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Decreased Binding and Autophosphorylation of the Epidermal Growth Factor Receptor in Ethanol-Fed Rats

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ABSTRACT. We have shown previously that binding and processing of epidermal growth factor are impaired in livers of ethanol-fed rats. In the current study, we examined these ethanol-induced alterations in greater detail by studying both high and low affinity epidermal growth factor binding as well as the ability of added ligand to stimulate receptor autophosphorylation. We also measured the binding of anti-receptor antibody to intact and permeabilized cells in order to determine more accurately the levels of receptor protein. Hepatocytes were isolated from ethanol-fed and pair-fed control rats. Ligand binding, antibody binding, and ligand-induced receptor autophosphorylation were measured in the respective cell populations. In ethanol-fed animals, binding to both high and low affinity states of the hepatic epidermal growth factor receptor was decreased by 40–50% ($P < 0.01$). This ethanol-induced decrease in ligand binding was accompanied by a reduced ability of epidermal growth factor to stimulate receptor autophosphorylation (32% decrease, $P < 0.01$). In contrast, binding of anti-receptor antibody was not altered in ethanol-fed animals. In conclusion, chronic ethanol feeding decreased epidermal growth factor binding with a concomitant decrease in the ability of the receptor tyrosine kinase to phosphorylate tyrosine residues. These changes were not accompanied by an actual decrease in receptor protein content. These findings could be relevant to modified responses to this growth factor in the livers of chronic ethanol-fed animals. BIOCHEM PHARMACOL 53;10:1445–1450, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. endocytosis; epidermal growth factor; receptors; autophosphorylation; ethanol; liver

Ethanol administration disorders protein trafficking pathways in the liver. Alterations in plasma membrane assembly [1] and RME[‖] of asialoglycoproteins and EGF [2, 3] have been demonstrated in hepatocytes from chronically ethanol-fed rats. RME for both of these ligands was impaired at numerous steps along the endocytosis pathway. Others have shown that ethanol treatment can affect signal transduction pathways involving cyclic AMP [4] and receptor tyrosine kinases [5–7].

EGF is thought to be an important factor in regulating liver cell metabolism and regeneration. The EGF receptor is a membrane protein that consists of an extracellular binding domain, a short transmembrane region, and intracellular tyrosine kinase and regulatory domains. Binding of EGF to its receptor causes receptor dimerization and autophosphorylation of tyrosine residues resulting from activation of the receptor tyrosine kinase [8, 9]. Subsequent phosphorylation of phospholipase C_γ, rasGAP, and phosphatidylinositol-3-kinase by the activated EGF receptor tyrosine kinase results in a number of signaling events leading to the initiation of DNA synthesis, cell growth, and cell division [10]. Thus, possible alterations in binding of EGF to its receptor by ethanol treatment could result in significant consequences in cellular function.

Previously, we have shown that chronic ethanol feeding of rats causes a 50–60% decrease in EGF binding to surface receptors of hepatocytes compared with pair-fed controls. This impairment of surface binding was not associated with changes in EGF-receptor affinity, suggesting a decrease in the number of functional surface receptors [3]. EGF receptors have been shown to have high and low affinity states for the binding of EGF [11]. It is thought that the high affinity state of the receptor is mainly responsible for the biological actions of EGF [12]. In the present study, we examined the effects of chronic ethanol feeding of rats on EGF binding to both the high and low affinity states of the EGF receptor in hepatocytes. We also examined the effects of chronic ethanol administration on the ability of the EGF receptor to autophosphorylate receptor tyrosine residues.

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‖ *Abbreviations:* EGF, epidermal growth factor; KRB, Krebs-Ringer buffer; PMSF, phenylmethylsulfonyl fluoride; RME, receptor-mediated endocytosis; TAME, Nα-*p*-tosyl-L-arginine methyl ester; TLCK, Nα-*p*-tosyl-L-lysine chloromethyl ketone, and TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone.

