Prolactin Binding to 7,12-Dimethylbenz(a)anthracene-induced Mammary Tumors and Liver in Diabetic Rats

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SUMMARY

Growth rates of 7,12-dimethylbenz(a)anthracene-induced mammary tumors and the specific 
$^{125}$I-labeled prolactin binding to membrane fractions prepared from livers and tumors were studied in rats made diabetic by streptozotocin injection. Growth was inhibited in a majority of tumors, and prolactin binding was reduced in both tumors and livers from diabetic animals. Prolactin binding to individual tumors varied over a wide range in both intact and diabetic animals. Scatchard analysis of binding data revealed that the apparent affinity of prolactin binding to liver and tumor membranes was similar ($K_a \approx 3.0 \times 10^8 M^{-1}$) and was not affected by diabetes.

We suggest that the reduction in prolactin binding to tumors may render these tissues less responsive to prolactin and thereby explain, at least in part, the observed inhibition of tumor growth in diabetic rats. However, some tumors in diabetic animals regressed despite relatively high levels of prolactin binding activity. Therefore, additional factors most certainly play important roles in the mechanism(s) by which the growth of 7,12-dimethylbenz(a)-anthracene-induced tumors is impaired in the diabetic rat.

INTRODUCTION

The growth rate of mammary tumors that have been induced in rats by the administration of DMBA$^2$ is affected by changes in the circulating levels of PRL and insulin. Most of these tumors grew faster in the presence of elevated circulating PRL and became static or regressed when plasma PRL was reduced below normal (13, 20). In addition, Cohen and Hilf (1) and Heuson and Legros (12) reported that a majority of DMBA-induced tumors regressed after the induction of diabetes, thus demonstrating that the growth of established tumors appears to require adequate levels of plasma insulin as well as PRL.

Binding sites for both insulin (14) and PRL (4, 5, 16, 18, 26) are present in plasma membrane-enriched subcellular fractions prepared from DMBA-induced tumors, and it is assumed that binding to these sites initiates the metabolic effects of the hormones. The PRL binding activity in these tumors may be hormonally controlled, since hormone binding was reduced after the administration of estrogen (18, 22), an antiestrogen (16), or testosterone (4) and after ovariectomy or hypophysectomy (5). These treatments also resulted in tumor regression. It was therefore suggested that the reduced PRL binding activity rendered the tumors less responsive to the hormone and that this, at least in part, explained the observed inhibition of growth of these PRL-dependent tumors.

Recent experiments in our laboratory showed that PRL binding activity was reduced in livers and R3230AC mammary carcinoma obtained from diabetic rats (27) and that DMBA-induced mammary tumors, which regressed following the induction of diabetes, contained fewer estrogen receptors (10). It seemed possible that PRL binding may also be impaired in DMBA-induced tumors following the induction of diabetes. Here we present the results of experiments designed to test this possibility.

MATERIALS AND METHODS

Animal Treatment and Tumor Responses. Female Sprague-Dawley rats were obtained from Charles River Breeding Laboratory, Wilmington, Mass. Mammary tumors were induced by the i.g. instillation of 5 weekly doses (5 mg) of DMBA in sesame oil (1.0 ml) beginning at 50 days of age. Tumors appeared 4 to 6 weeks following the last dose of DMBA and were measured 3 times weekly with calipers. Tumor size was expressed as the product of 2 perpendicular diameters. When tumors reached 2 sq cm, the animals were randomly assigned to diabetic or control groups. Tumor growth responses were expressed as the percentage of change in tumor size during the final 10 days of a 2- to 3-week treatment period and defined as follows: regressing, $\geq$20% decrease in tumor size; growing, $\geq$20% increase in tumor size; or static, $<$20% change in tumor size.

Diabetes was induced by a single i.v. injection of streptozotocin (55 to 60 mg/kg; The Upjohn Co., Kalamazoo, Mich.) as previously described (1). For the verification that the animals were diabetic, urinary glucose was monitored weekly with the use of Clinistix (Ames Co., Inc., Elkhart, Ind.), and plasma glucose concentrations were determined with the use of Glucostat reagents (Worthington Biochemical Corp., Freehold, N. J.).

At the end of the experiment, the rats were decapitated. Blood was collected for plasma glucose analysis and for the determination of plasma PRL by radioimmunoassay with the use of materials supplied by the National Pituitary Agency. Tumors and livers were excised, frozen in liquid
specific 125I-labeled PRL binding was defined as the difference observed between the number of cpm bound to the membrane particles in the presence and absence of an excess (2 μg/ml) of unlabeled ovine PRL. Radioactivity was quantitated in a Packard Autogamma Counter with 60% counting efficiency. 125I-Labeled PRL binding was further characterized in representative tumor and liver membrane preparations. The apparent affinity constant (Kd) and binding capacity (N) were determined by Scatchard analysis (25) of dose-response curves of the inhibition of 125I-labeled PRL binding by unlabeled PRL.

