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Prolactin Binding to Rat Mammary Tumor Tissue (38197)

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A single intravenous (iv) injection of 7,12 dimethylbenzanthracene (DMBA) to female rat results in the development of mammary tumors within one to 3 mo (1). Some of these tumors are dependent upon prolactin for growth (2) whereas others are not. In the case of human breast cancer, some tumors also may undergo remission with a change in hormonal milieu, such as occurs after ovariectomy of hypophysectomy which causes a decrease in circulating estrogen or prolactin levels respectively. The identification and quantitation of estrogen receptors in the cytosol of both experimental and human mammary tumors (3, 4) has been reported to be helpful in predicting the likelihood of response to endocrine ablative procedures. No comparable studies have appeared regarding the usefulness of identifying prolactin receptors in either human or experimental tumors. Therefore we examined DMBA-induced rat mammary tumors in order to determine whether the number of prolactin binding sites in membrane fractions obtained from these tumors correlated with the growth responsiveness to prolactin administration.

Materials and Methods. Female Sprague-Dawley rats, 55–60 days of age, were given a single iv injection of an emulsion containing 5 mg DMBA. Two and one half mo later, when tumors had developed in most of the injected rats, ten animals were injected subcutaneously daily for twelve days with 1 mg NIH ovine prolactin (oPRL, 26 IU/mg) dissolved in 0.85% saline made slightly basic with 0.1 *N* NaOH. Tumors were measured for length, width, and depth with calipers initially and every 4 days during the 12-day treatment period. Six days following the last oPRL injection, the animals were sacrificed and a total of 34 tumors (1–8 from each animal) were excised, weighed, and frozen. As an index of the degree to which each tumor responded to prolactin treatment, the difference in the sums of the three measurements (length, width, and depth) in centimeters at the beginning and end of the treatment period was calculated. This difference was defined as the growth index. Tumors were homogenized in 0.3 *M* sucrose, and a particulate membrane fraction was prepared as described previously (5). The membranes were then assayed for specific binding of ^{125}I-labeled oPRL and insulin. In addition, affinity constants (K_a) and binding capacities (N) of selected tumor membranes were determined by Scatchard analysis of displacement dose-response curves (6).

Results and Discussion. Tumors were ranked according to their responsiveness to prolactin, and these rankings were arranged in four groups. These groups together with the final tumor measurements, growth index, and specific binding of ^{125}I-labeled oPRL and insulin are presented in Table I. A definite trend in the binding of prolactin to membranes from rat mammary tumors and the response of these tumors to exogenous prolactin administration is apparent,

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TABLE I. Tumor Growth and Specific Binding of ^{125}I-Labeled oPRL and Insulin.

Rank	Final tumor size (l + w + d) (cm)	Growth Index (cm)	Specific binding[a] of ^{125}I-oPRL (%)	Specific binding[a] of ^{125}I-Insulin (%)
1–8	6.1 ± 1.1	2.2 ± 0.2	22.1 ± 2.4	2.2 ± 0.4 (7)[b]
9–17	5.0 ± 0.6	1.0 ± 0.6	12.7 ± 2.6	1.6 ± 0.2 (8)
18–26	3.3 ± 0.5	0.5 ± 0.0	10.0 ± 2.4	2.2 ± 0.4 (3)
27–34	2.8 ± 0.2	0.0 ± 0.0	7.3 ± 1.6	2.1 ± 0.5 (3)

[a] Total cpm minus nonspecific counts bound to membrane (counts in presence of excess unlabeled hormone) expressed as a percent of the total cpm added to the tube. Nonspecific binding was usually 5–8% of the total radioactivity added to the incubation tube.

[b] Due to the small size of some of the tumors, which yielded less membrane, the specific binding of only oPRL was determined. Numbers in parentheses indicate numbers of tumors in which insulin binding was assayed. In each column the mean ± SEM is indicated.

