27. J. W. Coleman, A. Foster, J. H. K. Yeung and B. K. Park, *Biochem. Pharmac.* **37**, 737 (1988).
28. J. W. Coleman, J. H. K. Yeung, D. H. Roberts, A. M. Breckenridge and B. K. Park, *Br. J. Clin. Pharmac.* **22**, 161 (1986).
29. K. K. Wong, S. J. Lan and B. H. Migdalof, *Biochem. Pharmac.* **30**, 2643 (1981).
30. O. H. Drummer, B. S. Workman, P. J. Miach, B. Jarrott and B. J. Louis, *Eur. J. clin. Pharmac.* **32**, 267 (1987).

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Phorbol ester-induced attenuation of tissue DNA synthesis: Antagonism by prolactin in liver and thymus

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Recent evidence suggests that prolactin (PRL) may play a central role in mammalian growth regulation. Administration of PRL to rats stimulates entry of cells into cell cycle. Ornithine decarboxylase (ODC, EC 4.1.1.17) is induced rapidly in a dose-dependent manner in liver, thymus, kidney, heart, spleen, and adrenal gland of rats within 6 hr of intraperitoneal PRL administration [1–3]. Plasminogen activator (PA, EC 3.4.21.31) is similarly induced in these tissues by PRL treatment [4]. Since the inductions of ODC and PA occur early during the G_1 phase of cell cycle, these observations suggest that PRL may regulate cell cycle progression in these tissues. Moreover, it has been demonstrated recently that PRL administration stimulates cell replication in rat liver as assessed by [^{3}H]thymidine ([^{3}H]TdR) incorporation into hepatocyte nuclei [5, 6]. Further, chronic PRL treatment causes hepatomegaly and increases the hepatic mitotic index [7]. Its administration subsequent to a hepatocarcinogen increases the expression of biochemical as well as histochemical markers characteristic of hepatic preneoplasia [7], suggesting that increases in PRL concentration may promote liver carcinogenesis as a direct result of its hepatotrophic effects. In addition, the role of PRL as an immunomodulatory hormone may be related to its ability to serve as a co-mitogen for lymphocytes [8].

The phorbol ester tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which directly activates protein kinase C (PKC, EC 2.7.1.37), stimulates *de novo* synthesis of ODC and PA activities in the liver [9–11] in a manner strikingly similar to the response produced by PRL. In the T51B liver cell line, activation of protein kinase C is positively coupled to cell cycle progression [12]. Recent evidence from our laboratory links the mitogenic action of PRL to the activation of PKC as an early event in liver replication in response to PRL or partial hepatectomy [13]. However, Tsukamoto and Kojo [14] have shown that phorbol ester treatment 8 hr after partial hepatectomy inhibits liver regeneration. Here, we report that TPA decreased DNA synthesis in liver, thymus, kidney, and heart. Further, co-administration of PRL abrogated the effects of TPA in liver and thymus, but not in kidney and heart.

Materials and Methods

Male weanling Sprague–Dawley rats (26- to 28-days-old, 60–75 g) were housed in a controlled environment at 23° with a 12-hr light/dark cycle. Food (LM485, Tekland, Madison, WI) and water were available *ad lib*. Rats received ovine PRL (5.5 mg/kg, i.p., NIDDK, Bethesda, MD), TPA (0.6 mg/kg, i.p.), or the combined regimen at 12-hr intervals for 48 hr. At 46 hr, 1.0 μCi/g of [^{3}H]TdR (70–75 Ci/mmol) was administered. No overt toxicity was caused by any of the treatment regimens. Treated and control animals continued to gain weight at similar rates over the 48-hr experimental period. Tissue DNA synthesis, assessed as the amount of [^{3}H]TdR incorporated into trichloroacetic acid precipitated DNA, was determined, as previously described [5], at 48 hr. This method for estimating DNA synthesis has been well characterized *in vivo* and *in vitro* and has been shown to positively correlate with other methods for determining cell proliferation responses [15–17]. We have demonstrated previously that PRL-stimulated [^{3}H]TdR incorporation in liver is time- and dose-dependent and specific for hepatic parenchymal cells [5, 7].

