Effect of 2-Acetylamino fluorene on the Binding of Epidermal Growth Factor to Microsomal and Golgi Fractions of Rat Liver Cells

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ABSTRACT

The livers of rats fed the hepatocarcinogen 2-acetylamino fluorene (0.02%) with chow showed a sharp decrease in the binding of epidermal growth factor to microsomes and Golgi fractions. The binding to the latter decreased from 15.3% specific binding per 0.1 mg protein in controls to 9.4% after 2 days and reached a nadir of 0.8% after 21 days. The binding to microsomes decreased from 26.3% specific binding per 0.5 mg protein in the controls to 17.4% after 4 days and reached a nadir of 7.5% after 48 days. The low binding which persisted until the end of the experiment (85 days) was due to the apparent decrease in the number of receptors without significant changes in their affinity. Also, there was only partial recovery in rats fed 2-acetylamino fluorene for 90 to 107 days and taken off the carcinogen for 30 to 75 days. In vitro, neither 2-acetylamino fluorene nor its metabolites hydroxy- and acetoxy-2-acetylamino fluorene significantly decreased epidermal growth factor binding to the isolated microsomal fraction.

INTRODUCTION

The receptors to EGF bind, in addition to EGF itself, some TGF, type I, produced by normal embryonic and some transformed cells (for reviews, see Refs. 6, 8, 9, 17, and 24). A decrease in the EGF binding to its receptors has been found in partially resected regenerating liver, in cells treated with phorbol esters, in certain experimental cancers, and in human hepatoma. Although alterations of the EGF binding to its receptors in the course of chemical hepatocarcinogenesis have not been studied systematically, such studies could be of interest since EGF is not only an almost ubiquitous growth factor but also one of the most potent stimulants of hepatocyte DNA synthesis. The potential importance of such a study for oncology has been stressed recently by the finding of the rat TGF, type I, produced by normal embryonic and some transformed cells (for reviews, see Refs. 6, 8, 9, 17, and 24). A decrease in the EGF binding to its receptors in the course of chemical hepatocarcinogenesis has not been studied systematically, such studies could be of interest since EGF is not only an almost ubiquitous growth factor but also one of the most potent stimulants of hepatocyte DNA synthesis. The potential importance of such a study for oncology has been stressed recently by the finding of the enzyme marker galactosyltransferase (4).

MATERIALS AND METHODS

Adult male Fischer rats (180 to 200 g) were fed a basal diet containing 0.02% 2-AAF. The rats were sacrificed by exsanguination under ether anesthesia after 2, 4, 8, 21, 34, 46, and 85 days. Control rats received regular laboratory chow. Each group consisted of 4 to 9 animals, and all the determinations were done in quadruplicates.

The livers of rats fed 2-acetylamino fluorene for 90 to 107 days and taken off the carcinogen for 30 to 75 days. In vitro, neither 2-acetylamino fluorene nor its metabolites hydroxy- and acetoxy-2-acetylamino fluorene significantly decreased epidermal growth factor binding to the isolated microsomal fraction.

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3 The abbreviations used are: EGF, epidermal growth factor; 2-AAF, 2-acetylamino fluorene; DMSO, dimethyl sulfoxide; TGF, transforming growth factor; hydroxy-AAF, hydroxy-2-acetylamino fluorene; acetoxy-AAF, acetoxy-2-acetylamino fluorene.
2-AAF and EGF Binding to Rat Liver

Chart 1. Binding of 125I-labeled EGF to microsomal (●) and Golgi (○) fractions of the rat liver cells. The animals were fed 2-AAF for 2 to 85 days. Day 0 shows the binding in the control group. Probability values are given for the differences from the control group. Bars, S.D.

RESULTS

Chart 1 shows an early, sharp decrease in the EGF binding to the liver fractions of rats fed 2-AAF. A decrease in the EGF binding to the Golgi fraction was statistically significant as early as 2 days after the beginning of the 2-AAF feeding: from 15.3 ± 2.6% in controls to 9.4 ± 1.2% in rats fed 2-AAF (p < 0.005). After 4 days, the binding decreased further to 3.5 ± 0.7%, by 8 days to 1.4 ± 1.2% (p < 0.001), reached a nadir of 0.8 ± 0.8% by 21 days (p < 0.001), and remained low thereafter to the end of the experiment.

A less marked decrease occurred in the EGF binding to the microsomal fraction (Chart 1). It reached statistical significance after 4 days, decreasing from 26.3 ± 5.8% to 17.4 ± 4.4% (p = 0.013). A nadir of 7.5 ± 5.5% was reached after 46 days (p < 0.001).

Charts 2 and 3 show the competition curves for microsomal and Golgi fractions for the control rats and rats fed 2-AAF (the latter, at the nadir of the EGF binding). The Scatchard plot of the EGF binding to microsomes presented a straight line. The binding
capacity was 0.96 pmol/mg in the control rats and 0.40 pmol/mg in the rats fed 2-AAF. The affinity of the receptors was the same in both groups (1.45 nM).

