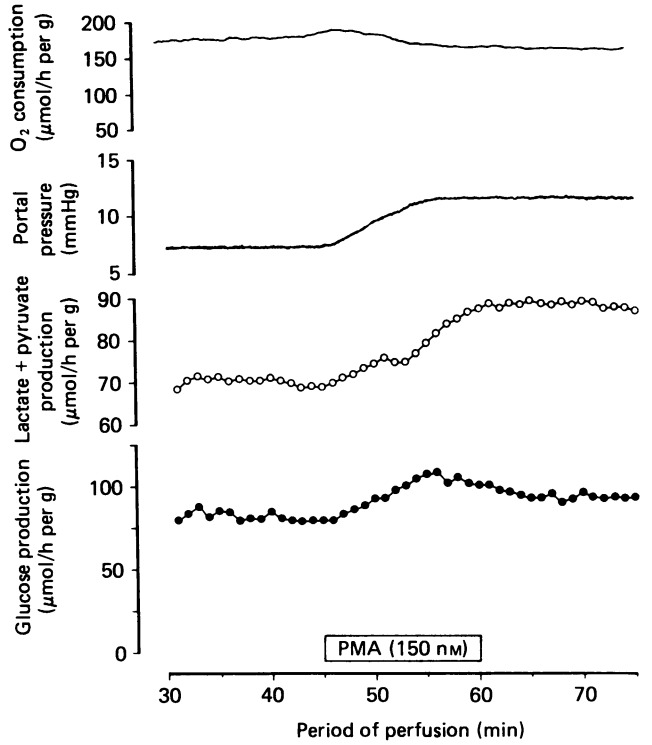

Fig. 3. Temporal changes in various parameters associated with infusion of PMA (150 nM) into a rat liver perfused with Ca^{2+}-depleted medium

Production of glucose (●) and lactate plus pyruvate (○) were monitored as described in the Materials and methods section.

rates of glucose production and glycolysis during PMA infusion, which was always observed in experiments with Ca^{2+}-replete medium, was blunted (cf. Figs. 2 and 3). Further, in experiments similar to that depicted in Fig. 3, inclusion of EGTA (1 mM) in the Ca^{2+}-free medium (i.e. livers were exposed to EGTA for 45 min before addition of PMA), did not significantly affect the extent to which the various parameters monitored were altered during PMA infusion (results not shown).

Since PMA increased the portal pressure, indicating vasoconstriction of the liver vasculature, and because such a vasoconstriction can alter the rate of glycogenolysis (Buxton *et al.*, 1986), experiments were performed to elucidate the effect of various PMA concentrations on the portal pressure in livers perfused in the presence and absence of Ca^{2+} in the perfusion medium. Fig. 4 demonstrates that, similarly to its effect on the rate of glucose production (Fig. 1), in rat livers perfused with Ca^{2+}-replete medium, increasing perfusate PMA concentrations elevated the portal pressure, which was maximally increased at 150 nM-PMA. However, at higher perfusate concentrations of PMA (e.g. 300 nM), the increase in portal pressure was significantly lower than the maximum observed at 150 nM-PMA (Fig. 4). When Ca^{2+} was omitted from the perfusion medium, the biphasic shape of the dose–response curve was preserved; however, PMA increased the portal pressure to a lesser extent as compared with rat livers perfused with Ca^{2+}-replete medium (Fig. 4).

Fig. 5 demonstrates that, besides the similarity between alterations in portal pressure and glucose production during PMA infusion (cf. Figs. 1 and 4), there is a very close correlation between the two events during PMA infusion into rat livers. Hence, when the maximal increase in glucose production during PMA infusion into the rat livers was expressed as a function of increase in the portal pressure, the resulting linear regression line had a correlation coefficient of 0.97 and a slope of 1.12 (Fig. 5).

