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7. Preliminary work suggests that the ventral gland secretion of females is little different from that of males. The TLC pattern appears identical, and the odorous component is found at the same R_F region. Both the female and male ventral gland responds to a wide range of gonadal hormones, including estrogen, progesterone, and testosterone.
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9. We wish to express appreciation to R. V. Coleman, R. Cocke, T. Mabry, and G. Vander Velde for valuable assistance during these studies. This research was supported by grants MH 14076-06 and NSF-USDP grant GU-1598 awarded to D.D.T. and NSF grant GB-30179X awarded to F.E.R.

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Prolactin Receptors in an Estrogen Receptor–Deficient Mammary Carcinoma

Abstract: *We demonstrate for the first time the presence of specific high-affinity receptors for prolactin in rat mammary carcinoma. There appear to be no significant differences between normal rat mammary tissue and the transplantable, estrogen receptor–deficient R3230AC tumor with regard to the number of binding sites, the affinity of the receptor for prolactin, or the specificity of binding.*

Although our understanding of hormone dependence in breast cancer is incomplete, prolactin and estrogen appear to be the principal hormones involved in breast tumor growth and regression (*1*). Recent efforts have focused on the presence of a cytoplasmic estradiol-binding protein (receptor), which correlates well with breast tumor regression following endocrine therapy (*2*). Nevertheless, it has been argued that prolactin might be the predominant hormone involved in mammary tumor growth and regression, and hence that the observations regarding estrogen receptors could be of secondary importance (*3*). Since techniques are now available for detecting specific membrane receptors for prolactin (*4*), we can now determine directly whether these prolactin receptors are present in mammary tumors and whether their presence is related to hormone dependence. Here we show that a rat mammary tumor which does not depend on either estrogen or prolactin for growth, and which has very little estrogen receptor, does possess prolactin receptors similar to those of the normal mammary gland.

Hilf *et al.* (*5*) originally described the transplantable rat mammary carcinoma R3230AC, which did not depend upon estrogen or prolactin for growth and yet would respond to these hormones with distinct alterations in certain enzymatic activities. Studies of this tumor revealed a marked deficiency in estrogen receptor content compared to hormone-dependent tumors or to normal estrogen target tissues (*6*) and suggested a possible causal relationship between the relative lack of estrogen receptor and the failure of the tumor to regress following ovariectomy (*7*). It thus became of interest to consider whether modified or deficient prolactin receptors might also be involved.

[^{125}I]Ovine prolactin was prepared by a soluble lactoperoxidase method (*8*). The iodinated prolactin was purified by diethylaminoethyl (DEAE) cellulose chromatography (*9*). Binding activity of this prolactin was checked independently with rabbit mammary gland particles according to Shiu *et al.* (*4*).

Tissue slices (1 mm thick, approximately 10 mg wet weight) were prepared from R3230AC rat mammary tumor grown in intact Fisher rats, or from freshly excised lactating rat mammary gland 18 hours after removal of pups. Five slices were placed in 1 ml of incubation medium [Medium 199 with 0.1 percent (wt/vol) bovine serum albumin, 5 m*M* $CaCl_2$, 5 m*M* Hepes buffer, *p*H 7.6] containing [^{125}I]-ovine prolactin (200,000 counts per minute at 55 to 77 μc/μg) and increasing quantities of nonradioactive prolactin (0 to 5000 ng). Experiments showed that both specific and nonspecific prolactin binding achieved a steady state within 2 hours of incubation when the slices were gently agitated at 22°C; a 3-hour incubation was routinely employed. The slices were then removed and washed four times with ice-cold Medium 199 containing 0.1 percent bovine serum albumin. Samples were assayed for radioactivity in a Nuclear-Chicago gamma counter, and the wet weight of each tissue slice was determined.

Figure 1 (left panel) is a representative Scatchard plot (*10*) of the binding data obtained by incubating normal rat mammary gland slices with increasing quantities of prolactin. The curved line represents the total prolactin binding. At low concentrations of prolactin, sites of high affinity and low capacity are revealed, whereas at progressively higher prolactin concentrations, binding of low affinity and high capacity predominates. We interpret the latter as nonspecific binding. Subtracting this

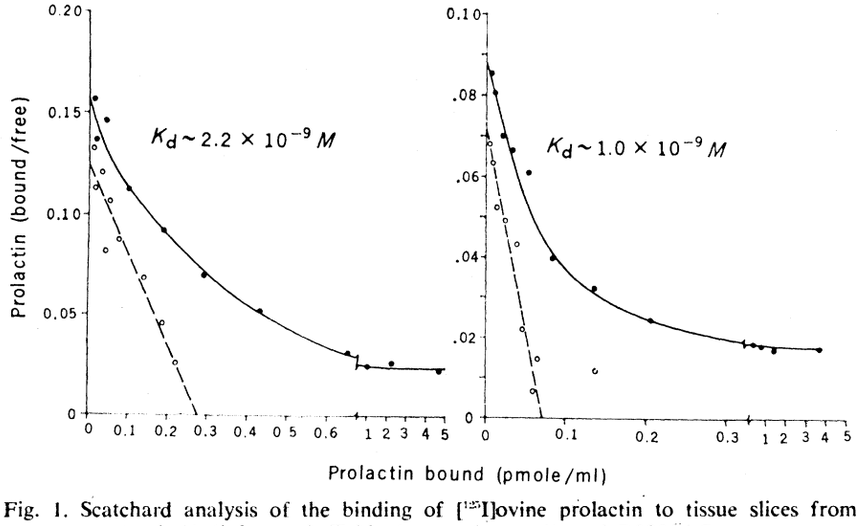

Fig. 1. Scatchard analysis of the binding of [^{125}I]ovine prolactin to tissue slices from rat mammary gland (left panel; Table 1, experiment 4) and R3230AC rat mammary carcinoma (right panel; Table 1, experiment 1). The high-affinity binding (dashed line) is derived from the total binding (solid line) as described in the text.

