Epidermal Growth Factor Receptor Number Decreases during Rat Liver Regeneration

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ABSTRACT The potential role of epidermal growth factor (EGF) in the regulation of rat liver regeneration was examined by assessing the binding of ^{125}I-EGF to hepatic membranes isolated at various times after partial hepatectomy. The results demonstrated a fall in ^{125}I-EGF binding detectable as early as 8 h after partial hepatectomy. The nadir in EGF binding, <40% of that observed in sham-operated control rats, was seen 36 and 48 h after partial hepatectomy. Scatchard analysis showed that the decrease in binding capacity was due to a fall in receptor number. The specificity of the observed loss of EGF receptors was substantiated in parallel studies of ^{125}I-insulin and ^{125}I-wheat germ lectin binding; the binding of these ligands did not decrease appreciably during liver regeneration. The data are consistent with the hypothesis that EGF or a similar substance is one component of the complex humoral signal that regulates liver regeneration.

INTRODUCTION

Advances in cell and organ culture techniques have led to the identification of numerous serum and cell-derived growth factors (1). The effect of these factors on proliferation and differentiation has been amply demonstrated in vitro, but their role in physiologic control of growth in vivo is largely undefined. Specifically, epidermal growth factor (EGF),[1] a well-studied mitogen (2), stimulates rat hepatic DNA synthesis both in vitro (3) and in vivo (4). However, there is no evidence demonstrating that EGF is involved in the regulation of the hepatocyte proliferation that rapidly and reproducibly follows partial hepatectomy. The present study examined the potential relationship between EGF and liver regeneration indirectly by determining the binding of ^{125}I-EGF to liver membranes at various times after partial hepatectomy and sham-operation. This approach was predicated upon the well-studied "down regulation" of EGF receptor number that attends cellular stimulation by EGF (2).

METHODS

Male Sprague-Dawley rats from Charles River Breeding Laboratories, Wilmington, Mass. (150–175 g) were fed and watered *ad lib.* pre- and postoperatively. Under ether anesthesia the median and left hepatic lobes were extruded and either excised (66–70% hepatectomy) or returned to the peritoneal cavity (sham-operation). Operations were performed at various times so that all rats in an experiment were killed between 8 and 9 a. m. Livers were homogenized in 0.25 M sucrose-10 mM Tris-HCl (pH 8.0) with a Brinkmann polytron apparatus (setting of 5, 20 s) Brinkmann Instruments, Westbury, N. Y.). Homogenates were centrifuged at 12,000 *g*,

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[1] *Abbreviation used in this paper*: EGF, epidermal growth factor.

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and the microsomal fraction was subsequently pelleted by centrifuging the supernate at 105,000 *g* (1 h, 50 Ti Rotor). Purified plasma membrane fractions were prepared by the method of Touster et al. (5) and the purity assessed by assay of 5′-nucleotidase activity (6).

Binding studies. Mouse EGF was purified to homogeneity (7) and iodinated with carrier-free $Na^{125}I$ (Union Carbide Corp., South Plainfield, N. J.) to a specific activity of 1.2 Ci/μmol (8). $Na^{125}I$ was removed by chromatography on Sephadex G-25 in 0.1 M sodium phosphate – 0.1% bovine serum albumin, pH 7.5. Radio-labeled ligands were incubated with 9–25 μg membrane protein (9) for 40 min at 24°C in 0.2 ml Dulbecco's phosphate-buffered saline containing 0.1% bovine serum albumin. Duplicate samples were collected on Millipore EGWP filters (Millipore Corp., Bedford, Mass.) (10). Each determination was corrected for nonspecific binding by subtraction of ^{125}I-ligand bound in the presence of 1 μM native ligand added 10 min prior to the labeled material. Porcine insulin (Eli Lilly & Co., Indianapolis, Ind.) and wheat germ agglutinin (Calbiochem-Behring Corp., San Diego, Calif.) were iodinated in a similar fashion (600 and 450 Ci/mmol, respectively). The assay concentration of all three labeled ligands was 2–5 nM.

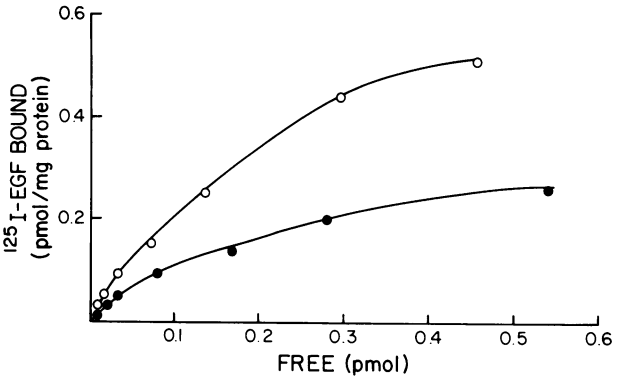

FIGURE 2 Specific ^{125}I-EGF binding at various ligand concentrations. Microsomal membranes (24 μg protein) prepared from sham-operated (○) and regenerating (●) rat livers 24 h after surgery were used. Scatchard analysis by linear regression in this and two similar experiments showed that the decrease in binding capacity was due to a fall in receptor number.

