**α-Lactalbumin in Human and Subhuman Primate Normal Mammary Tissue and in Human Breast Cancer as a Marker for Prolactin Activity**

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Abstract

Mammary tissue from virgin, nulliparous, and multiparous primates of various species has been shown to contain α-lactalbumin, a milk protein. Production of α-lactalbumin by these tissues in organ culture was maintained or increased under the influence of prolactin. These findings provide evidence that mammary tissues, even in animals that are not pregnant or lactating, are active and responsive to prolactin. The fact that α-lactalbumin was found in 48.5% of homogenates of histologically normal breast tissue from women with breast cancer, many of whom were menopausal, indicates that this protein can be produced in humans under conditions of relative hormonal dormancy.

Fewer specimens of human breast cancer (23.5% of homogenates and 21% of tissues in organ culture) were found to contain or produce α-lactalbumin. Studies on normal and cancer tissue from the same patients, 9 of 17 of whom had measurable quantities of α-lactalbumin in normal but none or much lower concentrations in cancer tissue suggest that during malignant transformation some epithelial cells may lose the ability to produce α-lactalbumin and by inference the sensitivity to prolactin. Even though some tumors may not contain α-lactalbumin, a significant minority of human tumors do. In addition, this milk protein was present in higher concentrations in cancer tissue than in normal tissue in two individuals, and prolactin more than doubled α-lactalbumin production in three cancers maintained in organ culture. Although the presence of α-lactalbumin in tumors may suggest a responsiveness to prolactin, the possibility that the actual growth of α-lactalbumin-containing or -producing tumors is dependent on prolactin must await clinical confirmation.

Introduction

Although prolactin is an important factor in controlling growth and maintenance of some experimentally induced mammary cancers in rats (11) and mice (16), a similar role for prolactin in human breast cancer has not been firmly established. To examine the possibility that prolactin may be important in human breast cancer, we have been measuring α-lactalbumin, a milk protein the production of which is responsive to prolactin in 7,12-dimethylbenz(a)-anthracene-induced rat tumors (9, 13). A radioimmunoassay for human α-lactalbumin has permitted measurement of much smaller quantities of this protein than was previously possible (3, 4). Progress toward our goal of determining whether prolactin affects the growth and maintenance of human breast cancer will be reviewed in this communication by the presentation of α-lactalbumin measurements in homogenate and organ culture studies.

It has been our feeling that an understanding of how prolactin affects α-lactalbumin production under normal circumstances is a necessary prerequisite to interpret adequately and put into perspective the observed effects in cancer tissue. Because it is not possible to obtain normal human breast tissue from healthy individuals for in vitro examination, we have developed an organ culture system to study the effects of prolactin on α-lactalbumin production in normal mammary tissue from several subhuman primate species (5, 6). Subhuman primates were chosen because of their close resemblance to humans in many respects including life span, menstrual cycle, and the possession of a single pair of thoracic mammary glands that produce immunologically similar milk proteins (3, 4). These studies have provided evidence that mammary tissue from nonpregnant, nonlactating subhuman primates is not dormant and that prolactin can stimulate α-lactalbumin production in vitro.

Methods of Procedure

Mammary tissue from primates, including examples of the Macaca nemestrina, Macaca mulatta (rhesus monkeys), and Papio species (baboons), was removed by bilateral mastectomy through a common midline incision. Ketamine hydrochloride with pentobarbital was used for anesthesia. Tissues were kept in sterile Hanks' balanced salt solution with antibiotics at 4°C until used. Within 3 to 24 hr after surgery, tissue either were frozen for later determination of α-lactalbumin content or were cut into cubes of approximately 2 cu mm for organ culture. A complete description of the organ culture system has been published (6). In essence 5 to 10 cubes of tissue were placed in each dish with Medium 199 supplemented with porcine insulin, hydrocortisone, and, in most studies, 20% pooled human female serum. The pool was obtained from a group of adult volunteers with unmeasurable serum α-lactalbumin. The pooled serum contained approximately 9 ng of prolactin per ml, so that the concentrations of human prolactin in individual dishes were about 1.8 ng/ml. Ovine prolactin (NIH-P-S-10) was added in concentrations of 100 and 1000 ng/ml.
ng/ml, but when filter sterilization was carried out it resulted in mean 33% losses (range, 28 to 40%) of ovine prolactin, so that the actual concentrations were 67 and 670 ng/ml, respectively. Cultures were maintained for up to 9 days at 37° in an atmosphere of 95% air and 5% CO₂. The medium was changed every 3 days and was stored at −20° until analyzed for α-lactalbumin content by the previously described radioimmunoassay (3, 4). Each α-lactalbumin determination represents a 3-day accumulation in medium. Homogenates were prepared as previously described (4, 6), and tissue proteins were measured by the method of Lowry et al. (7).

