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Chronic Ethanol Consumption Disrupts Complexation between EGF Receptor and Phospholipase C-$_{\gamma 1}$: Relevance to Impaired Hepatocyte Proliferation[1]

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We have previously shown that chronic ethanol consumption inhibits liver regeneration by impairing EGF receptor (EGFR)-operated phospholipase C-$_{\gamma 1}$ (PLC-$_{\gamma 1}$) activation and resultant intracellular Ca^{2+} signalling. Activation of PLC-$_{\gamma 1}$ by EGFR requires the EGFR to bind to PLC-$_{\gamma 1}$ after its translocation from cytosol to cytoskeleton. In order to understand the mechanism by which ethanol impairs PLC-$_{\gamma 1}$ activation, we examined the effect of alcohol on interactions between EGFR and PLC-$_{\gamma 1}$. In cultured hepatocytes from control rats, EGF rapidly induced tyrosine phosphorylation of both the EGFR and of PLC-$_{\gamma 1}$. EGF also stimulated PLC-$_{\gamma 1}$ translocation from cytosol to a cytoskeletal compartment where PLC-$_{\gamma 1}$ interacted with EGFR. In hepatocytes from rats fed ethanol for 16 weeks, the above reactions were substantially inhibited. Tyrphostin AG1478, an EGFR-specific tyrosine kinase inhibitor, mimicked the effects of chronic ethanol on EGFR phosphorylation, PLC-$_{\gamma 1}$ translocation and interactions between EGFR and PLC-$_{\gamma 1}$ in the cytoskeleton. Further, tyrphostin AG1478 also inhibited EGF-induced DNA synthesis. These results indicate that ethanol impairs EGFR-operated $[Ca^{2+}]_i$ signaling by disrupting the interactions between EGFR and PLC-$_{\gamma 1}$. **© 1999 Academic Press**

Binding of epidermal growth factor (EGF) to its cognate receptor on the cell surface leads to a variety of intracellular events that culminate in DNA synthesis and cell growth (1, 2). The first such event is receptor autophosphorylation on tyrosine residues, a reaction catalyzed by intrinsic receptor tyrosine kinase. This creates specific high-affinity binding sites for intracellular molecules involved in signal transduction and generation of second messengers (3, 4). One of the the best characterized signal transduction pathways that involves EFR receptor (EGFR) activation is intracellular calcium signaling {$[Ca^{2+}]_i$}. Thus, activation of EGFR generates inositol 1,4,5-trisphosphate {Ins(1,4,5)P_3} by activation of phospholipase C-$_{\gamma 1}$ (PLC-$_{\gamma 1}$), a process that involves translocation of PLC-$_{\gamma 1}$ from cytosol to cytoskeleton to interact with EGFR in hepatocytes (5). Ins(1,4,5)P_3 elevates $[Ca^{2+}]_i$ by releasing stored Ca^{2+} from endoplasmic reticulum (6). This process, combined with receptor-stimulated Ca^{2+} influx, leads to an increase in $[Ca^{2+}]_i$ that is involved with the production of many physiological responses in the cell, including growth and proliferation (7, 8).

Alcoholic liver disease is one of the most serious medical consequences of chronic alcohol use. A typical feature of the clinical outcome of this disorder is slow recovery after an episode of alcoholic hepatitis. This has been attributed, at least in part, to ethanol-induced impairment of liver regeneration. Thus many previous studies have shown experimentally that chronic ethanol consumption inhibits liver regeneration (9–13), but the precise molecular mechanism(s) by which ethanol impairs liver regeneration are poorly understood.

We and others have recently provided evidence that ethanol interferes with growth factor or cytokine-induced signal transduction pathways (14–24). The interruption of these signal transduction mechanisms has implications for the impairment of liver regeneration induced by long-term alcohol consumption. Thus we recently postulated that ethanol impairs liver regeneration by abrogating EGFR-operated $[Ca^{2+}]_i$ signaling (14). The targets for the toxic effect of ethanol on the $[Ca^{2+}]_i$ signaling pathway appeared to be at or proximal to the level of PLC-$_{\gamma 1}$ activation (14), but the exact components affected were not clear. The objectives of the present study, therefore, were (i) to examine the effect of chronic ethanol consumption on EGFR

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autophosphorylation and the subsequent association of the EGFR with PLC-$_{\gamma 1}$ and (ii) to characterize the relationship between changes in EGF-PLC-$_{\gamma 1}$ interactions and hepatocyte proliferation after chronic exposure of rats to alcohol.