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MATERIALS AND METHODS

Materials

Collagenase (type IV), Percoll, BSA (fraction V), HEPES, sodium orthovanadate, aprotinin, leupeptin, antipain, TLCK, TPCK, TAME, dithiothreitol, PMSF, pyrophosphate, pepstatin A, Triton X-100, EGTA, Protein G, Protein G Sepharose, and digitonin were purchased from the Sigma Chemical Co. (St. Louis, MO). Mouse EGF (receptor grade) and polyclonal anti-human EGF receptor antibody, which cross-reacts with rat EGF receptor, were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). ^{125}I-EGF was purchased from DuPont NEN Research Products (Boston, MA). Recombinant human EGF was obtained from Intergen (Purchase, NY) and used to determine nonspecific binding for the ^{125}I-EGF binding assays. Anti-phosphotyrosine antibody conjugated to horseradish peroxidase, RC20H, was from Transduction Laboratories (Lexington, KY). Hybond™ nitrocellulose membrane and ECL™ reagents were purchased from Amersham (Arlington Heights, IL). Eagle's medium (Gibco Laboratories, Gaithersburg, MD) was supplemented with 2.4 g/L of HEPES, pH 7.4, 0.22 g/L of sodium bicarbonate, and 0.1% BSA. All other chemicals were reagent grade.

Male Sprague-Dawley rats (100–125 g) were obtained from the Small Animal Supply Co. (Omaha, NE). Rat diets were formulated according to the method of Lieber and DeCarli [13] and purchased from Dyets Inc. (Bethlehem, PA). The ethanol diet contained 18% of total calories as protein, 35% as fat, 11% as carbohydrate, and 36% as ethanol. In the control diet, ethanol was replaced isocalorically with carbohydrates.

Ethanol Treatment of Rats

The ethanol feeding regimen has been described previously [2]. Briefly, animals were maintained initially on a Purina rat chow diet until they reached between 140 and 150 g. The rats were housed in individual cages and acclimated to the Lieber-DeCarli control diet for 3 days. Rats were then weight-matched and paired so that one rat received the liquid diet containing ethanol while the second animal was pair-fed the isocaloric control diet. The rats were pair-fed for 5–8 weeks. *Ad libitum* chow-fed rats were included as an additional control group to establish that any differences were a result of ethanol consumption and not due to other dietary factors. This project was approved by the Animal Studies Subcommittee of the Omaha Department of Veterans Affairs Medical Center. Animals were handled in accordance with applicable local and federal regulations concerning laboratory animals. The animals were housed in the Animal Research Facility at the Omaha Veterans Affairs Medical Center, which has been approved by the American Association for the Accreditation of Laboratory Animal Care.

Hepatocyte Preparation

Hepatocytes were prepared by the collagenase perfusion method of Seglen [14] with modifications previously described [2]. Briefly, livers were perfused with 0.02% collagenase (Type IV), and hepatocytes were shaken loose in KRB with 2.5 mM Ca^{2+} and 0.1% BSA. Hepatocytes were filtered through nylon mesh and purified by centrifugation with Percoll. Viability was greater than 80% as determined by trypan blue exclusion. Hepatocytes were preincubated in Eagle's medium, at cell densities of 5–10 million cells/mL, using a metabolic shaker at 37° for 30 min to increase and equilibrate the number of cell surface receptors. After preincubation, the cell suspensions were chilled to 0–4° on ice.

Ligand Binding Assays

Specific binding of ^{125}I-EGF to the surface of isolated hepatocytes was determined at 0–4° using the basic methods of Gladhaug and Christoffersen [11] with the following modifications. One million cells were suspended in 4 mL of Eagle's/BSA in a 25-mL Erlenmeyer flask. Appropriate dilutions of ^{125}I-EGF were added to each flask for final concentrations of 4.6 pM to 5 nM. Nonspecific binding was determined by adding at least 100-fold excess of unlabeled recombinant human EGF to another set of flasks with equal concentrations of hepatocytes and radiolabeled EGF. Hepatocytes were incubated overnight at 4° with gentle mixing. Under these conditions, no EGF degradation or internalization products were seen (data not shown). Cell suspensions were transferred to test tubes and flasks were rinsed with 1 mL of Eagle's/BSA; the rinse was added to the cells. The suspensions were centrifuged, and aliquots of the supernatant were removed and counted to determine free ^{125}I-EGF. The pellets were washed three times with 4 mL of ice-cold KRB/BSA and then counted. Specific binding was determined by subtracting nonspecific bound radioactivity from total bound radioactivity.