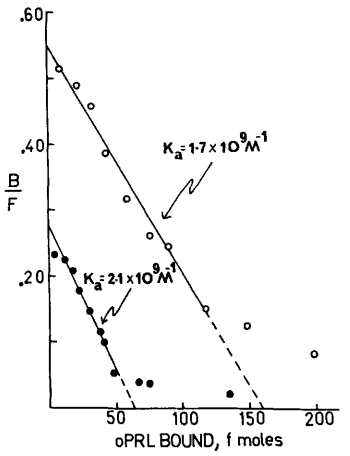

FIG. 1. Scatchard plot of displacement dose response curves of tumor membranes incubated with ^{125}I-oPRL and displaced with increasing concentrations of oPRL. The ordinate represents the ratio of membrane bound/free radioactivity; the abscissa is the amount of oPRL bound. Values for tube blanks were subtracted from total cpm bound to the membrane, Tube blanks were determined by incubating the standard assay mixture in the absence of membrane protein. The tube blanks represented approximately 5% of the total radioactivity added. The membrane preparation bindin the greatest amount of ^{125}I-oPRL which was obtained from the tumor with the greatest growth index is represented by (○). The membrane preparation with an intermediate degree of PRL binding and growth index (●). Binding constants were calculated for prolactin concentrations between 0.1 and 50 ng/ml in the incubation tube.

with those tumors showing the greatest response to prolactin also binding the greatest amount of ^{125}I-oPRL. The correlation coefficient for the growth index and specific binding for the 34 tumors examined was $r = 0.69$ ($P < 0.01$). When ^{125}I-hGH was used as tracer, a similar overall pattern was observed. The binding of insulin to tumor membranes was relatively constant in the 4 groups ranging from 1.6 to 2.2%.

Figure 1 is a Scatchard plot of displacement dose response curves for tumor membranes incubated with ^{125}I-oPRL. The affinity constants (K_a) for the 2 membrane preparations were similar. However, there was a 2.6-fold-difference in binding capacity of the 2 membrane preparations. The membrane preparation which was obtained from a tumor which had the grestest growth response to exogenous prolactin and which bound the greatest amount of ^{125}I-oPRL had a binding capacity of 530 fmoles/mg membrane protein ($K_a = 1.7 \times 10^9\ M^{-1}$), whereas the membrane with an intermediate growth index and prolactin bind had a binding capacity of 203 fmoles/mg ($K_a = 2.1 \times 10^9\ M^{-1}$). For membrane preparations which bound only small amounts of ^{125}I-oPRL, the binding was too low for a meaningful Scatchard analysis. In all membrane preparations examined, a second class of lower affinity binding sites were regularly observed.

The binding of ^{125}I-oPRL to the rat tumor membranes was higher than is gen-

erally observed in pregnant rat mammary tissue. Membrane fragments in the microsomal pellet (300 μg) from 20 day pregnant rats specifically bind approximately 2–3% of the ^{125}I-oPRL added (unpublished observations). The tumor tissues, on the other hand, had specific binding values ranging from 2 to 18% in the lower responding groups (27–35) and from 13.4% to 34.1% in the higher responding group (1–8). These values are more in line, but still slightly higher than the binding values for membranes from pregnant rabbit mammary glands which range from 10 to 15% per 300 μg (5). It is possible that the 12-day treatment with prolactin increased the amount of binding observed in rat mammary tissues, or that tumorous tissue inherently binds more prolactin.

Of the 34 tumors studied, there were some which did not fit into the overall pattern observed, i.e., some tumors responded well to PRL administration and yet had very low specific binding of ^{125}I-oPRL, and others which showed very little response to PRL had quite high binding of ^{125}I-oPRL. There are, of course, other factors which may influence tumor growth. The dependence of some DMBA induced mammary tumors upon estrogen has been documented (7), and it is quite probable tumors grow as a result of more than one stimulus.