nonspecific component from the total binding results in a linear plot (dashed line) indicative of a single class of high-affinity binding sites. The dissociation constant (K_d) is estimated from the slope, indicating the relatively high affinity of the hormone-receptor interaction (Table 1). The total number of high-affinity sites can be extrapolated at the abscissa. Table 1 shows the concentration of such sites in normal mammary gland with a mean of 2.8 femtomoles per milligram of tissue, wet weight.

A representative experiment showing prolactin binding to R3230AC tumor slices is presented in Fig. 1 (right panel). Five such experiments (Table 1) yield a mean value of 1.3 femtomoles of receptor per milligram, wet weight, of tissue. The range of K_d values is also remarkably similar to that for normal lactating mammary gland. Determining the DNA content for each sample (*11*), we find that mammary tissue contains 0.90 ± 0.39 femtomoles of prolactin receptor per microgram of DNA, while R3230AC mammary tumor has 0.61 ± 0.28 femtomoles of receptor per microgram of DNA.

Assuming 7 pg of DNA per cell, we calculate approximately 3800 and 2600 sites per cell for normal tissue and R3230AC tumor, respectively. It should be emphasized, however, that determination of the actual number of receptors on cells by a tissue slice technique assumes a homogeneous cell population within the tissue and free access of all cells to the hormone. Therefore, a more accurate assessment of the absolute number of sites per epithelial cell for these tissues will require cell suspension methods.

An alternative explanation for hormonal autonomy has been suggested by the demonstration of ectopic polypeptide hormone receptors in adrenal tumor cells (*12*). To rule out the possibility that as a result of malignant transformation modified prolactin receptor sites might be able to recognize and hence be stimulated by other circulating hormones, we examined the ability of several hormones to compete with prolactin for the receptor sites on the R3230AC tumor cell. Table 2 clearly shows that follicle-stimulating hormone, growth hormone, insulin, and epinephrine failed to compete. Rat and ovine prolactin are indistinguishable, as predicted from their biological behavior. These results demonstrate the hormone specificity of the prolactin receptor site in the R3230AC tumor.

Table 1. Characteristics of binding of [^{125}I]-ovine prolactin to slices of normal mammary tissue and R3230AC rat mammary carcinoma. Receptor concentration and K_d were determined as described in the text.

Experiment No.	Dissociation constant (K_d) ($\times 10^{-9} M$)	[Receptor] (femtomoles per milligram of tissue, wet weight)
	Rat mammary gland	
1	0.92	1.0
2	2.3	3.2
3	1.7	1.8
4	2.2	5.2
	R3230AC mammary carcinoma	
1	1.0	1.0
2	1.8	1.6
3	2.1	1.5
4	1.6	0.9
5	1.6	1.7

The presence of prolactin receptor sites in mammary tumors has not previously been reported. Our finding of prolactin receptor on R3230AC tumor cells with properties similar to the receptor of normal rat mammary gland shows that lesions in prolactin receptors cannot explain the hormone-independent growth of this tumor. The biochemical lesions leading to independence must lie distal to the prolactin binding step, or alternatively in a function mediated by estrogen receptor as previously suggested (*7*). We no longer believe, however, that there is necessarily a causal relationship between the relative lack of estrogen receptor and the hormone-independent growth of the tumor. Rather, the estrogen receptor may be a useful cell marker for hormone dependence, its absence being a signal that malignant transformation of the normal mammary cell has resulted in the deletion of certain components of the normal hormonal regulatory system. To understand the operation of this regulatory system in mammary tumor cells, we must now examine the role of prolactin receptors in tumors which are clearly dependent on hormones for growth.

Table 2. Hormone specificity of binding of [^{125}I]ovine prolactin to R3230AC rat mammary carcinoma. Slices of tissue were incubated as described in the text with [^{125}I]ovine prolactin (2.3 ng) in the presence of unlabeled competitor hormones (*13*). Results are expressed as mean percent reduction in binding compared to controls (no competitor added) $\pm$ standard error of the mean for at least five slices of tissue each. No competitor added = 208 counts per minute per milligram of tissue, wet weight.

Hormone competitor	Amount of competitor (ng)	Percent competition
Ovine prolactin	50	33 ± 3.6
	2000	65 ± 1.0
Rat prolactin	50	22 ± 4.1
	2000	54 ± 3.4
Rat follicle-stimulating hormone	50	10 ± 5.1
	2000	11 ± 2.4
Rat growth hormone	50	4 ± 6
	2000	10 ± 4.1
Insulin	50	10 ± 7.3
	2000	14 ± 1.4
Epinephrine	50	16 ± 2.2
	2000	12 ± 2.9

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14. Hormone sources: Rat growth hormone, NIAMD-Rat-GH-I-2; NIAMD-Rat prolactin I-1; NIAMD-Rat FSH-B-1; ovine prolactin, NIH-P-S-10; L-epinephrine, Nutritional Biochemical Corp.; insulin, Squibb. Supported in part by National Cancer Institute grant CA-11378 and contract NO1-CB-23862 and American Cancer Society grant BC23C. M.E.C. is supported by a postdoctoral fellowship from the Robert A. Welch Foundation.

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