Antibody Binding Assays

EGF receptor antibody binding to the hepatocyte surface was determined as follows. Cells (5×10^6) were centrifuged and washed two times with 4 mL of Eagle's/BSA. One million cells were brought up to 0.2 mL vol. with anti-human EGF receptor antibody (final dilution was 1:50) and incubated for 1 hr at 4° with gentle agitation. Then hepatocytes were washed and resuspended in 0.3 mL of Eagle's/BSA with 75 nM ^{125}I-Protein G, incubated for 1 hr at 4°, centrifuged, and washed three times with 1 mL KRB, and pellets were counted. Nonspecific binding was determined by the same method as above substituting nonimmune serum for the anti-EGF receptor antibody.

We also determined total receptor content in hepatocytes by binding the anti-EGF receptor antibody in cells permeabilized by digitonin. An aliquot of the cell suspen-

sions (typically 5×10^6 cells) was incubated with 0.1% digitonin on ice for 30 min. The cells were washed two times with 4 mL of Eagle's/BSA and treated as described above.

Western Blot Analysis

Aliquots of the cell suspensions (4×10^6 cells based on cell count) were pelleted and stored at −70° until ready to use. Cells were lysed with water, and an aliquot was mixed 1:1 with non-reducing sample buffer [0.18 M Tris, pH 6.8, 36% (v/v) glycerol, 2% (w/v) SDS, 0.64 mg/mL iodoacetic acid, 0.004% (w/v) bromophenol blue] to a final concentration of 2 million cells/mL. Samples were heated in a 65° water bath for 5 min, and 30-μL aliquots were loaded and separated by SDS–PAGE on 6% gels. Proteins were transferred to nitrocellulose at 10 V for 30 min. Blots were blocked with 3.5% (w/v) BSA in Buffer B (0.15 M NaCl, 50 mM Tris, pH 7.6), incubated with a 1:100 dilution of anti-human EGF receptor antibody for 2 hr, and washed two times for 30 min each with 1% (v/v) Tween 20 in Buffer B. Bound antibody was detected by incubation of the blots with ^{125}I-Protein G for 1 hr. Unbound radioactivity was removed by washing first with 1% Tween 20/Buffer B followed by washing with Buffer B alone. Blots were exposed to a Phosphor screen and analyzed by PhosphorImager using the ImageQuant program (Molecular Dynamics, Sunnyvale, CA). Results are reported as percent of values from control-fed animals for an equal number of cells for ethanol-fed, pair-fed, and chow-fed animals.