Membranes were also prepared from the livers of the animals receiving DMBA and specific binding determined; as mentioned, some rats had more than one tumor. The average growth index of the tumors for each rat was calculated and the rats were then ranked according to the amount of tumor growth. Table II shows this average growth index and the percent specific binding of ^{125}I-oPRL to liver membranes of these animals. Those rats which had tumors showing the larger growth response to prolactin had liver membranes which bound smaller amounts of ^{125}I-oPRL. The correlation coefficient for the average growth index of the tumors in each rat the % specific binding of ^{125}I-oPRL to liver membranes of the 10 rats was $r = -0.69$ ($P < 0.05$). This is just the reverse of the correlation for PRL binding to mammary tumors and growth response of the tumors. We have observed that binding of ^{125}I-oPRL to rat liver membranes is low in fetal and immature stages and increases at puberty in females and also during pregnancy (8). In the present study the specific binding of ^{125}I-oPRL ranged from 3.2% in the rat with the largest growth index (1.6 cm) to 24.9% in the rat with the smallest growth index (0.2 cm). The explanation for this intriguing negative correlation remains unclear, but there may be competition for circulating prolactin between liver and mammary tumor tissue.

TABLE II. Tumor Growth and Specific Binding of ^{125}I-oPRL in Liver Membranes of DMBA-Treated Rats.

Rank	Growth index of tumors[a] (cm)	Specific binding of ^{125}I-oPRL (%)
1–4	1.4 ± 0.1	4.8 ± 1.0
5–7	0.6 ± 0.1	10.0 ± 3.5
8–10	0.4 ± 0.1	17.2 ± 5.5

[a] Some rats had more than one tumor, when this occurred, the growth indices of all the tumors from one rat were averaged. In each column the mean ± SEM is indicated.

We have shown that specific binding of ^{125}I-oPRL to a particulate fraction in the total microsomal pellet from rat mammary tumor tissue induced by DMBA is strongly correlated with the tumor response to exogenous prolactin, and the dependence of these tumors upon prolactin has been well documented (2). It is possible that this or a similar method of determining prolactin dependence may be potentially useful for assessing the responsiveness of human breast cancer to prolactin. Tumor tissues obtained by a simple breast biopsy could be examined in the same manner for prolactin binding sites. One might anticipate that tumors which contain relatively large numbers of prolactin binding sites might be more susceptible to therapeutic maneuver intended to lower serum prolactin concentrations such as hypophysectomy or treatments with drugs like Br-ergocryptine or L-DOPA.

Summary. Specific binding of ^{125}I-ovine prolactin was measured in mammary tumors from rats given 7, 12 dimethylbenzanthracene. A significant correlation was noted between the binding of prolactin to particulate membrane fragments in the total microsomal pellet and the growth response of these tumors to the administration of prolactin, that is the tumors showing the greatest response to prolactin also bound the greatest amount of ^{125}I-ovine prolactin ($r = 0.69$). There was a negative correlation between the average growth response of all tumors in one rat and the amount of ^{125}I-ovine prolactin specifically bound to liver membranes of that animal ($r = -0.69$). This or a similar method of determining prolactin dependence may be useful in assessing prolactin responsiveness in human breast cancer.

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1. Huggins, C., Grand, L. C., and Brillante, F. B., Nature **189**, 204 (1961).

2. Talwalker, P. K., Meites, J., and Mizuno, H., Proc. Soc. Exp. Biol. Med. **116**, 531 (1964).

3. McGuire, W. L., Huff, K., Jennings, A., and Chamness, C. G., Science **175**, 335 (1972).

4. Jensen, E., *in* "Proceedings of the 4th International Congress of Endocrinology" (Scow, R., ed.), Excerpta Medica, Amsterdam (1974).

5. Shiu, R. P. C., Kelly, P. A., and Friesen, H. G., Science **180**, 968 (1973).

6. Scatchard, G., Ann. N. Y. Acad. Sci. **51**, 660 (1949).

7. McGuire, W. L., and Julian, J. A., Cancer Res. **31**, 1440 (1971).

8. Kelly, P. A., Posner, B. I., Tsushima, T., and Friesen, H. G., Endocrinology, in press.

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