MATERIALS AND METHODS

Chronic alcohol consumption model. Male Wistar rats, initially weighing 180–220 g, were fed commercial rat pellets, with ethanol in the drinking water for 16 weeks, as described previously (14). Control rats were either pair-fed the same diet with ethanol-fed rats where ethanol is isocalorically substituted by dextrose in food or were allowed ad libitum access to the commercial rat pellets. This study was approved by the Animal Ethics Committee of the Western Sydney Area Health Service and experiments, conformed to the highest international standards of humane care.

Isolation and culture of hepatocytes. After 16 weeks on the diets, hepatocytes were isolated from ether-anesthetized rats by non-recirculating collagenase perfusion through the portal vein, as described previously (25). Freshly isolated hepatocytes were plated onto collagen I-coated culture dishes and then cultured at 37°C in a humidified atmosphere of air containing 5% CO_2. Three hours after plating, the medium was changed to remove unattached hepatocytes.

Preparation of cytosol and cytoskeleton fractions. After 15–18 h culture hepatocytes were treated with EGF (200 ng/ml) for various times, as indicated in figure legends. Cells were chilled on ice and washed twice with ice-cold PBS to terminate the reaction. Cell cytosol and cytoskeleton fractions were prepared as described by Yang et al (5). Cell lysates were sonicated briefly, clarified by centrifugation and diluted to a protein concentration of 2 mg/ml.

Immunoprecipitation and immunoblotting. Both cytosol and cytoskeleton fractions were precleared by incubating for 1 h at 4°C with 50 μl of a 50% slurry of protein A-Sepharose (Sigma Chemical Co., St. Louis, MO), after which the beads were pelleted. Precleared samples were incubated with the appropriate primary antibodies for 45 min followed by incubation by rotation with 50 μl of 50% protein A-Sepharose for 1 h at 4°C. Immunoprecipitates were washed three times with ice-cold RIPA buffer and once with PBS. Immunoprecipitates or plasma membrane samples were denatured and resolved by 7.5% SDS-polyacrylamide gel electrophoresis (PAGE). Proteins were transferred to nitrocellulose membranes. Blots were blocked with 3% BSA in tris-buffered saline (TBST: 50 mM Tris pH 7.4, 200 mM NaCl and 0.1% Tween-20) when RC20 (Transduction Laboratories, Lexington, KY) was used to examine the phosphorylation status of EGFR or PLC-$_{\gamma 1}$, or with 5% milk power in TBST for the other antibodies. The blocked nitrocellulose membranes were probed with either HRPO-conjugated RC20 for phosphorylation examination or other primary antibodies followed by HRPO-linked anti-rabbit or anti-mouse secondary antibodies (Amersham Australia, Sydney). Blots were developed by the ECL method and semi-quantified by scanning laser densitometry. Antibodies against EGFR and PLC-$_{\gamma 1}$ were from Santa Cruz Biotecnology (Santa Cruz, CA).

DNA synthesis. DNA synthesis was determined by a [^{3}H]thymidine incorporation assay, essentially as described previously (25). Hepatocytes were pretreated with 1 μM tryphostin AG1478 for 3 h to 12 h followed by addition of EGF (10 ng/ml) to cell culture. [^{3}H]thymidine (2 μCi/ml, sp act 40 Ci/mmol) was added to the medium 48 h after addition of EGF, and hepatocytes were harvested 24 h later for DNA extraction. The specific activity of the extracted DNA was determined with an LKB RackBeta (Model 1215) liquid scintillation spectrometer with dpm package (LKB, Turka, Finland); an external standard and the channels ratio method was used to correct for quenching. Values were expressed as disintegrations per minute per microgram of DNA (dpm/μg DNA).