Autophosphorylation of EGF Receptor

Hepatocytes were prepared as above. Immediately following preincubation, 3 million cells in 1 mL KRB were incubated with 5 nM EGF at room temperature for 2 min. Cells were centrifuged immediately, the supernatant was discarded, and 1.2 mL of lysing buffer (50 mM HEPES, 150 mM NaCl, 1% Triton, 10% glycerol, 1.5 mM $MgCl_2$, 100 mM EGTA, 0.1 mM PMSF, 35 mM sodium orthovanadate, 95 mM sodium fluoride, 30 mM pyrophosphate, 1 mM dithiothreitol, 10 μg/mL aprotinin, 1 μg/mL of each of the following; leupeptin, antipain, pepstatin A, TAME, TLCK, TPCK) was added and mixed at 4° for 1 hr. Lysates were centrifuged at 10,000 *g* for 15 min, and the EGF receptor was immunoprecipitated by the following method. Anti-human EGF receptor antibody (10 μg) was added to 1 mL of the supernatant and mixed overnight at 4°. All of the antibody mix was added to 50–100 μL of packed Sepharose Protein G beads and mixed for 2 hr at 4°. Sepharose beads were centrifuged and washed twice, each time with three different immunoprecipitation wash buffers (wash No. 1 was 0.05 M Tris, pH 7.4, 0.5 M NaCl, 1 mM EDTA, 0.5% Triton X-100, 1 ng/mL aprotinin, 0.25% BSA; wash No. 2 was 0.05 M Tris, pH 7.4, 0.15 M NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 1 ng/mL aprotinin, 0.25% BSA; wash No. 3 was 10 mM Tris, pH 7.4). SDS sample buffer (150 μL of 0.25 mM Tris containing 2% SDS, 2% β-mercaptoethanol, 0.002% bromophenol blue, 20% glycerol) was added to the Sepharose beads, and samples were placed in a 65° water bath for 5 min. Proteins were resolved on 6% SDS–PAGE gels, and transferred to Hybond membranes using a semi-dry transfer apparatus, 10 V for 30 min. Duplicate blots were blocked in either 1% or 3.5% BSA overnight. One blot was probed with anti-human EGF receptor antibody as described above. The second blot was probed with a 1:2500 dilution of anti-phosphotyrosine antibody conjugated to horseradish peroxidase (RC20H) for 20 min at 37° and then washed with several changes of wash buffer (0.1 M Tris, pH 7.5, 0.1 M NaCl, 0.1% Tween 20). ECL reagents were mixed 1:1, wash buffer was decanted, and the blot was covered by the ECL mixture for 1 min. The blot was patted dry and exposed to Fuji X-ray film (Japan) for 30 and 60 sec. Images were quantitated by a densitometer (ImageQuant, Molecular Dynamics).

RESULTS

Cellular signaling by EGF is initiated via binding to its surface receptor, which activates an intrinsic receptor tyrosine kinase and results in autophosphorylation of tyrosine residues on the receptor [8, 9]. We initially examined the effects of chronic ethanol administration on EGF receptor autophosphorylation by stimulating hepatocytes from ethanol-fed and pair-fed controls with EGF (5 nM) for 2 min at room temperature. Treatment of the cells with EGF resulted in a marked increase in phosphorylation of tyrosine residues on the EGF receptor, whereas untreated cells showed a minimal level of EGF receptor phosphorylation (Fig. 1A). This EGF-stimulated autophosphorylation of the receptor was attenuated markedly in hepatocytes isolated from ethanol-fed rats as compared with the pair-fed controls (Fig. 1A). Additional time points (30 sec to 5 min) were examined, and a similar pattern of reduced autophosphorylation was observed at all times (data not presented). Thus, the receptor phosphorylation levels we report here most likely represent a steady-state condition in these cells. When blots of the immunoprecipitates of the EGF-receptor were probed with the anti EGF-receptor antibody to determine total receptor content, bands of similar intensities were observed in samples from the ethanol-fed and control rats (Fig. 1B), indicating minimal changes in receptor content between the two groups of animals. After the immunoblots were quantified by densitometric analysis, autophosphorylation was normalized per receptor number. When values from 9 pairs of animals were averaged, autophosphorylation of the EGF receptor tyrosine residues per receptor number was decreased by 32.0 ± 7.6% in hepatocytes from the ethanol-fed animals ($P < 0.002$). Associated with the decrease in EGF-receptor autophosphorylation, a reduction in hepatocyte surface binding of EGF was observed under these experimental conditions. After a 2-min exposure to EGF (5 nM), hepatocytes from the controls bound 193 ± 19.8 fmol/million cells compared