In an attempt to isolate the glycogenolytic effect of PMA from its vasoconstriction action, rat livers were perfused with the vasodilator sodium nitroprusside. However, sodium nitroprusside (10 μM) at concentrations previously demonstrated to block hepatic vasoconstriction owing to nerve stimulation (Hartmann *et al.*, 1982) did not attenuate either the PMA (150 nM)-mediated increase in portal pressure or its stimulation of glycogenolysis (results not shown). In support of our observations with nitroprusside and PMA, Dale & Obianime (1985) have demonstrated that in guinea-pig lung parenchymal strips nitroprusside failed to block PMA-mediated contraction.

In order to elucidate the direct effects of PMA on liver parenchymal cells, experiments with isolated hepatocytes were performed. These experiments demonstrated that, although the α-adrenergic agonist phenylephrine stimu-

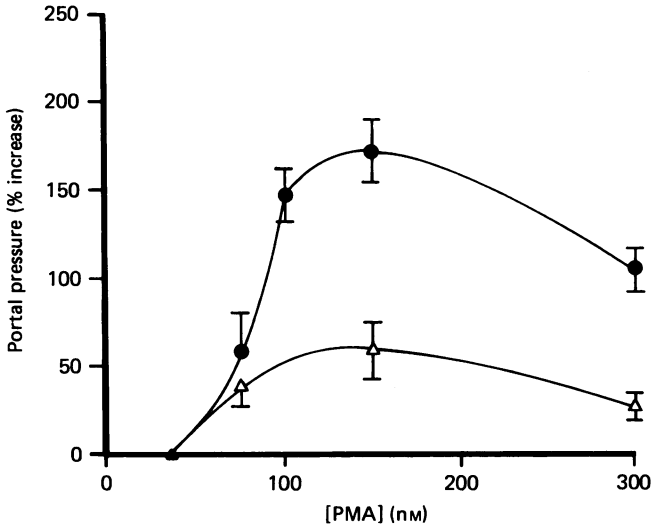

Fig. 4. Effect of perfusate PMA concentration on portal pressure in rat livers perfused in the presence (●) and absence (△) of Ca^{2+}

Percentage increases in portal pressure over control values of 5.1 ± 1.0 mmHg ($n = 44$) are represented as means ± S.E.M. ($n > 4$). Experimental protocol and conditions were similar to those described in the legends of Figs. 1 and 2 and in the Materials and methods section.

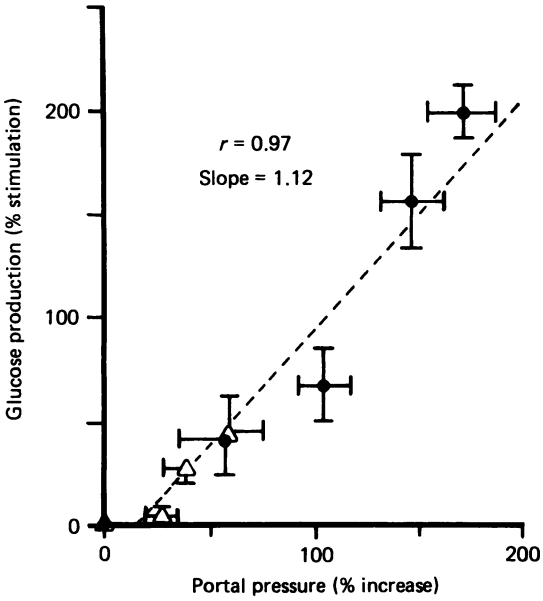

Fig. 5. Relationship between increase in portal pressure and stimulation of glucose production in livers perfused with various concentrations of PMA in the presence (●) and absence (△) of Ca^{2+}

Livers from fed rats were perfused with various PMA concentrations (see Figs. 1 and 4) in the presence and absence of Ca^{2+}. Data are presented as means ± S.E.M. ($n > 4$) for percentage increases in portal pressure and glucose production during PMA infusion.

lated the rate of glucose production from hepatocytes, neither PMA nor the synthetic diacylglycerol, 1-oleoyl-2-acetyl-*rac*-glycerol, at concentration of up to 1.25 μM and 12.5 μM respectively, affected the rate of glucose production (Fig. 6).