Expression of data and statistical analysis. Results are expressed as mean ± SD. Analysis of variance (ANOVA) and Fisher's least significant method were used for comparisons among more than two means, and the Student's t test was used for comparison between two means. A P value less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Activation of the EGF receptor stimulates the intrinsic tyrosine kinase activity of the receptor and triggers intracellular signals through multiple pathways that eventually lead to mitogenesis (1). Among the signals generated, the best understood mechanism is that for EGFR-induced activation of PLC-$_{\gamma 1}$, with its resultant production of Ins(1,4,5)P_3 and Ins(1,4,5)P_3-dependent increase of $[Ca^{2+}]_i$. In many cell types, activation of PLC-$_{\gamma 1}$ is initiated by its phosphorylation, a direct consequence of the association of PLC-$_{\gamma 1}$ with EGFR that is mediated by binding of the Src homology (SH2 domain) of PLC-$_{\gamma 1}$ to specific motifs located in the EGFR C-terminals. However, in highly differentiated cell types like hepatocytes, EGF-induced PLC-$_{\gamma 1}$ activation appears to occur in the cytoskeleton where an inhibitory guanine nucleotide-binding regulatory protein ($G_{\alpha i}$-protein) is also involved (5); we have recently indentified that the isoform involved is $G_{\alpha i2}$ (Zhang BH and Farrell GC, unpublished data).

As an initial step after EGF binding, EGFR phosphorylation is essential for EGFR activation. In order to examine the effect of ethanol on EGFR activation, hepatocytes were treated with EGF (200 ng/ml) for various times and EGFR was immunoprecipitated from cell subfractions. As expected, EGFR phosphorylation was induced by EGF in a time-dependent manner in cytoskeleton fractions (Fig. 1). In cytoskeleton fractions of hepatocytes from pair-fed rats, a detectable increase in EGFR phosphorylation was evident at 30 sec after EGF stimulation, rising to a peak at 1 min. Thereafter a decline in phosphorylation was evident, but levels remained for more than 10 min (1B). There was no significant difference in EGFR phosphorylation between pair-fed and ad libitum-fed rats (data not shown). In contrast, EGFR phosphorylation was significantly decreased at all time points after EGF stimulation in skeleton fractions from ethanol-fed rats compared with pair-fed control rats (1A). The reduction in EGF-induced EGFR phosphorylation in hepatocytes from ethanol-fed rats was not due to changes in EGFR immunoprecipitation because EGFR immunoblots of these precipitates demonstrated identical amounts of EGF protein in ethanol-fed and pair-fed hepatocytes (Figs. 1C and 1D). In contrast to the cytoskeleton fractions, there was no detectable EGFR or EGFR phosphorylation at any time after EGF stimulation in the cytosol fraction of hepatocytes from either ethanol-fed or pair-fed rats (data not shown).

In order to confirm a role of tyrosine kinase in EGFR phosphorylation, hepatocytes were preincubated for 30

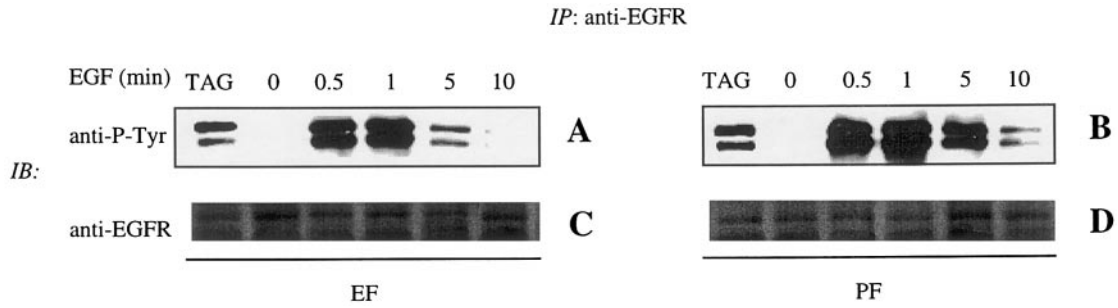

FIG. 1. EGF-induced EGFR phosphorylation in hepatocytes from ethanol-fed and pair-fed rats. Hepatocytes were treated with EGF (200 ng/ml) for the times indicated. Equal amounts of cell skeleton fractions from ethanol-fed (EF, panels A and C) and pair-fed (PF, panels B and D) rats were immunoprecipitated (IP) with anti-EGFR antibody. The immunoprecipitates were then separated by SDS–PAGE, following by immunoblotting (IB) with anti-phosphotyrosine (anti-P-Tyr) (panels A and B) and anti-EGFR antibodies (panels C and D), respectively. TAG: tyrphostin AG1478; hepatocytes in this group were pretreated with 1 μM tyrphostin AG1478 for 30 min followed by EGF stimulation for 1 min. Illustrations are immunoblots for an individual experiment, representative of at least 3 replicates.