Results and Discussion

Data presented in Fig. 1 demonstrate the effects of PRL and TPA administration on [^{3}H]TdR incorporation in various PRL-responsive tissues. Administration of PRL significantly increased hepatic DNA synthesis ($P < 0.01$, Fig. 1A). Treatment with TPA attenuated [^{3}H]TdR incorporation ($P < 0.01$) in liver compared to vehicle-treated controls, an effect consistent with the observations reported by Tsukamoto and Kojo [14]. However, the ability of TPA to decrease [^{3}H]TdR in liver was inhibited by PRL administration. In the thymus, a tissue in which PRL functions as a co-mitogen, the effect of TPA on decreasing [^{3}H]TdR incorporation was similarly antagonized by PRL treatment (Fig. 1B). In both the liver and thymus, PRL and TPA administration significantly ($P < 0.05$) increased [^{3}H]TdR incorporation compared to TPA treatment alone. In contrast, the TPA-produced decrease in [^{3}H]TdR incorporation in kidney and heart was not restored to control levels by co-administration of PRL (Fig. 1, panels C and D). In these tissues, the level of [^{3}H]TdR incorporation produced by the combined regimen did not differ from the effect of TPA administration alone.

Administration of PRL to rats increased hepatic DNA synthesis, similar to the previously reported effect of lactogen treatment in liver [5]. We reported recently that PRL administration is coupled to translocation of PKC from the cytosol to the hepatic membrane first detected by 15 min [13]. In addition, we found that partial hepatectomy increases serum PRL within 1 min with significant PKC translocation detectable at 30 min, the earliest time measured. TPA, which is thought to directly activate PKC by substituting for endogenous diacylglycerol, stimulates the expression of biochemical markers of G_1 cell cycle progression [10], but in the liver appears to inhibit entry into S phase. This interpretation is supported by the present study, and also by the reported ability of phorbol ester to inhibit DNA synthesis in regenerating rat liver [14]. In the latter, TPA had no effect on control DNA synthesis. This apparent discrepancy may be due to the treatment protocol employed by these investigators who administered phorbol esters as a single dose 16 hr prior to harvesting the liver for

