affect glutathione levels. The results presented in this paper show that there is no clear mechanistic link between chemically-induced growth arrest and changes in glutathione status. Therefore caution should be exercised when attempts are made to extrapolate findings concerning the relationship between mechanisms of cell growth modulation and glutathione status from one cell type to others.

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Effect of phorbol ester on rat liver regeneration

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Liver regeneration after partial hepatectomy has been employed as a model system to investigate the regulatory mechanism *in vivo* of cellular proliferation. We have shown that DNA synthesis in regenerating rat liver is primarily regulated by catecholamines, which are released from the splanchnic nerves and function via hepatic α_1-receptors [1, 2]. Recent *in vitro* observations using isolated liver cells suggest that protein kinase C [3] activated by phorbol 12-myristate 13-acetate (PMA) interferes with the signal transduction of α_1-receptors [4–8]. It is well documented that PMA prevents calcium mobilization [7, 9] and turnover of inositol phospholipids [7, 10], which are involved in α_1-adrenergic action [11, 12]. However, to our knowledge, *in vivo* experiments concerning the action of PMA on DNA synthesis have not yet appeared. In the present paper, we evaluated the effect of PMA on liver regeneration in an attempt to clarify the function of the tumor promoter and to explore the relationship between cellular proliferation and protein kinase C activity in the whole animal.

Materials and methods

Partial hepatectomy was performed according to the procedure of Higgins and Anderson [13]. Activities of thymidylate synthetase (TS) and thymidine kinase (TK) and DNA content were determined as previously described [1, 2, 14]. Protein content was measured by the method of Lowry *et al.* [15]. PMA or 4α-phorbol (0.25 mg/kg), dissolved in 50% ethanol, was injected intraperitoneally 8 hr after partial hepatectomy. The same quantity of PMA was injected into normal rats 16 hr before killing them. Other treatments and materials used were similar to those in our previous reports [1, 2, 14].

Results and discussion

As in our previous studies [1, 2, 14, 16], we evaluated regenerative responses of the liver by measuring the activities of hepatic thymidylate synthetase (TS; EC 2.1.1.45) and thymidine kinase (TK; EC 2.7.1.21), which are rate-determining enzymes in DNA synthesis [1, 2]. These measurements were made 24 hr after partial hepatectomy when the activities of these enzymes reach almost maximal levels [1, 2]. DNA content of the liver was also quantitated. The TS level at 24 hr following partial hepatectomy increased 7.8 times compared to that of the non-operated rats (shown in Table 1). The TK level of the control animals (partial hepatectomy only) also increased 13-fold over that of the non-operated group. When PMA (0.25 mg/kg) was administered intraperitoneally to rats 8 hr after partial hepatectomy, the enzymatic activities of TS and TK 24 hr following partial hepatectomy increased by 2.8 and 5.8 times, respectively, compared with the non-operated group. However, these levels were significantly lower than those of the control animals. The DNA content of the liver was also diminished by the treatment with PMA, as shown in Table 1. The biologically inactive analogue of PMA, 4α-phorbol (0.25 mg/kg, i.p.), given 8 hr after partial hepatectomy, produced no detectable effect on either the activities of TS and TK or the liver DNA content in 24 hr-regenerating liver compared with the control group. These results suggest that protein kinase C activated by PMA prevented the rise of the activities of TS and TK with concomitant inhibition of DNA synthesis in liver regeneration.