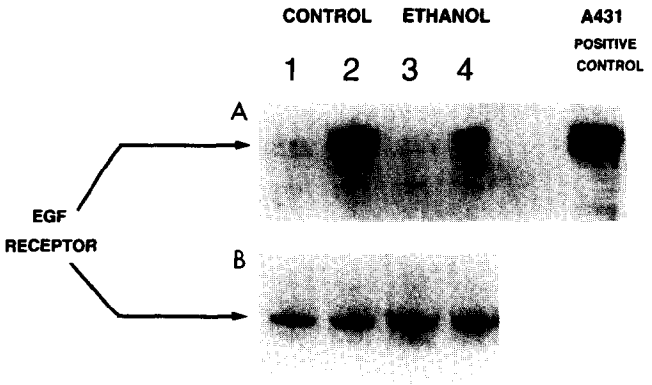

FIG. 1. Effects of ethanol on EGF receptor autophosphorylation. Hepatocytes (3 × 10^6 cells) from ethanol-fed (lanes 3 and 4) rats and pair-fed control (lanes 1 and 2) rats were incubated with (lanes 2 and 4) or without (lanes 1 and 3) EGF (5 nM) for 2 min at room temperature. Cells were lysed and EGF receptor was immunoprecipitated and subjected to 6% SDS–PAGE and then transferred to Hybond. (A) Autoradiograph of tyrosine phosphorylated EGF receptor. Blots were incubated with an anti-phosphotyrosine antibody conjugated to horseradish peroxidase (1:2500 dilution). Detection was by an ECL detection kit. Data were analyzed by densitometric analysis. (B) Autoradiograph of immunoreactive EGF receptor. Blots were incubated with anti-EGF receptor antibody (1:100 dilution), and then the antibody receptor complex was incubated with ^{125}I-Protein G. Data were analyzed by PhosphorImager analysis. Both autoradiographs are of one representative experiment.

with 110 ± 16.2 observed for the ethanol-fed animals ($P < 0.001$; N = 9). This 43% reduction in EGF binding correlated quite well with the 32% decrease in receptor autophosphorylation observed with the ethanol-fed rats.

Since impaired autophosphorylation was associated with decreased EGF binding, we investigated in more detail the effects of chronic ethanol feeding on EGF binding in the hepatocytes. Saturation binding of ^{125}I-EGF to hepatocytes from ethanol-fed and control-fed animals showed that ethanol treatment decreased binding of EGF to surface receptors (Fig. 2, inset). EGF binding in cells from control rats was not significantly different from binding in cells from chow-fed animals (data not shown). Scatchard analysis of ^{125}I-EGF binding at concentrations from 4.6 pM to 5 nM was consistent with the presence of high and low affinity sites for hepatocytes from both ethanol and pair-fed control animals (Fig. 2). This is in agreement with the study by Gladhaug and Christoffersen [11] examining the kinetics of EGF binding in rat hepatocytes. The K_d values from cells from the ethanol-fed rats were unchanged for the high affinity sites compared with control cells, while the K_d was decreased for the low affinity sites (Table 1). Scatchard analysis of saturation curves of ^{125}I-EGF binding to the EGF receptor from 10 separate experiments showed a significant decrease in B_{max} values by 50% for both high and low affinity sites in cells from ethanol-fed animals when compared with pair-fed controls (Table 1). These results at first inspection suggest a decrease in receptor number. However, the binding of an anti-EGF receptor antibody to surface EGF receptors as well as to the total EGF receptor pool (surface plus intracellular) was unchanged in hepatocytes from ethanol-fed rats compared with pair-fed controls (Table 2). Furthermore, estimation of receptor content by western blot analysis (Fig. 3) and subsequent quantification of the blots by densitometry indicated a relative EGF receptor content in the ethanol-fed animals to be 100 ± 1.7% of the controls (N = 8). Thus, the decrease in B_{max} of EGF binding was not associated with any apparent decrease in EGF receptor number in the hepatocytes from the ethanol-fed animals.

DISCUSSION

The results of this study demonstrated that chronic ethanol administration interferes with EGF signaling in the liver. A significant impairment of EGF-stimulated autophosphorylation of the EGF receptor was observed in hepatocytes isolated from ethanol-fed rats. This reduction in the autophosphorylation of the EGF-receptor was also accompanied by a decrease of EGF binding to its surface receptor. Levels of immunodetectable receptor protein in the cells were not altered.