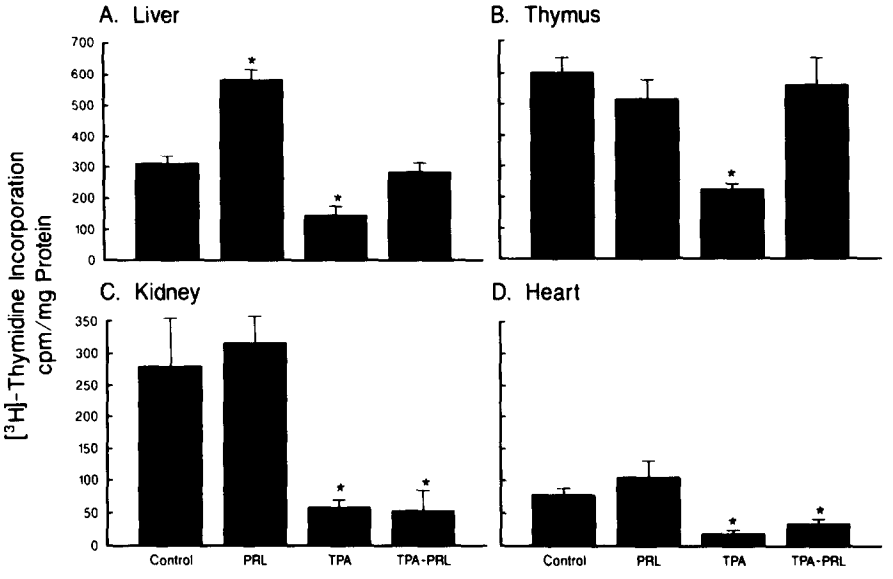

Fig. 1. Effects of PRL and TPA on [^{3}H]TdR incorporation in PRL-responsive tissues. Rats received either PRL (5.5 mg/kg, i.p.), TPA (0.6 mg/kg, i.p.), or the combined regimen at 12-hr intervals for 48 hr. At 46 hr 1.0 μCi/g body weight of [^{3}H]TdR was administered i.p. Tissue homogenates were assessed in triplicate for [^{3}H]TdR incorporation into DNA precipitated with trichloroacetic acid. Data are the means ± SEM from six to ten animals per treatment group. This experiment was repeated three times with identical results. Statistical differences were determined by the Newman–Keuls studentized range for multiple comparisons. Key: (*) $P < 0.01$ vs vehicle-control.

determination of DNA synthesis at 24 hr [14].

Numerous reports in the literature demonstrate the ability of TPA to initiate entry of liver cells into the G_1 phase of the cell cycle. However, the inhibition by TPA of entry into the S phase suggests that activation of PKC, while required, is not sufficient to drive mitogenesis in the liver. It is also possible that episodic TPA treatment for 48 hr decreases cellular PKC activity [18]. The demonstrated ability of PRL to antagonize the inhibitory effect of TPA in liver suggests that, in addition to its coupling to PKC, PRL provides other intracellular signals which are requisite for a hepatic mitogenic response. This suggestion is supported by the inability of tumor promoting phorbol esters to fully mimic PRL-stimulated mitogenesis in the Nb2 node lymphoma cells in culture [19]. These cells are dependent upon stimulation by a lactogenic hormone for mitogenesis [20]. Addition of 20 nM TPA stimulates proliferation in a manner similar to low concentrations of PRL. However, TPA is unable to stimulate proliferation to the same level as that detected with an optimal concentration of PRL [19, 21].

Similar to the ameliorating effects of PRL demonstrated in the liver, PRL was able to overcome the inhibitory effects of TPA on [^{3}H]TdR incorporation in thymus. This suggests that other signals generated as the result of PRL interaction with its receptor are able to overcome the inhibitory effects of TPA. On the other hand, in kidney and heart, administration of PRL to TPA-treated animals did not alter the reduced level of [^{3}H]TdR produced by phorbol ester treatment.

In summary, administration of TPA dramatically reduced the ongoing rate of DNA synthesis assessed by [^{3}H]TdR incorporation in liver, thymus, heart, and spleen. Co-administration of PRL restored radiolabel incorporation to control levels in the liver and thymus but not in the kidney or heart. We suggest that prolonged activation of PKC by phorbol esters, while capable of stimulating entry into cell cycle, may block cellular transit into S phase. Further, since PRL is able to counteract TPA inhibition of DNA synthesis in tissues in which PRL is thought to participate in mitogenesis, PRL treatment may set in motion other tissue specific biochemical signals coupled to proliferation.

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REFERENCES

1. Richards JF, Ornithine decarboxylase activity in tissues of prolactin-treated rats. *Biochem Biophys Res Commun* **63**: 292–299, 1975.
2. Russell DH, Larson DF, Cardon SB and Copeland JG, Cyclosporine inhibits prolactin induction of ornithine