min with tyrphostin AG1478, an EGFR-specific tyrosine kinase inhibitor. As shown in Fig. 1A and 1B, EGFR phosphorylation after 1 min stimulation by EGF was considerably reduced by 1 μM tyrphostin AG1478. Higher concentration of tyrphostin AG1478 (10 μM) abolished the EGF-induced EGFR phosphorylation (data not shown). Similar observations were made when a broad tyrosine kinase inhibitor genistein was tested (data not shown). These results indicate that EGFR phosphorylation can be attributed to activation of the intrisic EGFR tyrosine kinase.

EGFR activation induces translocation of PLC-$_{\gamma 1}$ from the cytosol to the cytoskeleton and this translocation is critical for the activation of PLC-$_{\gamma 1}$ (5). We therefore investigated the effect of long-term ethanol feeding on PLC-$_{\gamma 1}$ translocation by immunoprecipitating PLC-$_{\gamma 1}$ from both cytosol and cytoskeleton fractions following EGF stimulation. The resultant immunoblots are shown in Fig. 2. In unstimulated cells, we confirmed the previous observation that PLC-$_{\gamma 1}$ is predominantly located in the cytosol (Figs. 2A and 2B). In control (pair-fed) hepatocytes, addition of EGF produced a decrease in the amount PLC-$_{\gamma 1}$ in the cytosol fractions that was detected 30 sec after EGF addition (Fig. 2B). The level of cytosolic PLC-$_{\gamma 1}$ was lowest at 1 min followed by recovery to the basal level after 10 min. This decrease in cytosolic PLC-$_{\gamma 1}$ was accompanied by a corresponding increase in the amount of PLC-$_{\gamma 1}$ in cytoskeleton fractions in control hepatocytes, indicating that translocation of PLC-$_{\gamma 1}$ from cytosol to