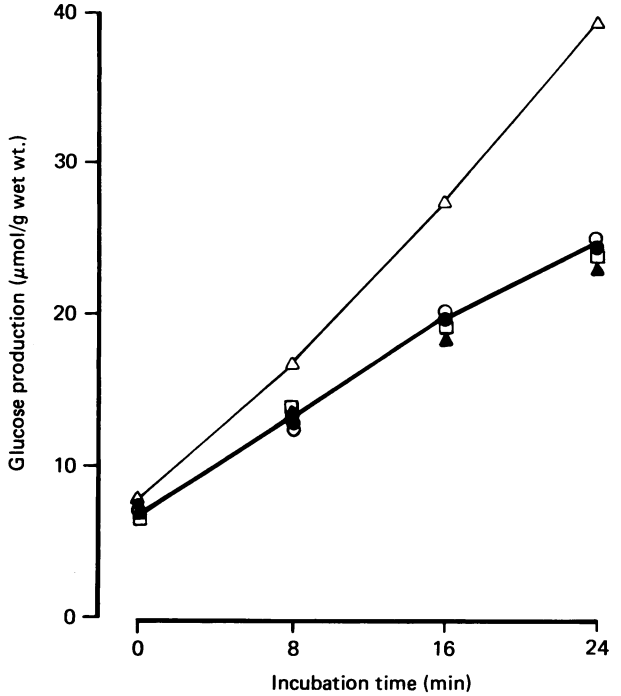

Fig. 6. Effect of PMA, 1-oleoyl-2-acetyl-*rac*-glycerol and phenylephrine on the time course of glucose production by hepatocytes isolated from fed rats

Hepatocytes (5 ml) were incubated in Krebs–Henseleit bicarbonate buffer at 30 °C as described in the Materials and methods section. PMA [○, 150 nM; □, 1.25 μM), 1-oleoyl-2-acetyl-*rac*-glycerol (▲; 12.5 μM) and phenylephrine (△; 10 μM) were added at zero time; ●, control. Samples were withdrawn at times indicated and analysed for glucose content.

Finally, experiments were performed to determine if PMA infusion into livers resulted in generation of cyclo-oxygenase products, as suggested by Garcia-Sainz & Hernandez-Sotomayor (1985). Hence, livers were perfused under exactly the conditions used in Fig. 2. Perfusate samples were collected for 1 min periods just before and at various times during PMA infusion into the livers. The data in Table 1 indicate that PMA did not affect the rates of production of either PGE_2 or 6-oxo-$PGF_{1\alpha}$ by the livers. It should be noted that, similarly to the data of Garcia-Sainz & Hernandez-Sotomayor (1985), infusion of indomethacin (100 μM) into livers for a 10 min period before initiation of PMA (150 nM) infusion blocked the PMA-mediated stimulation of glucose production and increase in portal pressure. However, on initiation of indomethacin (100 μM) infusion the portal pressure was transiently increased. The maximum increase in portal pressure (25%) was observed 1.5 min after initiation of indomethacin and was accompanied by a 25% increase in the rate of hepatic glucose production. On continued infusion of indomethacin (10 min), the rate of glucose production and the portal pressure reverted to control values (i.e. those before indomethacin infusion).

DISCUSSION

The data presented here clearly demonstrate that, in isolated perfused livers derived from rats fed *ad libitum*,

Table 1. Effect of PMA on prostaglandin production from perfused rat livers

Rat livers were perfused by the protocol described in the legend to Fig. 1 and as illustrated in Fig. 2. Effluent perfusate samples were collected for the control period before initiation of PMA infusion and at various times during the course of PMA infusion into the livers. All samples were collected for a 1 min period. Control samples were collected just before initiation of PMA infusion into the livers. The data are presented as means ± S.E.M. for determinations in six separate livers. Rates of production of PGE_2 and 6-oxo-$PGF_{1\alpha}$ were not significantly different from control values, as assessed by unpaired Student's *t* test.