The PRL binding data were expressed as cpm/100 μg membrane protein and presented as the mean ± S.E. of the observations in each group. In certain cases, data were expressed as cpm/unit of 5'-nucleotidase activity (see "Other Procedures"). Means were compared by Student's t test.

RESULTS

The data presented were collected in 2 separate experiments. The plasma concentrations of glucose and PRL observed are shown in Table 1, Experiment 1. As expected, both plasma glucose and urinary glucose (data not shown) were elevated in animals treated with streptozotocin. Immunoreactive plasma PRL concentrations in diabetic rats (13 ± 1 ng/ml) did not differ significantly from those observed in control animals (23 ± 8 ng/ml). Plasma PRL was not determined in Experiment 2, but plasma and urinary glucose values were similar to those in Experiment 1 (data not shown).

As illustrated in Table 1 and Chart 1, tumor growth was impaired in diabetic rats. In Experiment 1, 25% of the tumors in diabetic animals regressed and only 25% were classified as growing during the last 10 days of treatment. In contrast, 64% of the tumors in control animals were growing. No spontaneously regressing tumors were observed in the control group. The results obtained in Experiment 2 were similar, except that the incidence of tumor regression in the diabetic group was even greater (52%) than that observed in Experiment 1. This value is similar to that reported previously by Cohen and Hilf (1).

The effect of diabetes on specific 125I-labeled PRL binding to liver is shown in Table 2. Specific binding to liver membranes from control rats was consistently found to be 2- to 3-fold greater than that observed in liver membranes from diabetic rats. However, the absolute level of binding on protein basis was higher in Experiment 1 than in Experiment 2. Since different batches of labeled hormone were used in these experiments, it is probable that variations in the specific activity and/or the quality of the 125I-labeled PRL account for this variation; PRL binding to tumor membranes was also greater in Experiment 1. When the binding data were expressed on the basis of 5'-nucleotidase activity, similar differences were noted between control and diabetic animals (Table 2, Experiment 1).

Similarly, 125I-labeled PRL binding activity observed in tumors from diabetic rats and expressed in terms of the membrane protein content was significantly reduced in both Experiments 1 and 2 (35 and 45%, respectively), compared to that observed in tumors from controls (Table 2). When the binding activity was expressed on the basis of 5'-nucleotidase activity (Table 2, Experiment 1), this differ-

Table 1

| Plasma glucose, plasma prolactin, and DMBA-induced tumor growth in diabetic and control rats |
| --- | --- | --- | --- |
| **Experiment 1** | **Tumor growth** |  |  |
|  | Plasma glucose (mg/100 ml) | Plasma prolactin (ng/ml) | Growing | Static | Regressing |
| Control | 106 ± 7 (6) | 23 ± 8 (6) | 15/24 | 9/24 | 0/24 |
| Diabetic | 606 ± 44 (5) | 13 ± 1 (5) | 4/16* | 8/16 | 4/16* |
| **Experiment 2: tumor growth** |  |  |  |
|  | Growing | Static | Regressing |
| Control | 9/11 | 2/11 | 0/11 |
| Diabetic | 0/23* | 11/23* | 12/23* |

a Mean ± S.E.

b Numerator, number of tumors in each growth classification; denominator, total number of tumors in each treatment group.

* Numbers in parentheses, number of observations.

<sup>a</sup> p < 0.05 versus control, χ² analysis.

<sup>b</sup> p < 0.01 versus control t test.

**Other Procedures.** 5'-Nucleotidase activity was determined by the method of Widnell and Unkeless (28), except that trichloroacetic acid was used to precipitate the protein prior to the quantitation of P1, as described by King (17). In addition, 10 mM sodium potassium tartrate was added to the assay incubation mixture to inhibit nonspecific phosphatase activity (24). One unit of enzyme activity was defined as 1 μmole P1 released per min. Protein was determined by the method of Lowry et al. (19) with the use of bovine serum albumin as the standard.

<sup>2</sup> Our preparations of enzymatically 125I-labeled prolactin were shown to retain biological activity when assayed in a mouse mammary gland explant system by Dr. David Borst and Dr. Byron Doneen in the laboratory of Dr. Howard Bern.
ence was magnified (65% reduction in tumors from diabetic animals) due to a 2-fold increase in the enzyme activity in tumor membranes from diabetic hosts.

Protein content, PRL binding, and 5'-nucleotidase activities were determined in subcellular fractions prepared by differential centrifugation of tumor homogenates (24). Diabetes did not change their distribution among subcellular fractions of tumors (Table 3) or of liver (data not shown). When compared to controls, PRL binding was consistently reduced (50 to 70%) in all particulate subcellular fractions of tumors from diabetic animals, while a 2- to 3-fold increase in the activity of 5'-nucleotidase was noted in all fractions from tumors from diabetic animals.