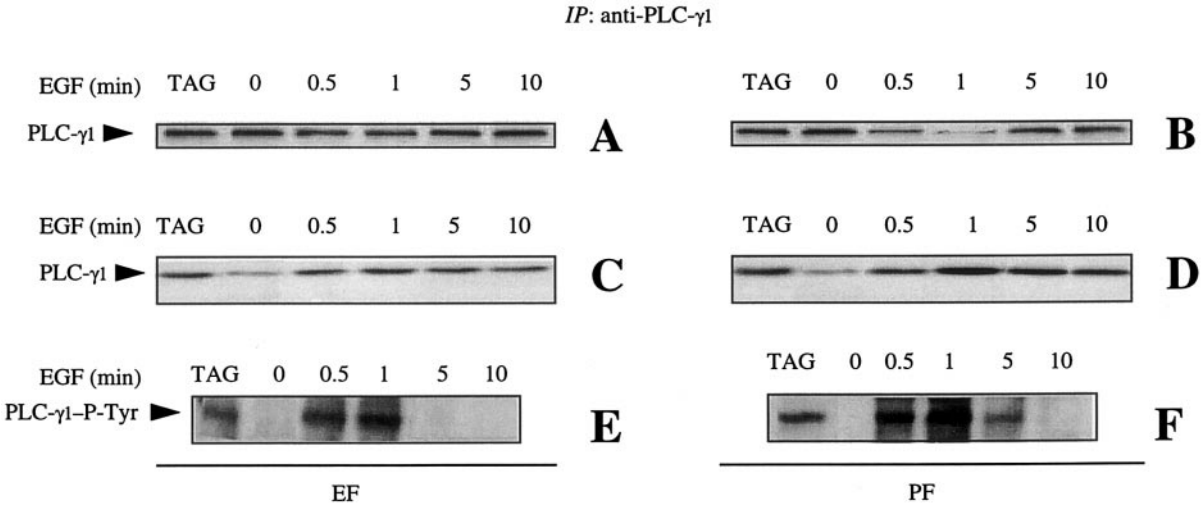

FIG. 2. EGF-induced translocation of PLC-$_{\gamma 1}$ from cytosol to cytoskeleton in hepatocytes from ethanol-fed and pair-fed rats. Hepatocytes from ethanol-fed (EF, panels A, C and E) and pair-fed (PF, panels B, D and F) rats were treated with EGF (200 ng/ml) for the indicated times. Cytosol and cytoskeleton fractions were prepared as described in "Methods". PLC-$_{\gamma 1}$ was immunoprecipitated (IP) from equal amounts of fractions and separated on SDS–PAGE. After transfer to nitrocellulose, the immunoblots were probed with anti-PLC-$_{\gamma 1}$ antibody for both cytosol (A and B) and cytoskeleton (C and D) samples or with antiphosphotyrosine antibody (PLC-$_{\gamma 1}$-P-Tyr) for skeleton samples (E and F). TAG: tyrphostin AG1478; hepatocytes were pretreated with 1 μM tyrphostin AG1478 for 30 min followed by EGF stimulation for 1 min.

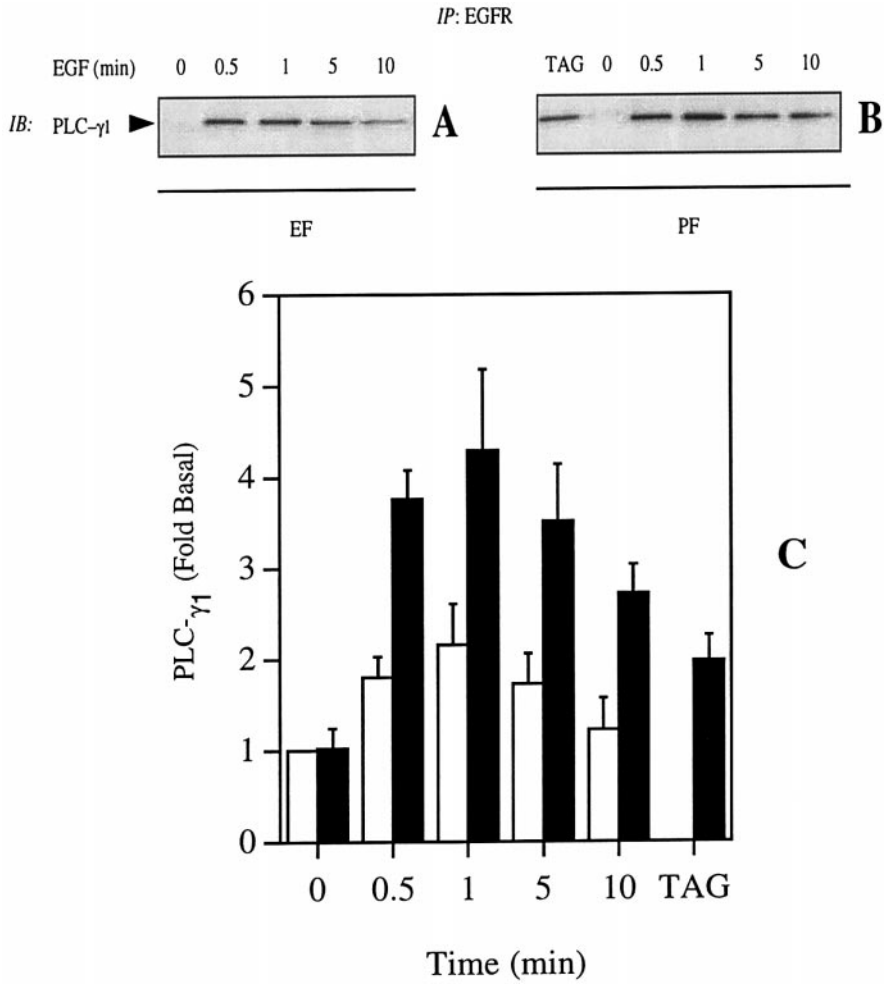

FIG. 3. EGF-induced interactions of EGFR with PLC-$_{\gamma 1}$ in cytoskeleton of hepatocytes from ethanol-fed and pair-fed rats. EGFR was immunoprecipitated from cytoskeleton fractions of hepatocytes from ethanol-fed (EF, panel A) and pair-fed (PF, panel B) rats. Proteins were separated by SDS–PAGE and transferred to nitrocellulose. After transfer, nitrocellulose membranes were blotted with anti-PLC-$_{\gamma 1}$ antibody. Autographs were quantified by scanning laser densitometry, and the results were presented as fold increase over non-stimulated cells from ethanol-fed rats (C) EF: □. PF: ■. The data shown represent the means ± SD from at least three separate experiments. TAG: tyrphostin AG1478; hepatocytes were pretreated with 1 μM tyrphostin AG1478 for 30 min followed by EGF stimulation for 1 min.