Condition	Prostaglandin production (pg/min per g wet wt.)	
	PGE_2	6-Oxo-$PGF_{1\alpha}$
Control	151.0±29.5	4.07±0.15
PMA (150 nM)		
1 min	183.8±49	4.15±0.17
2 min	206.3±45	4.06±0.15
3 min	187.6±28	4.38±0.2
9 min	207.6±43	4.36±0.38
15 min	194.9±23	4.12±0.7

PMA (150 nM) infusion increases by 3-fold the rate of glycogenolysis and also the portal pressure (Figs. 1, 2 and 4). The increase in portal pressure observed on PMA infusion into rat livers is indicative of vasoconstriction of the liver vasculature. Indeed, studies from Hidaka's laboratory have demonstrated that protein kinase C can phosphorylate both the myosin light chain (Endo *et al.*, 1982), and the myosin light-chain kinase (Ikebe *et al.*, 1985). Such a phosphorylation of myosin light chains in the liver vasculature, owing to activation of protein kinase C by PMA, would result in vasoconstriction and the attendent increase in portal pressure observed in our studies. Furthermore, the observation that PMA caused a slow but sustained vasoconstriction of the hepatic vasculature is consistent with the findings of Rasmussen *et al.* (1984) and Forder *et al.* (1985), who also observed vasconstriction in rabbit ear arteries treated with PMA. In this respect, it should be noted the time course of increase in portal pressure during PMA infusion in livers (Fig. 2), and the time course of phosphorylation of myosin light-chain kinase by protein kinase C (Ikebe *et al.*, 1985), are very similar. Interestingly, in our studies the PMA-mediated increases in portal pressure and glucose production were not reversed on withdrawal of PMA from the perfusion medium (see Fig. 2). This may be the result of a slow dissociation of PMA from protein kinase C or of irreversible binding of PMA to protein kinase C. An explanation for the transient stimulation of glucose production by PMA in the studies of Kimura *et al.* (1984) and Garcia-Sainz & Hernandez-Sotomayor (1985), as compared with the sustained response demonstrated here, is not obvious, but may be related to the fact that Kimura *et al.* (1984) and Garcia-Sainz & Hernandez-Sotomayor (1985) employed a constant-pressure perfusion system, as opposed to the constant-flow system used in the present study.

At present an explanation for the biphasic shape of the dose–response curves for the rates of glucose production and the increase in portal pressure as a function of PMA concentration (Figs. 1 and 4) is not obvious, but may be representative of a 'toxic' effect of high PMA concentrations on tissue function (e.g. constriction), which would probably not be evident on events such as phosphorylation. However, the concentration of PMA (150 nM) which elicited maximal responses in our studies is very similar to that shown to be effective in inhibiting Ca^{2+} mobilization in response to α-adrenergic stimulation in hepatocytes (Cooper *et al.*, 1985), and phosphorylating proteins in hepatocytes (Garrison *et al.*, 1984).

For reasons noted below, it would appear that the increased glycogenolysis observed during infusion of PMA into the perfused livers is the result of increased vasoconstriction of the liver vasculature, and not a direct effect of PMA on parenchymal cells. First, a very close correlation between increase in portal pressure and increase in the rate of glucose production during PMA infusion was observed (Fig. 5). Secondly, in hepatocytes isolated from livers of fed rats, although the α-adrenergic agonist phenylephrine stimulated the rate of glucose production (Fig. 6), neither PMA (in the range 15 nM–1.25 μM; see, e.g., 150 nM and 1.25 μM in Fig. 6) nor the synthetic diacylglycerol 1-oleoyl-2-acetyl-*rac*-glycerol (in the range 1–12.5 μM; see, e.g., 12.5 μM in Fig. 6) affected the rate of glucose production. This observation is consistent with the findings of Cooper *et al.* (1985) and Lynch *et al.* (1985), that PMA and 1-oleoyl-2-acetylglycerol do not alter glycogen phosphorylase activity in isolated hepatocytes. In addition, Cooper *et al.* (1985) did not observe any alterations on either Ca^{2+} influx or Ca^{2+} efflux in isolated hepatocytes. Similarly, in our studies involving perfusion of livers with Ca^{2+}-free medium, analysis of the effluent perfusate by atomic absorption spectrometry did not show any alterations in Ca^{2+} content of the effluent perfusate during PMA infusion (results not shown). This last observation indicates that PMA neither mobilized intracellular Ca^{2+} nor increased its efflux, since such events, which occur, for example, during phenylephrine infusion (see, e.g., Blackmore *et al.*, 1979), would have been detected as an increase in Ca^{2+} concentration in the effluent perfusate (Blackmore *et al.*, 1979).