The growth responses and PRL binding activities observed in individual tumors in Experiments 1 and 2 are shown in Chart 1. PRL binding varied widely among tumors within treatment groups in both experiments. However, individual values observed in tumors from diabetic animals generally fell within the lower range of values observed in the control group. The large variation in PRL binding activity confirms our previous observations (26).

Scatchard plots of binding data obtained in experiments with the use of membranes from tumors and livers from animals in Experiment 2 are shown in Chart 2. 125I-labeled PRL bound to both liver and DMBA-induced tumor membranes with a similar high affinity (Kd = 3 \times 10^{-9} M^{-1}), which was not changed in diabetes. In contrast, the 125I-labeled PRL binding capacity of membranes from the liver of a control rat (203 fmoles/mg protein) was approximately 6-fold that observed in liver membranes from a diabetic rat (35 fmoles/mg protein). Likewise, the PRL-binding capacity of a tumor obtained from a diabetic rat (46 fmoles/mg protein) was reduced compared to that observed in a tumor from an intact host [187 fmoles/mg protein (Chart 2)]. Similar results were obtained in other experiments with tissues from diabetic and control rats.

DISCUSSION

Sustained growth of DMBA-induced mammary tumors in the rat is dependent upon the maintenance of adequate circulating levels of both PRL and insulin. Reduction to subnormal levels of the plasma concentrations of either hormone inhibits the growth of most of these hormone-dependent tumors. On the basis of our findings that PRL binding activity was reduced in livers and R3230AC tumors removed from diabetic rats (27), we reasoned that insulin insufficiency might similarly affect PRL binding to DMBA-induced tumors. Assuming that this hormone binding is physiologically significant, it might be expected that these tumors would then be less responsive to endogenous PRL, thus explaining the observed impairment of tumor growth in the diabetic animal.

The results reported here generally support this idea. Tumor growth was inhibited by streptozotocin treatment, and mean PRL binding to tumors was reduced in diabetic rats as compared to that observed in tumors from control animals (Tables 1 and 2). The wide variation observed in PRL binding to membranes from regressing tumors in diabetic rats is similar to that reported to be associated with tumor regression following the administration of high doses of estrogen (18, 26) or androgens (4). These findings there-
Prolactin Binding to DMBA Tumors in Diabetes

Tumor tissue (1.0 g) was homogenized in a Polytron homogenizer (3 × 15 sec at Setting 9 with cooling at 0° between each burst), and subcellular fractions were prepared by differential centrifugation (24). The protein values represent the total protein recovered in each fraction.

Table 3

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Prolactin Binding (cpm/100 µg protein)</th>
<th>5'-Nucleotidase (units/mg protein)</th>
<th>Protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
<td>Control</td>
</tr>
<tr>
<td>1,500 x g</td>
<td>2,582 ± 527 (3)</td>
<td>767 (2)</td>
<td>0.023 ± 0.008 (3)</td>
</tr>
<tr>
<td>15,000 x g</td>
<td>3,946 ± 933 (3)</td>
<td>2,273 ± 422 (3)</td>
<td>0.029 ± 0.004 (3)</td>
</tr>
<tr>
<td>100,000 x g</td>
<td>8,478 ± 1,612 (3)</td>
<td>4,718 ± 182 (3)</td>
<td>0.068 ± 0.011 (3)</td>
</tr>
</tbody>
</table>

Superнатant | 0 | 0 | ND* | ND | ND | ND |

* One unit of 5'-nucleotidase activity is defined as 1 µmole P1 released per min.
* Mean ± S.E.
* Numbers in parentheses, number of observations.
* ND, not determined.

The effect of diabetes on PRL binding activity in liver membranes confirms our earlier findings (27) and extends those of others who reported increased insulin binding (6), decreased lectin binding (3), and altered enzyme activities (2) in the membranes from livers of streptozotocin-induced diabetic rats. Ovariectomy (9) and hypophysectomy (5, 22, 23) result in similar reductions in liver PRL binding, perhaps due to a suppression of plasma PRL (23). The results of our experiments suggest that the effect of diabetes does not involve alterations in plasma PRL and that insulin is important in the control of PRL binding activity in these tumors and liver.

The actual physiological sequelae of the reduction in PRL binding activity in liver and tumor remain unknown. Experiments designed to measure responses to PRL in tissues with altered binding activities must be done to study this. The fact that tumor regression does occur in conjunction with reduced PRL binding in hormone-dependent tumors should provide impetus for future study in this area.

ACKNOWLEDGMENTS

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DIABETIC
REFERENCES


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