RESULTS

Fig. 1 depicts the results of experiments in which the membrane binding of ^{125}I-EGF was assessed after partial hepatectomy or sham-operation. The comparison indicates that a fall in EGF binding capacity is detected by 8 h and progresses during the initial 36 h. Since the onset of DNA synthesis occurs ~16 h after partial hepatectomy (11), the decrease in EGF binding precedes the onset of DNA synthesis. Examination of EGF binding in sham-operated rats at 8, 16, 24, and 36 h demonstrates that the binding averaged from 95–110% that observed in unoperated control rats; therefore, the operative stress itself does not alter EGF binding.

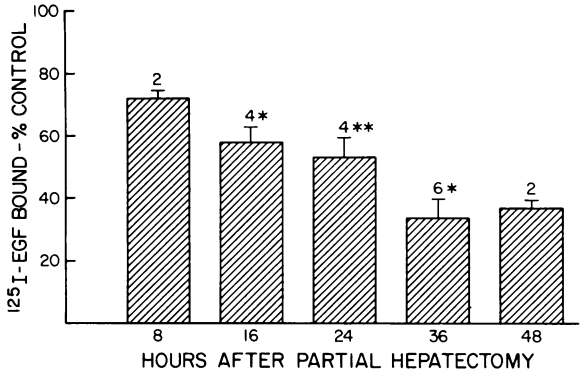

FIGURE 1 EGF binding to hepatic microsomal membranes during liver regeneration. The specific ^{125}I-EGF binding at the indicated times following partial hepatectomy is expressed as the percent binding of the matched sham-operated and control rats. The height of the bar represents the mean of *n* separate observations. The brackets represents the range when $n = 2$ and the SEM when the $n = 4$–6. The level of significance was determined in the latter by analysis of variance. $^*P < 0.01$. $^{**}P < 0.02$.

Fig. 2 shows the binding of ^{125}I-EGF at various concentrations in membranes prepared simultaneously from rats 24 h after sham-operation and partial hepatectomy. Scatchard analysis by linear regression indicates that the difference in binding capacity is produced by a 50% reduction in receptor number; the calculated binding constants, 1.7 and 1.4 nM (sham and regenerating), are equivalent.

To determine whether a general decrease in hormone receptor or membrane glycoprotein synthesis occurred during the growth process, the binding of two other ^{125}I-labeled ligands was assessed 36 h after partial hepatectomy and sham-operation. The binding of ^{125}I-insulin was increased slightly (Table I). Insulin concentrations fall during regeneration (12) and the slightly increased insulin binding may reflect the de-

TABLE I
Ligand Binding during Regeneration

^{125}I-Ligand	Binding	*n*	*P*
	%		
EGF	37±11	4	<0.02
Insulin	134±38	4	NS
Wheat germ agglutinin	99±13	4	NS

The binding of ^{125}I-labeled ligands to membranes isolated 36 h after partial hepatectomy and sham-operation. The cumulative percent change (mean±SEM) in ligand binding (regenerating/sham-operation) in the four separate experiments in which the binding of all three ligands was studied is shown.

crease in portal vein insulin concentration. The binding of ^{125}I-wheat germ agglutinin was determined to assess membrane glycoprotein content. (The EGF receptor appears to be a glycoprotein [2]). Lectin binding was equivalent in sham and regenerating liver membranes (Table I).

The binding of ^{125}I-EGF in microsomal and purified plasma membrane fractions was compared to determine whether the decrease was confined to a loss of plasma membrane receptors. The EGF binding capacity (per microgram protein) of plasma membranes from both sham and regenerating livers was increased proportionally (8–10-fold) when compared with microsomal preparations from the same livers. Thus, the "down regulation" of ^{125}I-EGF binding during regeneration was observed when purified plasma membrane fractions were examined; again there was no decrease in insulin or lectin binding (data not shown). A 20-fold increase in the specific activity of 5′-nucleotidase above that found in homogenates confirmed the purity of the plasma membrane preparations.

DISCUSSION

Experiments utilizing cultured cells have demonstrated that one consequence of EGF binding is the loss of cell-surface EGF receptors (2) and internalization of the EGF-receptor complex (13). In homogeneous cultured cell populations, the fall in EGF receptor number may approach 90%, but the mitogenic action has been demonstrated at lower receptor occupancy (14). During regeneration the maximum fall was 65%. This may reflect the heterogeneous cell population or the fact that 100% receptor occupancy is unnecessary for the proliferative response.