cytoskeleton had been induced by EGF (Fig. 2D). However, such translocation of PLC-$_{\gamma 1}$ was impaired in cells from ethanol-fed rats (Fig. 2C). Thus, the increase in cytoskeletal PLC-$_{\gamma 1}$ after 1 min of EGF stimulation was significantly reduced in hepatocytes from ethanol-fed rats compared with that of control cells (Fig. 2C c.f. Fig. 2D). It should be noted that the total amount of PLC-$_{\gamma 1}$ protein (cytosolic plus cytoskeletal fractions) was not altered by ethanol consumption.

It is evident from the results in Figs. 1 and 2 that the kinetics of translocation of PLC-$_{\gamma 1}$ from cytosol to cytoskeleton resemble those of the increase in EGFR phosphorylation. One explanation for this would be that EGFR phosphorylation is a factor that determines PLC-$_{\gamma 1}$ translocation. This hypothesis was therefore examined by studying whether chemical inhibition of EGFR phosphorylation produced the expected inhibition of PLC-$_{\gamma 1}$ translocation. To ascertain this, hepatocytes were pre-incubated with 1 μM tyrphostin AG1478 for 30 min. As shown in Fig. 2, such inhibition of tyrosine phosphorylation by tyrphostin AG1478 inhibited the translocation of PLC-$_{\gamma 1}$ stimulated by EGF.

Previous studies in hepatocytes have shown that only cytoskeletal PLC-$_{\gamma 1}$ is a substrate for EGFR tyrosine kinase (5). As shown in Fig. 2F, phosphorylation of PLC-$_{\gamma 1}$ was induced in cytoskeleton fractions of control hepatocytes with similar kinetics to those of EGFR phosphorylation. In contrast, EGF stimulated phosphorylation of PLC-$_{\gamma 1}$ was impaired in hepatocytes from ethanol-fed rats (Fig. 2E). Pretreatment of cells with 1 μM tyrphostin AG1478 also impaired EGF-induced PLC-$_{\gamma 1}$ phosphorylation (Fig. 2E, 2F). Taken together, the above results indicate that impairment of EGFR phosphorylation caused by chronic alcohol con-

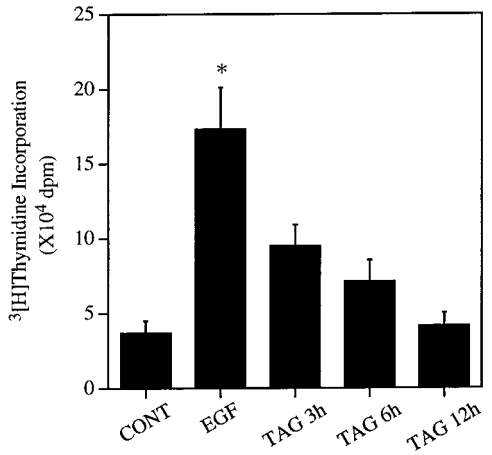

FIG. 4. Effects of tyrphostin AG1478 on EGF-induced hepatocyte proliferation. Hepatocytes were treated with 1 μM tyrphostin AG1478 (TAG) for 3, 6 or 12 h followed by stimulation with of EGF (10 ng/ml). 48 h after stimulation, [methyl-^{3}H]thymidine (2 μci/ml) was added to the cell culture. Incubation was continued for a further 24 h, after which cells were processed for determination of [^{3}H]thymidine incorporation into hepatic DNA. Data shown are the mean $\pm$ SD of three experiments, each performed in triplicate. *$P < 0.01$, as compared with other groups.

sumption could be responsible for the impairment of both PLC-$_{\gamma 1}$ translocation and phosphorylation.

