Effect of Prolactin on Lactalbumin Production by Normal and Malignant Human Breast Tissue in Organ Culture

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ABSTRACT

To determine whether lactalbumin production by normal and neoplastic human mammary tissue is under the same control, the effect of prolactin treatment was studied in organ culture. Of 9 premenopausal normal breast samples, 6 produced lactalbumin in culture, and all 6 responded to prolactin treatment over 4 days. One biopsy of pregnant breast tested also responded to prolactin treatment, producing 200 times more lactalbumin in culture than did normal breast. Two of 4 normal postmenopausal biopsies produced lactalbumin, and one increased synthesis and release after prolonged exposure to prolactin. Of 10 scirrhous carcinomas, 6 produced lactalbumin, but none responded to prolactin treatment. In 2 premenopausal patients, normal breast tissue responded to different concentrations of prolactin, which were without effect on malignant tissue from the same breast.

In summary, lactalbumin production in the samples that we have studied can be stimulated in normal but not in malignant breast tissue. This may indicate an absence or deficiency of prolactin receptors in malignant tissue.

INTRODUCTION

Lactalbumin is a major protein component of human milk and acts as the specifier or B protein for the lactose synthetase system (1, 2). It is secreted with other components of milk during lactation but can also be found in the serum during pregnancy and lactation, and at much lower concentrations in nonpregnant premenopausal female serum (7, 12). It is found in males with gynecomastia, and elevated levels are found in females receiving large doses of phenothiazines (7). Reports of lactalbumin in normal male sera (7, 13) probably reflect their serum, although whether this is derived from the tumor or in males with gynecomastia, and elevated levels are found in females receiving large doses of phenothiazines (7). Reports of lactalbumin in normal male sera (7, 13) probably reflect those reports. It has been demonstrated that prolactin and estradiol are required for maximal stimulation of lactalbumin from normal rodent mammary gland (4) and that prolactin is of prime importance in primate mammary tissue (6, 8). The aim of this study was to determine whether lactalbumin synthesis and release was stimulated by prolactin in the normal and neoplastic gland in vitro. The study is part of a continuing investigation of the hormone response mechanisms of human breast carcinomas and their relation to the outcome of endocrine therapy. Some aspects of this work have already been published in abstract form (9, 10).

MATERIALS AND METHODS

Tissue Samples. The tumor tissue used in these studies was from excision biopsies at mastectomy from a site distant to the primary lesion. These tissues were histologically assessed for abnormalities which would exclude them from the study. Normal pregnant breast tissue was removed with an excision biopsy of a fibroadenoma from a 7-month pregnant woman.

Hormones. Ovine prolactin (NIH-P-S-12) was donated by the National Institute for Arthritis, Metabolism, and Digestive Diseases, Bethesda, Md. Concentrations varying from 0.5 to 50 µg/ml were dissolved in culture medium brought to pH 9.0 with 0.1 N NaOH, which was then returned to pH 7.4 with 0.1 N HCl.

Organ Culture. Tissue was collected fresh from theatre in Roswell Park Memorial Institute 1640 medium (Flow Laboratories, Paisley, Scotland) buffered with 20 µM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and containing penicillin (200 units/ml), streptomycin (250 µg/ml), and kanamycin (100 µg/ml). Excess fat was removed, and the tissue was cut into 1- to 2-cm explants were placed at the liquid-gas interface on a supportive, permeable membrane (defatted lens tissue) on an expanded stainless steel grid in a compartment of a 100- x 100-mm Petri plate (Flow Laboratories). One ml of culture medium with or without prolactin was aliquoted into each compartment, and the cultures were incubated for periods up to 10 days at 37°C in a humidified atmosphere of 95% air: 5% CO2. The culture medium was serum-free Medium 199 (Wellcome Reagents Limited, London, England) buffered with 20 µM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and containing sodium bicarbonate (1.1 g/liter) and penicillin (200 units/ml) and supplemented with D-glucose (1.5 g/liter; Sigma Chemical Company, London, England).

Radioimmunoassay of Lactalbumin. Lactalbumin levels were measured in cytosol extracted from a 7-month pregnant woman.
EDTA and 0.5 mM dithiothreitol (Sigma). The cytosol fraction was obtained by spinning down the particulate cell components at 100,000 x g for 1 hr at 4°. Because it was impractical to weigh each set of 6 explants before culture, the results were expressed per ml of culture medium. The initial cytosol levels were expressed per 50 mg, wet weight, for ease of comparison, this being the mean amount of tissue per culture.