It is unclear whether receptor loss and/or internalization are obligatory steps in the mitogenic response; however, in the absence of pharmacologic perturbation, these events follow EGF binding and, conversely, may reflect EGF action. A simple explanation of the present data is that partial hepatectomy increases plasma EGF concentration, which leads to "down regulation." The putative increase in plasma EGF might be effected by a regulated release of EGF or, alternatively, since two-thirds of the liver has been removed, a decrease in the degradation of EGF may result in elevation of the concentration perfusing the liver remnant. Several alternative explanations must be considered. These include (*a*) the stimulation of hepatocytes by other humoral factors that may bind to the EGF receptor and trigger its internalization (15), (*b*) a modulation in apparent receptor number produced by hormones (16) or factors (17) that do not bind directly to the EGF receptor, (*c*) an intracellular process that results in the loss of EGF receptors. The latter possibility might occur as a consequence of growth and has been suggested as a mechanism underlying certain types of viral transformation (18).

Liver regeneration clearly provides an in vivo model of proliferation in which to study the "down regulation" of EGF receptor number. Resolution of the mechanism of receptor loss awaits further research including accurate measurement of EGF plasma concentration. However, since exogenous EGF leads to DNA synthesis (3, 4) and lipid accumulation (19), two phenomena that occur during regeneration, it is tentatively concluded that EGF binding to specific receptors plays a physiologic role in the complex response to partial hepatectomy.

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REFERENCES

1. Golde, D. W., H. R. Herschman, A. J. Lusis, and J. E. Groopman. 1980. Growth factors. *Ann. Intern. Med.* **92:** 650–662.
2. Carpenter, G., and S. Cohen. 1979. Epidermal growth factor. *Annu. Rev. Biochem.* **48:** 193–216.
3. Richman, R., T. H. Claus, S. J. Pilkis, and D. L. Friedman. 1976. Hormonal stimulation of DNA synthesis in primary cultures of adult rat hepatocytes. *Proc. Natl. Acad. Sci. U. S. A.* **73:** 3589–3593.
4. Bucher, N. L. R., U. Patel, and S. Cohen. 1978. Hormonal factors and liver growth. *Adv. Enzyme Regul.* **16:** 205–213.
5. Touster, O., N. N. Aronson, Jr., J. T. Dulaney, and H. Hendrickson. 1970. Isolation of rat liver plasma membranes. *J. Cell Biol.* **47:** 604–618.
6. Koide, Y., H. S. Earp, S. H. Ong, and A. L. Steiner. 1978. Alterations in the intracellular distribution of cGMP and guanylate cyclase activity during rat liver regeneration. *J. Biol. Chem.* **253:** 4439–4445.
7. Savage, C. R., Jr., and S. Cohen. 1973. Proliferation of corneal epithelium induced by epidermal growth factor. *Exp. Eye Res.* **15:** 361–366.
8. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labeled human growth hormone of high specific activity. *Nature (Lond.).* **194:** 495–496.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
10. O'Keefe, E., M. D. Hollenberg, and P. Cuatrecasas. 1974. Epidermal growth factor: characteristics of specific binding in membranes from liver, placenta, and other target tissues. *Arch. Biochem. Biophys.* **164:** 518–526.
11. Bucher, N. L. R., and R. A. Malt. 1971. Regeneration of

Liver and Kidney. Little Brown and Co., Boston, Mass. 1–159.
12. Bucher, N. L. R., and M. N. Swaffield. 1976. Synergistic action of glucagon and insulin in regulation of hepatic regeneration. *Adv. Enzymol. Regul.* **13**: 281–293.
13. Das, M., and C. F. Fox. 1978. Molecular mechanism of mitogen action: processing of receptor induced by epidermal growth factor. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 2644–2648.
14. Hollenberg, M. D. 1979. Epidermal growth factor-Urogastrone, a polypeptide acquiring hormonal status. *Vitam. Horm.* **37**: 69–110.
15. Todaro, G. J., J. E. DeLarco, and S. Cohen. 1976. Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor to cells. *Nature (Lond.).* **264**: 26–31.
16. Baker, J. B., G. S. Barsh, D. H. Carney, and D. D. Cunningham. 1978. Dexamethasone modulates binding and action of epidermal growth factor in serum-free cell culture. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 1882–1886.
17. Wrann, M., C. F. Fox, and R. Ross. 1980. Modulation of epidermal growth factor receptors on 3T3 cells by platelet-derived growth factor. *Science (Wash. D. C.).* **210**: 1363–1365.
18. Blomberg, J., F. H. Reynolds, Jr., W. J. M. Van de Ven, and J. R. Stephenson. 1980. Abelson murine leukaemia virus transformation involves loss of epidermal growth factor-binding sites. *Nature (Lond.).* **286**: 504–507.
19. Heimberg, M., I. Weinstein, V. S. LeQuire, and S. Cohen. 1965. The induction of fatty liver in neonatal animals by a purified protein (EGF) from mouse submaxillary gland. *Life Sci.* **4**: 1625–1633.