decarboxylase in rat tissues. *Mol Cell Endocrinol* **35**: 159–166, 1984.
3. Russell DH and Larson DF, Prolactin-induced polyamine biosynthesis in spleen and thymus: Specific inhibition by cyclosporine. *Immunopharmacology* **9**: 165–174, 1985.
4. Buckley AR, Putnam CW and Russell DH, Rapid elevation of plasminogen activator activity in rat tissues by prolactin. *Biochem Biophys Res Commun* **122**: 1005–1011, 1984.
5. Buckley AR, Putnam CW, Montgomery DW and Russell DH, Prolactin administration stimulates rat hepatic DNA synthesis. *Biochem Biophys Res Commun* **138**: 1138–1145, 1986.
6. Murphy LJ, Bell GI and Friesen HG, Growth hormone stimulates sequential induction of "c"-myc and insulin-like growth factor I expression *in vivo*. *Endocrinology* **120**: 1806–1812, 1987.
7. Buckley AR, Putnam CW and Russell DH, Prolactin is a tumor promoter in rat liver. *Life Sci* **37**: 2569–2575, 1985.
8. Montgomery DW, Zukoski CF, Shah GN, Buckley AR, Pacholczyk T and Russell DH, Concanavalin A-stimulated murine splenocytes produce a factor with prolactin-like bioactivity and immunoreactivity. *Biochem Biophys Res Commun* **145**: 692–698, 1987.
9. Weiner RA and Byus CV, Induction of ornithine decarboxylase by 12-*O*-tetradecanoylphorbol-13-acetate in rat tissues. *Biochem Biophys Res Commun* **97**: 1575–1581, 1980.
10. Buckley AR, Putnam CW and Russell DH, *In vivo* induction of rat hepatic ornithine decarboxylase and plasminogen activator by 12-*O*-tetradecanoylphorbol-13-acetate. *Biochim Biophys Acta* **841**: 127–130, 1985.
11. Butler AP and McDonald FF, Transient induction of ornithine decarboxylase in RNA in rat hepatoma cells treated with 12-*O*-tetradecanoylphorbol-13-acetate. *Biochem Biophys Res Commun* **147**: 809–817, 1987.
12. Boynton AL, Kleine LP, Whitfield JF and Bossi D, Involvement of the Ca^{2+}/phospholipid-dependent protein kinase in the G_1 transit of T51B rat liver epithelial cells. *Exp Cell Res* **160**: 197–205, 1985.
13. Buckley AR, Putnam CW, Evans R, Laird HE, Shah GN, Montgomery DW and Russell DH, Hepatic protein kinase C: Translocation stimulated by prolactin and partial hepatectomy. *Life Sci* **41**: 2827–2834, 1987.
14. Tsukamoto I and Kojo S, Effect of phorbol ester on rat liver regeneration. *Biochem Pharmacol* **36**: 2871–2872, 1987.
15. Fisher B, Gunduz N, Saffer EA and Zheng S, Relation of estrogen and its receptor to rat liver growth and regeneration. *Cancer Res* **44**: 2410–2415, 1984.
16. Sand TE, Bronstad G, Digernas V, Killi A, Amara W, Refsnes M and Christoffsen T, Quantitative aspects of the effects of insulin, epidermal growth factor and dexamethasone on DNA synthesis in cultured adult rat hepatocytes. *Acta Endocrinol (Copenh)* **109**: 369–377, 1985.
17. Okamoto Y, Nishimura K, Nakagama M, Nakagawa M and Nakano H, Protein kinase C in the regenerating rat liver. *Biochem Biophys Res Commun* **151**: 1144–1149, 1988.
18. Besterman JM, Duronio V and Cuatrecasas P, Rapid formation of diacylglycerol from phosphatidylcholine: A pathway for generation of a second messenger. *Proc Natl Acad Sci USA* **83**: 6785–6789, 1986.
19. Buckley AR, Montgomery DW, Kibler R, Putnam CW, Zukoski CF, Gout PW, Beer CT and Russell DH, Prolactin stimulation of ornithine decarboxylase and mitogenesis in Nb2 node lymphoma cells: The role of protein kinase C and calcium mobilization. *Immunopharmacology* **12**: 37–51, 1986.
20. Gout PW, Beer CT and Nobel RL, Prolactin-stimulated growth of cell cultures established from malignant Nb rat lymphomas. *Cancer Res* **40**: 2433–2436, 1980.
21. Russell DH, Buckley AR, Montgomery DW, Larson NA, Gout PW, Beer CT, Putnam CW, Zukoski CF and Kibler R, Prolactin-dependent mitogenesis in Nb2 node lymphoma cells: Effects of immunosuppressive cyclopeptides. *J Immunol* **138**: 276–284, 1987.