**Light Microscopy.** After cessation of culture, the tissue was fixed in 4% formaldehyde in 0.85 m NaCl, processed through graded alcohol and xylene, and embedded in paraffin. Hematoxylin- and eosin-stained sections were examined for viability and alterations in tissue morphology due to prolactin treatment.

Little or no degeneration was seen in normal breast tissue for any length of culture. The carcinomas were variable, with some degeneration occurring in those that were poorly differentiated. There were no specific changes in the normal or neoplastic tissue in the presence of prolactin.

**RESULTS**

**Concentrations of Lactalbumin in Cytosol Fractions.** No lactalbumin was detected in normal pre- and postmenopausal breast tissue. In the normal pregnant breast, the value was 3.06 ng/50 mg tissue, and in the fibroadenoma from the pregnant breast the concentration was 0.48 ng/50 mg. The lactalbumin content of malignant breast tissue ranged between nondetectability and 0.626 ng/50 mg.

**The Effect of Culture with Prolactin on Normal Pre- and Postmenopausal Human Breast Tissue.** Five of 9 premenopausal breast cultures and 2 of 4 postmenopausal cultures had measurable lactalbumin in the culture supernatant of the control dishes, whereas none was detectable in the cytosol of the original tissue. There was significant stimulation of lactalbumin production by ovine prolactin in 4 of 5 premenopausal cultures; in the fifth, stimulation was significant at the 10% level. In one other culture of premenopausal breast, lactalbumin was not detectable in the control cultures but was present in the prolactin-stimulated cultures after 4 days of incubation (see Chart 1A).

**Postmenopausal breast tissue produced low concentrations of lactalbumin in culture but was not stimulated by prolactin over a 4-day incubation period (Chart 1B). However, when postmenopausal tissue was exposed to a higher concentration (20 μg/ml) or was cultured for a 10-day period, significant stimulation of lactalbumin occurred (Chart 2).**

**The Culture of Pregnant Breast Tissue with Prolactin.** The amount of lactalbumin released by normal pregnant breast tissue in culture was 600-fold greater than could be accounted for by the initial cytosol content. The pregnancy-primed fibroadenoma produced 60 times the cytosol lactalbumin content and was also stimulated by prolactin, although this did not reach significance at the 5% level (Chart 3). These levels of lactalbumin released by pregnant breast are a factor 10² to 10³ greater than those found in normal breast cultures.

**The Culture of Malignant Breast Tissue from Pre- and Postmenopausal Patients.** Three of 6 tumors from premenopausal patients released lactalbumin into the culture medium, but this was not influenced by prolactin. The amount of lactalbumin released was lower than in most normal premenopausal breast cultures (Chart 4A).

Three of 6 tumors from postmenopausal patients also released lactalbumin, and this also was not influenced by prolactin (Chart 4B).

In order to determine if higher concentrations of prolactin would stimulate lactalbumin production by malignant breast tissue, 2 tumors were exposed to concentrations between 0.5 and 50.0 μg/ml. Normal tissue from a site distant to the tumor was treated similarly. There was stimulation of normal tissue of between 21 and 178% across the range of concentrations of prolactin, but there was no effect on the malignant breast tissue (Chart 5).

Incubation of malignant breast tissue with prolactin for up to 10 days had no effect on lactalbumin synthesis or release.

**DISCUSSION**

The term "normal" breast tissue used in these experiments must be qualified since such tissue was always taken from breasts which contained malignant tumor. A sample taken

**Effect of Prolactin on Lactalbumin Release**

![Graph showing effect of prolactin on lactalbumin release](chart2.png)

Chart 2. Effect of long-term incubation with 2 dose levels of ovine prolactin on lactalbumin release from normal human postmenopausal breast tissue. The medium was changed every 2 days and the results represent the amount of lactalbumin released in each 2-day period. The results are expressed as mean ± S.E. for 8 replicates in control and prolactin (2 μg/ml) cultures and 6 replicates in the prolactin (20 μg/ml) cultures.
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The hormones in the basal medium. Our own medium contained no serum and no other hormones, but we are currently investigating the effects of other hormones on lactalbumin production by normal and malignant human mammary tissue.