The Scatchard plot of the EGF binding to the Golgi fraction was curvilinear with the following characteristics. The high-affinity receptors showed \( K_h = 0.40 \text{nM} \) in both control and 2-AAF-fed rats; the binding capacity was 0.43 pmol/mg in control rats, and 0.13 pmol/mg in rats fed 2-AAF; the low-affinity component showed \( K_L = 1.58 \text{nM} \) in control rats and 3.42 nM in rats fed 2-AAF, with the corresponding binding capacity of 1.25 and 0.65 pmol/mg.

In another series of experiments, 2-AAF was given for a period of 90 to 107 days, after which it was removed from the diet for an additional period of 30 to 75 days. Chart 4 shows that, in both microsomal and Golgi fractions, the binding of EGF remained sharply reduced in comparison to the control rats and was only slightly higher than in the rats sacrificed after 85 days of 2-AAF feeding. In control rats, in rats fed 2-AAF for 85 days, and in the “on-off” group, the EGF binding to the microsomes was 26.3 ± 5.8%, 8.4 ± 2.1%, and 15.5 ± 1.9% per 0.5 mg of protein, and to the Golgi fraction, it was 15.3 ± 2.6%, 1.6 ± 0.4%, and 2.6 ± 0.7% per 0.1 mg of protein.

In vitro experiments with the EGF binding to normal liver microsomes incubated with 2-AAF, hydroxy-AAF, and acetoxy-AAF showed that, in the presence of 5% DMSO alone, the binding was 85% of the control. 2-AAF and its metabolites in the concentrations of 25 to 100 μg/ml had no effect on the binding, whereas in the concentration of 200 μg/ml, they decreased the binding to 76.5% of the control.

**DISCUSSION**

In the described experiments (repeated 3 times), the hepatocarcinogen 2-AAF caused an early, sharp, and sustained decrease in the EGF binding to microsomal and Golgi fractions in rats. Only one class of receptors was found in the microsomes, and they exhibited a sharp decrease in their number without changes in their affinity. The Golgi fraction showed, on the other hand, the presence of 2 classes of receptors. In rats fed 2-AAF, the number of high-affinity receptors decreased, but their affinity did not change. The low-affinity receptors demonstrated a decrease in their number and affinity.

Endoplasmic reticulum (the main component of the microsomes) is the putative site of the synthesis of proreceptors with the final glycosylation and processing taking place in the Golgi system (25). We could find no references concerning the differences in the binding of EGF by different intracellular receptors, but it was reported recently in relation to insulin that the affinity of the Golgi receptors was much higher than that of plasma membranes (21). It is possible that the higher affinity of the Golgi receptors for EGF found in our experiments can be explained by the higher purity of the fraction, but the latter cannot possibly explain the presence of 2 classes of receptors in the Golgi fraction in comparison to one class in the microsomes. In any case, in further experiments with hepatocarcinogens, the possibility of differential changes in different intracellular receptors should be taken into account.

The total or partial loss of the EGF receptors was reported in chemically or spontaneously transformed liver cells, in W8 cells transformed by 2-AAF (15), and in human hepatoma (7). In rat hepatomas, both EGF binding to microsomes and EGF-induced receptor autophosphorylation were diminished or absent (3). A significant decrease in the affinity of EGF receptors is caused by phorbol esters (15, 19).

The changes found in the EGF binding to subcellular fractions are among the earliest reported. Other known early changes include the induction of resistance to cytotoxicins observed after 24 hr (5), increased activity of hepatic acid DNase and β-glucuronidase seen after 3 days (2), and electron-microscopic changes in endoplasmic reticulum and the Golgi system described after 1 week (16). Numerous biochemical changes (22) appear much later; no hyperplasia appears histologically before 4 weeks, and no nodules appear before 6 weeks.

Several possible explanations of the decreased EGF binding in the course of hepatocarcinogenesis can be offered. One possibility is a true decrease in the number of receptors. This seems likely in view of the similar finding in several experimental hepatomas (3). Another possibility is the production by the cells of TGF, type I (23), competing with EGF for its receptors (20). In our experiments, acid-ethanol extracts of the livers of rats fed 2-AAF did not possess any activity competing with EGF; therefore, this possibility seems unlikely. An equally unlikely explanation is the production of TGF, type II, since the latter does not decrease and, on the contrary, increases the number of EGF receptors and suppresses their down-regulation (1). Finally, an intriguing possibility is the dissociation of the ligand-binding and tyrosine-phosphorylating domains of the receptors: peptides derived from the cytoplasmic domain of the EGF receptor exhibited homology to certain oncoprotein sequences (13). This possibility, though, seems less likely in view of the reported decrease in the receptor autophosphorylation in experimental hepatomas (3).

Receptors to EGF share with the receptors to insulin, somatomedin-C, and platelet-derived growth factor a common property: tyrosine phosphorykase. Our studies showed that the changes in the insulin binding were less pronounced than, but highly positively correlated with, the changes in the EGF binding. Therefore, it seems probable that the changes reported here are not specific for EGF receptors only.

Several observations point to possible intimate relations among EGF, EGF receptors, and carcinogenesis, e.g., a correlation between the tumorigenicity and a decrease in EGF binding in several rat liver cell lines (15), extensive homology of TGF,
type I, to EGF (20), and a homology of certain avian oncogene proteins to some proteins derived from the EGF receptor (13). Our observations of the early appearance of the changes in EGF binding in the course of chemical hepatocarcinogenesis point to the same direction and deserve further study.

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