Vol. 164, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1989 Pages 290-294

AN EARLY ELEVATION OF DIACYLGLYCEROL AND PHOSPHATIDATE IN REGENERATING LIVER

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Received August 14, 1989

SUMMARY: Liver diacylglycerol and phosphatidate are elevated following partial hepatectomy. These increases precede those in DNA synthesis and triacylglycerol accumulation. Possible factors involved in the increase in the lipids and the possible role of the lipids in liver regeneration are discussed.

Diacylglycerol and phosphatidic acid have been implicated in the hormonal control of many processes, including cell division. Diacylglycerol has been shown to be mitogenic for cultured fibroblasts (1); this effect is thought to be mediated by the activation of protein kinase C. The tumor promoter, phorbol myristate acetate, which mimics diacylglycerol as an activator of protein kinase C, causes increased DNA synthesis in hepatocytes (2). Phosphatidate has recently been shown to be mitogenic for cultured fibroblasts (3-5) and for normal mouse mammary epithelial cells (6). Although many hormones and growth factors elevate diacylglycerol and phosphatidate content *in vitro*, these lipids have not been measured *in vivo* during accelerated cell division.

When rats are subjected to partial hepatectomy, the remaining liver lobes hypertrophy rapidly, restoring the original mass of the liver within weeks (7). We have measured the diacylglycerol and phosphatidate content of the remnant lobes, and find that both lipids are elevated rapidly following partial hepatectomy.

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0006-291X/89 $1.50

MATERIALS AND METHODS

Diolein and phosphatidic acid were from Avanti (Pelham, AL). Halothane was from Ayerst Labs (Atlanta, GA). HPLC solvents were from Burdick and Jackson-Baxter. Ammonium dihydrogen phosphate, tributylamine, methylamine, hydrazine and 1-butanol were from Aldrich. Glycerol-3-phosphate dehydrogenase was from Boehringer and NAD, NADH and triolein were from Sigma. Diacylglycerol kinase was from Lipidex (Westfield, NJ).

Surgery. Partial hepatectomies were performed on 200-250 g male Sprague-Dawley rats as described by Higgins and Anderson (7) using halothane as the anesthetic. Surgery was performed between 7-10 am. Rats were sham hepatectomized by laparotomy and externalization of the liver without excision. At varying times after surgery, animals were anesthetized with halothane and the right lobe of the liver was quickly removed and freeze-clamped with liquid N_2. Frozen livers were stored at -70°C for 1-7 days until extraction.

Lipid extraction. Frozen livers were pulverized in a mortar with liquid N_2 and 500 mg of liver powder was extracted either by the Bligh and Dyer procedure (8)(for diacylglycerol and triacylglycerol) or by a modified Rose and Osklander (9) procedure as described in Ref. 10 (for phosphatidate). Lipid extracts were filtered, dried under N_2, resuspended in $CHCl_3$, and stored under N_2 at -20°C for 1-2 days until assay.

Lipid assays. Diacylglycerol was measured by the diacylglycerol kinase assay (11) as modified by Wright et al. (12). Phosphatidate was measured by a new procedure (10) involving enzymatic determination of the glycerol-3-phosphate produced by transacylation with methylamine. Triacylglycerol was measured by densitometry after separation by TLC (hexane/diethyl ether/acetic acid, 80:20:2; Whatman K6F plates). The lipids were visualized by dipping the plate in $CuSO_4$ (5% w/v)-phosphoric acid (4% w/v) and charring at 190°C for 15 min. Under these conditions, lipids with saturated fatty acids char as well as unsaturated lipids (13). Lipid phosphorus was measured by the method of Anderson and Davis (14).

RESULTS

As shown in Figure 1, diacylglycerol content rose rapidly in the right lobe of livers from partially hepatectomized animals. Diacylglycerol content was significantly greater ($p < 0.01$, t test) in partial hepatectomized than control

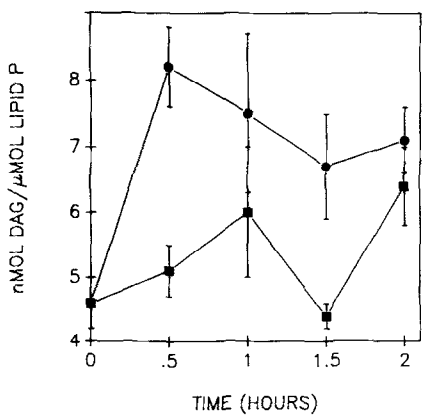

Figure 1. **The time course of 1,2-diacylglycerol accumulation in partially hepatectomized and sham-operated rats.**

Data points represent the mean ± SEM of duplicate measurements from 12 (0 h), 7 (0.5, 1, 2 h) or 8 (1.5 h) animals. Partially hepatectomized rats - open symbols; sham-operated rats - filled symbols. Rats sacrificed at "0 h" were not operated on prior to removal of the liver.