Normal postmenopausal breast tissue responded to prolactin only at high concentration or if incubated for prolonged periods. This may reflect a priming effect of other endogenous hormones in the premenopausal women not present after the menopause. There is no doubt from our experiments that the postmenopausal breast can produce milk proteins and can be stimulated. It should also be noted that none of this group of patients was on hormonal replacement therapy. This can also be seen clinically since hyperprolactinemia can induce lactation in postmenopausal women and in some men.

When examining the results of normal breast overall, it is apparent that lactalbumin production varies greatly in premenopausal breast but, in the few samples we have studied, has been low, when detectable, in postmenopausal breast. It also appears that the more lactalbumin released by the control cultures, the greater the response to the prolactin stimulus is likely to be. In our previous study of sera from normal women (13), this variation in lactalbumin levels was also evident. We are currently investigating the possibility that lactalbumin release may vary throughout the menstrual cycle and that variations in response to prolactin in culture may reflect differences in mammary responsiveness in vivo and depend on the period of the cycle when the tissue was removed.

Holdaway and Friesen (3) have demonstrated greater than 1% specific prolactin binding in 20% (8 of 41) human mammary tumors and have suggested that this is evidence for prolactin receptors in breast cancer tissue. Since we have not shown stimulation of lactalbumin production by prolactin, even at high concentrations, or over prolonged periods of culture in malignant tumors, this suggests either that we have missed tumors with prolactin receptors or that, if the receptor is present, subsequent steps along the pathway of its biochemical action adjacent to the normal breast was examined histologically and always appeared normal. This does not constitute proof of normality, and all that should be said concerning these results is that malignant breast tissue did not respond to prolactin, whereas breast tissue taken distant from the site of the cancer did respond to prolactin over a 4-day culture period for premenopausal breasts and over a longer culture period for postmenopausal breast tissue.

Kleinberg (5) has recently reported a lack of response of normal human breast tissue to prolactin, but his results do not conflict with our own since he used postmenopausal tissue incubated for 3 days in the presence of ovine prolactin, 1 µg/ml, and a response would not be expected. However, in contrast to the experiments reported here, he has shown stimulation of lactalbumin production by 1 µg prolactin in 3 malignant tumors out of a series of 33, 7 of which had detectable lactalbumin in the base-line medium. His cultures contained serum, insulin and hydrocortisone. It is possible that this prolactin stimulation may reflect a priming of synergistic effect of

Chart 3. Effect of ovine prolactin (PRL) (2 µg/ml) on lactalbumin release from normal pregnant breast (A) and a fibroadenoma (B) from the same breast. The cultures were incubated for 4 days at 37°. Only small amounts of tissue were available, consequently only 2 replicates were possible for cultures of normal pregnant breast, and these are represented as individual dots. The results for the fibroadenoma are expressed as mean ± S.E. for 4 observations.

Chart 4. Effect of ovine prolactin (PRL) (2 µg/ml) on lactalbumin release from malignant human premenopausal breast tissue (A) and postmenopausal breast tissue (B). The cultures were incubated for 4 days at 37°, and the results are expressed as mean ± S.E. for the number of observations shown per experiment (n).

Chart 5. Effect of different concentrations of prolactin on lactalbumin release from normal and malignant premenopausal breast tissue taken simultaneously from the same breast. —, normal tissue; — — — —, malignant tissue. The results are expressed as percentage change over control for each tissue with 6 observations per treatment. Significance was tested, using the Student's t test. *, p < 0.05; **, p < 0.02; ***., p < 0.01; ****., p < 0.001.
are defective. It is also possible that while normal mammary gland survives well and responds to prolactin stimulation under our culture conditions these may not be suitable for malignant tissue. Histologically, the tumors appeared well preserved after 4 days of culture, and the addition of serum to the medium did not alter the tumor responsiveness to prolactin.

The high ratio of culture supernatant lactalbumin to cytosol lactalbumin in the pregnant and normal tissues suggests that lactalbumin is rapidly exported from cells. The detection of lactalbumin in tumor cytosol but not in the cytosol of adjacent breast probably reflects the greater cellularity of the malignant tissue.

Although full lactation requires the action of several hormones, our results indicate a specific defect of prolactin responsiveness in the tumors that we have studied.

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