The inhibitory action of PMA on liver regeneration may be explained on the ground that activated protein kinase C phosphorylates and desensitizes α_1-receptors [10], which primarily regulate DNA synthesis in liver regeneration [1, 2]. It is well established [11, 12] that stimulation by an α_1-agonist gives rise to inositol turnover to produce inositol triphosphate, a calcium mobilizing agent [17], and diacyl glycerol, a physiological activator of protein kinase C [3]. Based on the present results, protein kinase C may play a pivotal role as a negative rather than a positive messenger, providing an immediate feedback control that prevents over-response via the α_1-receptor. This may be compatible with the report [18] that describes the reduced responsiveness of the liver cell toward α_1-agonist at 72 hr after partial hepatectomy, when regenerative responses pass through the maximal point at 24–48 hr and are declining. The present paper is the first whole animal study showing the role of protein kinase C as a negative regulator of liver cell proliferation. This is in sharp contrast to *in vitro* experiments showing that PMA functions as a typical tumor

Table 1. Effect of phorbol ester on 24 hr-regenerating rat liver

Treatment	TS activity (pmol/min/mg protein)	TK activity (pmol/min/mg protein)	DNA content (mg/liver)
Control (partial hepatectomy only)	50.97 ± 4.10	218.10 ± 25.41	6.88 ± 0.11
Partial hepatectomy + PMA	20.13 ± 3.33*	93.55 ± 21.45*	5.97 ± 0.28*
Partial hepatectomy + 4α-phorbol	51.74 ± 4.32	216.05 ± 30.00	6.99 ± 0.21
Non-operated	7.07 ± 0.64*	16.19 ± 1.13*	ND†
Non-operated + PMA	6.15 ± 0.56*	13.93 ± 2.77*	ND

PMA or 4α-phorbol (0.25 mg/kg) dissolved in 50% ethanol was injected intraperitoneally at 8 hr after partial hepatectomy. At 24 hr after partial hepatectomy the liver was perfused *in situ* with 0.9% NaCl, immediately excised, and homogenzed with 4 vol. of 50 mM Tris–HCl buffer, pH 7.5, containing 0.25 M sucrose, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride and 1 mM EDTA. The supernatant fraction of the centrifugation at 36,000 *g* for 30 min at 4° was used to determine the TS and TK activities. Liver DNA content, TS and TK activities, and protein concentration were determined as previously described [1]. In (non-operated + PMA) rats, the same quantity of PMA was injected at 16 hr before killing. Values are expressed as mean ± SE of seven to twenty rats.

* Significantly different from control ($P < 0.05$, Student's *t*-test).

† Not determined.

promoter and induces DNA synthesis in T51B rat liver cell [19]. Further studies are necessary to explain the contradictory behavior between the cultured and the native liver cell toward the tumor promoter. Investigation on this problem will provide further insights into the signal network of tumorigenesis as well as on the limits of using cultured cells as a model.

On the other hand, PMA (administered at 0.25 mg/kg, i.p., at 16 hr before killing) exerted no appreciable effect on normal rat liver (resting in G_0 state) as shown in Table 1. This fact contrasts with the case of phenobarbital, which is also classified as a tumor promoter and increases the activity of TS of normal rat liver [16]. PMA seems to affect the potentiated cell probably in G_1 and/or S phase. Since α_1-agonist regulates DNA synthesis in the hepatocyte at about 12 hr after partial hepatectomy [1, 2], when the liver cell seems to enter G_1 and/or S phase [20] and is ready for the signal of the splanchnic nerve, the effect of PMA as a negative agent may be visualized explicitly in our system. Although the present results indicate the inhibition of liver regeneration by PMA, it is not unambiguously established whether protein kinase C exerts this effect through the desensitization of only α_1-receptors, because protein kinase C also desensitizes other calcium-mobilizing hormone receptors such as EGF [21, 22], vasopressin and angiotensin II [7], whose contributions in liver regeneration are not well characterized.

In summary, the administration of phorbol 12-myristate 13-acetate (PMA) prevented effectively rat liver regeneration which occurs after partial hepatectomy. Regenerative response was monitored by the activity of thymidylate synthetase and thymidine kinase, which are rate-determining enzymes in DNA synthesis, as well as liver DNA content. The present paper is the first *in vivo* study suggesting that protein kinase C activity plays a role as a negative regulator of cellular proliferation.

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