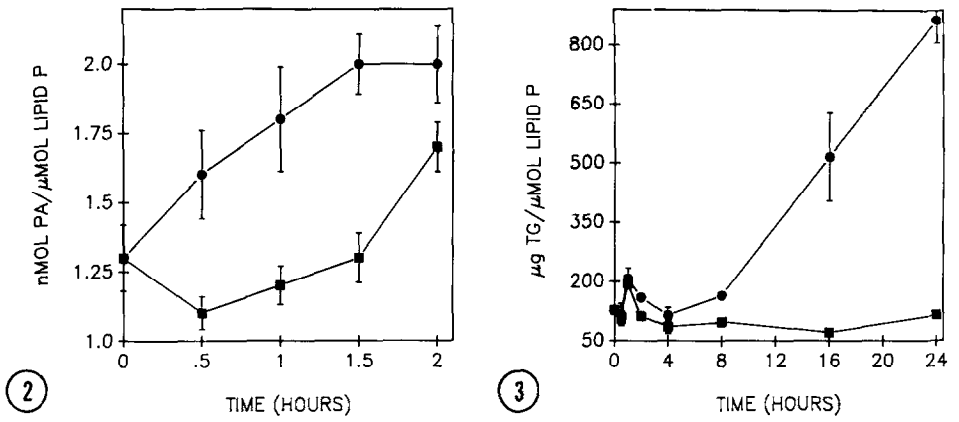

Figure 2. **The time course of phosphatidate accumulation in partially hepatectomized and sham-operated rats.**

Data points represent the mean ± SEM of duplicate measurements from 6 animals. Symbols and conditions as in Fig. 1.

Figure 3. **The time course of triacylglycerol accumulation in partially hepatectomized and sham-operated rats.**

Data points represent the mean ± SEM of duplicate measurements from 3 animals. Symbols as in Fig. 1.

(unoperated) rats at all time points. Diacylglycerol in livers from sham-operated rats was somewhat variable but differed from control ($p < 0.05$) only at 2 h after surgery. The diacylglycerol in partially hepatectomized rats was significantly greater ($p < 0.01$) than in sham-operated rats at 0.5 and at 1.5 h.

Phosphatidate also accumulated rapidly after partial hepatectomy (Fig. 2). Phosphatidate content was significantly greater ($p < 0.05$) in partially hepatectomized than control (unoperated) rats at all time points. Phosphatidate in livers from sham-operated rats was greater than control ($p < 0.05$) only at 2 h after surgery. Phosphatidate in livers from partially hepatectomized rats was significantly greater ($p < 0.05$) than in sham-operated rats at all time points. The content of both lipids was normalized to total lipid phosphorus, which is unchanged by partial hepatectomy (data not shown). Triacylglycerol accumulated much later (Fig. 3), producing a visibly fatty liver, in agreement with the findings of others (15). At 1 h, triacylglycerol levels were elevated significantly ($p < 0.05$) above control (unoperated) in both hepatectomized and sham-operated animals. Thus, this seems to represent a response to surgical stress, not to partial hepatectomy *per se*. Diacylglycerol and phosphatidate accumulation (Figs. 1 and 2) are evident in partially hepatectomized animals before this small peak of triacylglycerol accumulation. At 8, 16 and 24 h, triacylglycerol is significantly elevated from control (unoperated) values; triacylglycerol content in sham-operated animals remains at control levels or below at all times other than 1 h. Accumulation of diacylglycerol and phosphatidate also precedes the increase in DNA synthesis, which starts at about 12 h post-hepatectomy (16).

DISCUSSION

The signals initiating the intense proliferative response following partial hepatectomy are unknown, but norepinephrine (17), vasopressin (18), insulin, glucagon (19) and epidermal growth factor (20) appear to be necessary for regeneration. Hepatic denervation or blockade of α_1-adrenergic receptors inhibits liver regeneration (17). Plasma norepinephrine levels are increased after partial hepatectomy (17) and norepinephrine has been shown to elicit increased DNA synthesis in cultures of rat hepatocytes (21). In the vasopressin-deficient Brattleboro rat, regeneration is impaired; this defect is remedied by vasopressin treatment (18). Vasopressin, too, is mitogenic for hepatocytes (22). Diacylglycerol and phosphatidic acid accumulation is elicited by both norepinephrine and vasopressin in isolated hepatocytes (23). Both of these lipids have been shown to be mitogenic in some cell types (1-6). Our finding that diacylglycerol and phosphatidate accumulate in regenerating liver provides further support for a role for Ca^{2+}-mobilizing hormones in this response. Although the source of these lipids is unknown at present, phosphatidylcholine and/or phosphatidylinositol bisphosphate appear to be likely candidates (23). Rapid breakdown of these lipids has been shown in response to Ca^{2+}-mobilizing hormones in hepatocytes (23). Diacylglycerol and phosphatidate accumulate significantly (Figs. 1-3) before triacylglycerol, but may play a role in de novo triacylglycerol synthesis in early regenerating liver.

It is interesting to note that translocation of liver protein kinase C from cytosol to particulate fractions has been reported at 30-60 min following partial hepatectomy (24). The time course of this response is consistent with that of diacylglycerol accumulation (Fig. 1). The changes in diacylglycerol and phosphatidate are among the earliest observed after partial hepatectomy and may be related to the rapid induction of the protooncogene fos seen at 10-60 min following hepatectomy (25,26). This protooncogene has been shown to be induced by Ca^{2+}-mobilizing hormones and by phosphatidate (3). The proposal that diacylglycerol and/or phosphatidate play a role in liver regeneration awaits further experimentation.

ACKNOWLEDGMENTS

We would like to thank Mangalika Aluthge for her excellent technical assistance and Carolyn Strickland for preparing the manuscript.

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