In a previous study, we reported that chronic ethanol feeding decreased the ability of EGF to bind to the surface membrane of hepatocytes [3]. Scatchard analysis of the binding data revealed a decrease in the B_{max} without any changes in the affinity of the EGF receptor, suggesting a possible reduction in the number of surface receptors. The results of this study confirm the significant decrease in B_{max} for EGF binding and show that both the high and low affinity sites of the receptor were affected similarly by ethanol treatment. However, immunochemical analyses of the EGF-receptor indicated that hepatocytes from ethanol-fed and control animals contained equal numbers of surface

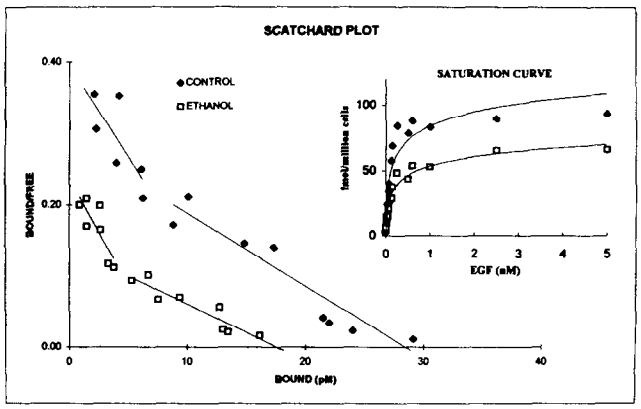

FIG. 2. Scatchard analysis of ^{125}I-EGF binding to hepatocytes from ethanol-fed and pair-fed control rats. Hepatocytes (1 × 10^6 cells) were incubated overnight at 4° with concentrations of ^{125}I-EGF from 4.6 pM to 5 nM, and binding was determined as described under Materials and Methods. Inset. Saturation curve of ^{125}I-EGF binding data. Values given are specific binding calculated as total ^{125}I-EGF binding minus binding in the presence of excess unlabeled EGF. Data are from one representative experiment.

TABLE 1. Comparison of B_{max} and K_d values from ligand binding of ^{125}I-EGF (4.6 pM to 5 nM) calculated from Scatchard plots

	B_{max} (fmol/million cells)		K_d (pM)	
	High affinity	Low affinity	High affinity	Low affinity
Control	52.0 ± 4.3	129 ± 8.9	34.0 ± 3.1	290 ± 37.0
Ethanol	26.0 ± 3.0*	66 ± 1.4*	37.5 ± 7.6	158 ± 24.0*

Data are means ± SEM for 10 determinations from ethanol-fed and pair-fed control rats. See Fig. 2 for representative Scatchard plot and saturation curve.
*Significantly different from controls, $P < 0.01$.

and intracellular EGF receptors. Therefore, a reduction of the B_{max} for EGF binding without the concomitant changes in receptor immunoreactive material (i.e. receptor protein content) suggests that the EGF receptor may be inactivated in hepatocytes from ethanol-fed rats. In other words, it appears that a pool of EGF receptors that are unable to bind EGF exists in hepatocytes from ethanol-fed rats. A similar apparent inactivation of the hepatic asialoglycoprotein receptor by ethanol feeding has also been reported recently by our laboratory [15]. Weigel and coworkers [16–18] have suggested that recycling receptors may undergo an inactivation and reactivation process during the course of endocytosis, and they hypothesize that this inactivation–reactivation cycle may regulate ligand binding properties of the receptor. Studies from our laboratory with both the EGF receptor and the asialoglycoprotein receptor show that ethanol treatment may interfere with this cycle in a way that results in an accumulation of inactivated receptors. An accumulation of inactive receptors in the cell could alter the ligand–receptor segregation steps that operate at such high efficiency in the hepatocytes, and would thus contribute to altered protein trafficking in these cells. Further studies are underway to investigate this intriguing possibility.