PLC-$_{\gamma 1}$ translocation from cytosol to cytoskeleton is an initial step for EGFR-PLC-$_{\gamma 1}$ interaction. To examine the interactions between EGFR and PLC-$_{\gamma 1}$ in hepatocytes, EGFR was immunoprecipitated from the cytoskeleton fraction, separated by SDS–PAGE and blotted with anti-PLC-$_{\gamma 1}$ antibody. In unstimulated hepatocytes either from ethanol-fed or pair-fed rats, there were only negligible amounts of PLC-$_{\gamma 1}$ attached to EGFR (Fig. 3). Addition of EGF induced a rapid increase in the amount of PLC-$_{\gamma 1}$ detected in EGFR precipitates, and maximum levels were observed at 1 min (Figs. 3B and 3C). In cells from ethanol-fed rats, significantly less PLC-$_{\gamma 1}$ was bound to EGFR at each time (Figs. 3A and 3C). Pretreatment of hepatocytes with tyrphostin AG1478 also diminished the association of EGFR with PLC-$_{\gamma 1}$.

The above results indicate that chronic ethanol consumption in rats inhibits EGFR phosphorylation and subsequent formation of EGFR-PLC-$_{\gamma 1}$ complexes. To elucidate whether inhibition of EGFR phosphorylation and disruption of EGFR-PLC-$_{\gamma 1}$ complex formation could contribute to the impairment of liver regeneration induced by ethanol, we mimicked these effects with the EGFR tyrosine kinase inhibitor tyrphostin AG1478 and studied the effect of such inhibition on EGF-stimulated hepatic DNA synthesis. As expected, DNA synthesis of hepatocytes in primary culture was greatly stimulated by EGF. However, treatment of cells with 1 μM tyrphostin AG1478 induced a time-dependent inhibition of DNA synthesis (Fig. 4). Thus, pretreatment with 1 μM tyrphostin AG1478 for 3 h inhibited by 44.5% hepatocyte DNA synthesis induced by EGF. Longer incubation (eg. 12 h) with tyrphostin AG1478 essentially abolished hepatocyte proliferation.

The findings that ethanol impairs EGFR autophosphorylation and the subsequent interactions of EGFR with PLC-$_{\gamma 1}$ suggest that membrane-embedded receptors and receptor-induced protein-protein interactions are the main targets of long-term ethanol consumption. Several recent observations lend support to this proposal. Thus chronic alcohol consumption has been shown to impair interleukin-6 receptor-induced activation of signal transducer and activator transcription factor 3 (Stat 3) (18), as well as to abrogate insulin receptor-induced insulin receptor substrate-1 (IRS-1) phosphorylation and the subsequent interaction of IRS-1 and phosphatidylinositol-3 kinase (PI-3 kinase) (19, 20). Chronic ethanol consumption has also been demonstrated to impair TGF-α-stimulated receptor autophosphorylation (24). In addition, Saso et al have reported that chronic alcohol consumption impairs EGFR phosphorylation and the subsequent association of EGFR with growth factor receptor binding protein 2 (Grb2), suggesting that ethanol also impairs Ras-mitogen-activated protein kinase (MAPK) signal pathways (17). A recent study by Chen et al (26) provides clear evidence that chronic alcohol consumption blocks all three MAPK cascades. Taken together, these studies imply that alcohol may interfere with membrane structure/protein function interfaces, thereby leading to impairment of transmembrane signaling. How alcohol impairs EGFR signaling by interrupting membrane structure/function, and the implications for MAPK pathways that control hepatocyte function and proliferation are important directions for further study.

In summary, the present results provide a partial mechanistic explanation for the finding of our previous study that chronic ethanol consumption impairs hepatocyte proliferation by impairing EGFR-induced PLC-$_{\gamma 1}$ activation and resultant $[Ca^{2+}]_i$ signaling. Thus it is now clear that chronic alcohol consumption impairs EGF-induced EGFR autophosphorylation, and a key implication of that impairment is decreased translocation of PLC-$_{\gamma 1}$ from cytosol to cytoskeleton. An additional consequence of altered EGFR autophosphorylation appears to be diminished association of EGFR with PLC-$_{\gamma 1}$. The proposal that decreased formation of EGF-PLC-$_{\gamma 1}$ complexes is responsible for decreased PLC-$_{\gamma 1}$ activation and hepatocyte proliferation was given credence by studies in which a specific EGFR tyrosine kinase inhibitor produced the identical effects, decreased PLC-$_{\gamma 1}$ migration, decreased EGFR-PLC-$_{\gamma 1}$ complexation and inhibition of EGF-induced hepatocyte proliferation. Further studies are required to establish how alcohol impairs the tyrosine kinase activity of hepatocyte mitogenic receptors.