It is likely that vasoconstriction of liver vasculature may redirect flow through the liver. Since α-adrenergic stimulation, which increases portal pressure (Hartmann *et al.*, 1982), has been demonstrated to constrict the portal venules and sinusoids involved in regulating hepatic flow (Reilly *et al.*, 1981), it is conceivable that the PMA-mediated increase in portal pressure may also involve similar changes. Redistribution of flow may result in local hypoxia, with concomitant increase in flow to other regions of the liver. Hypoxia in the liver has been demonstrated to convert phosphorylase *b* into phosphorylase *a* (Hems & Whitton, 1980; Theen *et al.*, 1982), most probably by an increase in AMP content of the hypoxic tissue (Hems & Whitton, 1980). Because alterations in AMP contents in localized hypoxic regions of perfused livers would be masked by the presence of normoxic tissue zones in the liver (see, e.g., Buxton *et al.*, 1986), measurements of AMP content were not performed in the present study. However, the decrease in the rate of O_2 consumption by the livers in the presence of PMA (Figs. 2 and 3) supports the notion that portions of the

liver were not accessible to the perfusate, and therefore were hypoxic.

In keeping with the contention that vasoconstriction of the hepatic vasculature may result in increased glycogenolysis, Buxton *et al.* (1986) have demonstrated that the potent vasoactive phosphoacylglycerol, alkylacetylglycerophosphocholine, stimulates glucose production from perfused livers by this same mechanism.

Although indomethacin has been demonstrated to block the effect of PMA on glycogenolysis in perfused rat livers (Garcia-Sainz & Hernandez-Sotomayor, 1985), it is not likely that cyclo-oxygenase products are involved in mediating the PMA-stimulated glycogenolysis. First, indomethacin is known to be a Ca^{2+} antagonist and therefore interferes with biological processes involving Ca^{2+} (Northover, 1977); stimulation of hepatic glycogenolysis by PMA is sensitive to Ca^{2+} antagonists (e.g. verapamil; see Kimura *et al.*, 1984) and perfusate Ca^{2+} concentration (Figs. 1 and 3). Secondly, PMA infusion into livers did not affect the rate of production of either PGE_2 or 6-oxo-$PGF_{1\alpha}$ (Table 1). Additionally, Brass & Garrity (1985) have demonstrated that indomethacin and other cyclo-oxygenase inhibitors alter glycogenolysis independently of their ability to inhibit the cyclo-oxygenase. Finally, since indomethacin has been shown to inhibit cyclic-AMP-dependent and -independent protein kinases (Ahmet & Goucli, 1980; Kantor & Hampton, 1978), it is possible that it may also inhibit protein kinase C or other cellular protein kinases involved in the PMA-mediated biological response.

The observation that in livers perfused with Ca^{2+}-depleted medium the ability of PMA to increase perfusion pressure was attenuated (Fig. 5) indicates that extracellular Ca^{2+} is required for maximal vasoconstriction in response to activation of protein kinase C. This observation corroborates the findings by Rasmussen *et al.* (1984), who also observed that constriction of rabbit ear arteries in response to PMA treatment was Ca^{2+}-dependent. Additionally, the demonstration that PMA does not affect either the Ca^{2+} fluxes across plasma membrane or cytosolic free Ca^{2+} concentrations in hepatocytes (Cooper *et al.*, 1985), and our finding that stimulation of glycogenolysis by PMA in livers perfused with Ca^{2+}-depleted medium was decreased (Fig. 1), further support the notion that vasoconstriction of the hepatic vasculature in response to PMA regulates the rate of glucose production by the liver.

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