The ethanol-induced impairment of EGF binding was accompanied by a corresponding decrease of EGF-stimulated autophosphorylation of the EGF receptor. Since EGF binding initiates the autophosphorylation of the receptor and the magnitude of impairment of these two processes was similar, it is likely that reduced EGF binding is a major contributing factor responsible for decreased autophosphorylation observed in the hepatocytes from ethanol-fed rats. However, we cannot at this time completely rule out an effect of ethanol treatment on the autophosphorylation process directly independent of binding. For example, ethanol feeding could alter kinase activity directly or, alternatively, could affect phosphatase activity in the hepatocyte, both of which could influence the phosphorylation state of the receptor.

Autophosphorylation of the EGF receptor results in activation of receptor tyrosine kinase activity, which in turn results in phosphorylation and subsequent activation of key intracellular enzymes, including phospholipase C_γ, rasGAP, and phosphatidylinositol kinase, along the signaling cascade elicited by EGF binding [10]. Thus, interference with this key initial event in EGF signaling by ethanol treatment would likely result in altered biological responses to the growth factor in hepatocytes of ethanol-fed rats. Consistent with this conclusion are the findings of Carter and Wands [19] who have shown that ethanol metabolism inhibits hormone-stimulated (including EGF) DNA synthesis in cultured hepatocytes and of Gerhart *et al.* [20] who reported that ethanol treatment alters some of the early effects of EGF on liver glycolysis. Furthermore, the impairment of EGF signaling by ethanol observed in this study supports the notion that altered signal transduction elicited

TABLE 2. Binding of the anti-EGF receptor antibody to hepatocytes from ethanol-fed and pair-fed control rats

	Antibody binding (fmol Protein G bound/million cells)	
	Surface	Total
Control	239 ± 18	786 ± 68
Ethanol	318 ± 51	755 ± 64

An aliquot of cells (5×10^6) was pretreated with 0.1% digitonin. Cells were incubated with a 1:50 dilution of antibody, and bound IgG was detected with ^{125}I-Protein G as described under Materials and Methods. Surface receptor number refers to femtomoles Protein G bound to intact cells while the total receptor number indicates the amount bound to cells permeabilized by digitonin treatment. Data are means ± SEM for 17 determinations. No significant difference between control and ethanol-fed animals was observed.

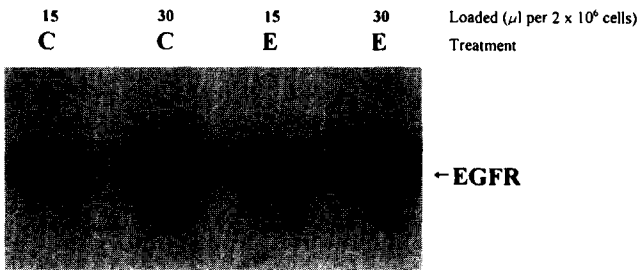

FIG. 3. Western blot analysis of EGF receptor in hepatocytes from ethanol-fed and control rats. Aliquots of total cellular protein (15 or 30 μL of 2×10^6 cells/mL final concentration) from ethanol-fed (E) and pair-fed control (C) rats were resolved by 6% SDS–PAGE. The proteins were transferred to nitrocellulose paper, probed with polyclonal anti-EGF receptor antibody, and detected with [^{125}I]Protein G as described under Materials and Methods. The autoradiograph is from one representative experiment. Densitometric quantitation of the data from 8 pairs of ethanol and pair-fed control animals showed no significant difference in the amount of EGF receptor present in hepatocytes from either treatment.

by ethanol may play a significant role in ethanol-induced liver injury [4, 21, 22].

In summary, chronic ethanol administration impaired the binding of EGF to both the high and low affinity states of the EGF receptor without causing a change in receptor content. Decreased EGF binding was associated with a reduction in receptor autophosphorylation, implying that ethanol treatment impairs the ability of EGF to activate receptor tyrosine kinase. This apparent inactivation of the EGF receptor and its inability to stimulate receptor kinase activity could lead to impaired functions of the signaling cascade associated with EGF stimulation, thus leading to impairments in hepatocellular reparative and regenerative processes.

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