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REFERENCES

1. Carpenter, G., and Cohen, S. (1990) *J. Biol. Chem.* **265,** 7709–7712.
2. Fantl, W. J., Johnson, D. E., and Williams, L. T. (1993) *Annu. Rev. Biochem.* **62,** 453–481.
3. Ullrich, A., and Schlessinger, J. (1990) *Cell* **61,** 203–212.
4. Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R., and Soltoff, S. (1991) *Cell* **64,** 281–302.
5. Yang, L. J., Rhee, S. G., and Williamson, J. R. (1994) *J. Biol. Chem.* **269,** 7156–7162.
6. Berridge, M. J. (1993) *Nature* **361,** 315–325.
7. Moolenaar, W. H., Tertoolen, L. G. J., and de-Laat, S. W. (1984) *J. Biol. Chem.* **259,** 8066–8069.
8. Rozengurt, E. (1986) *Science* **234,** 161–166.
9. Diehl, A. M., Chacon, M., and Wagner, P. (1988) *Hepatology* **8,** 237–242.
10. Diehl, A. M., Thorgeirsson, S. S., and Steer, C. J. (1990) *Gastroenterology* **99,** 1105–1112.
11. Duguay, L., Coutu, D., Hetu, C., and Joly, J. G. (1982) *Gut* **23,** 8–13.
12. Orrego, H., Crossley, I. R., Saldivia, V., Medline, A., Varghese, G., and Israel, Y. (1981) *J. Lab. Clin. Med.* **97,** 221–231.
13. Wands, J. R., Carter, E. A., Bucher, N. L. R., and Isselbacher, K. J. (1979) *Gastroenterology* **77,** 528–531.
14. Zhang, B. H., Horsfield, B. P., and Farrell, G. C. (1996) *J. Clin. Invest.* **98,** 1237–1244.
15. Zhang, B. H., and Farrell, G. C. (1997) *Gastroenterology* **113,** 641–648.
16. Yang, S. Q., Lin, H. Z., Yin, M., Albrecht, J. H., and Diehl, A. M. (1998) *Am. J. Physiol.* **275,** G696–G704.
17. Saso, K., Moehren, G., Higashi, K., and Hoek, J. B. (1997) *Gastroenterology* **112,** 2073–2088.
18. Chen, J., Bao, H., Sawyer, S., Kunos, G., Gao, B. (1997) *Biochem. Biophys. Res. Commun.* **239,** 666–669.
19. Sasaki, Y., and Wands, J. R. (1994) *Biochem. Biophys. Res. Commun.* **199,** 403–409.
20. Mohr, L., Tanaka, S., and Wands, J. R. (1998) *Gastroenterology* **115,** 1558–1565.
21. Saso, K., Higashi, K., Nomura, T., Hoshino, M., Ito, M., Moehren, G., and Hoek, J. B. (1996) *Alcohol. Clin. Exp. Res.* **20,** 330A–334A.
22. Patel, B. C., D'Arville, C., Iwahashi, M., and Simon, F. R. (1991) *Am. J. Physiol.* **261,** G199–G205.
23. Zeldin, G., Yang, S. Q., Yin, M., Lin, H. Z., Rai, R., and Diehl, A. M. (1996) *Alcohol. Clin. Exp. Res.* **20,** 1639–1645.
24. Tuma, D. J., Todero, S. L., Barak-Bernhagen, M., Casey, C. A., Sorrell, M. F. (1998) *Alcohol* **15,** 233–238.
25. Zhang, B. H., and Farrell, G. C. (1995) *Gastroenterology* **108,** 477–486.
26. Chen, J., Ishac, E. J. N., Dent, P., Kunos, G., and Gao, B. (1998) *Biochem. J.* **344,** 669–676.