Human tissues, both cancer and normal, were obtained from patients with breast cancer at New York University Hospital at the time of biopsy or mastectomy. Tissues were handled under sterile conditions as previously described (4). Sections of tissue were stored at −20° for later α-lactalbumin analysis or were prepared for organ culture studies or both, depending on the amount of tissue available. The organ culture studies were similar to those described above, but studies were carried out for only 3 days.

Results

Studies in Subhuman Primates. α-Lactalbumin was found in homogenates of normal mammary tissue from most subhuman primates, including nulliparous, multiparous, and virgin animals. Table 1 lists α-lactalbumin concentrations in tissues from several species. The highest levels were found in three 4-year-old animals of the M. nemestrina variety, all of which were nulliparous but were cycling regularly. α-Lactalbumin was also detected in tissue from 11 adult baboons, all but one of which had a history of pregnancy. Much lower concentrations of α-lactalbumin were found in samples of mammary tissue from 4 of 5 rhesus monkeys, none of which had a history of pregnancy.

That prolactin is capable of maintaining existing or stimulating additional or new production of α-lactalbumin in mammary tissue from nonpregnant, nonlactating primates has recently been determined from studies in this laboratory (5, 6). For example, simultaneous examination of the α-lactalbumin content in culture medium and homogenates of tissue taken from 3 monkeys of the M. nemestrina variety revealed that α-lactalbumin was consistently higher in dishes containing ovine prolactin than in controls. Chart 1 illustrates results of the effects of ovine prolactin (1000 ng/ml) on α-lactalbumin in medium and tissue homogenates after 9 days in culture. Prolactin at 100 ng/ml produced 1.4- to 15-fold increases above control (not shown in the chart). Although there was a decline in the total amount of α-lactalbumin production with time, prolactin continued to stimulate increased quantities of α-lactalbumin compared to controls (6). Uptake of [3H]lysine into trichloroacetic acid-precipitable protein was observed in both controls and prolactin-treated tissues at each time period. That no significant differences were noted suggests that the effect of prolactin on α-lactalbumin production is specific.

Studies on mammary tissue from 7 adult nonpregnant, nonlactating baboons (Chart 2) showed that α-lactalbumin was present in the medium at 3 days in each case even in the absence of added prolactin; the mean was 93.7 ng/ml (range, 12.7 to 243 ng/ml). Increased α-lactalbumin production was noted in 3 of 7 cases in the presence of 100 ng of prolactin per ml but in 7 of 7 cases with 1000 ng/ml (mean, 309 ng/ml; range, 18.3 to 583 ng/ml). After 6 days in culture, α-lactalbumin in control dishes fell by half to a mean of 47.6 ng/ml (range, 13.2 to 117 ng/ml), while in the presence of 100 ng of prolactin per ml α-lactalbumin was higher (mean, 242.5; range, 23.1 to 997 ng/ml) than both the 3- and 6-day controls in 4 of 7 and 6 of 7 instances. Chart 2. Effect of ovine prolactin (1000 ng/ml) on α-lactalbumin production in culture medium (A) and tissue homogenates (B) of mammary tissue from 3 nulliparous subhuman primates (M. nemestrina). Results express accumulated α-lactalbumin in medium during Days 7 to 9 in culture and in tissue homogenates kept in culture for 9 days.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>α-Lactalbumin in mammary tissue homogenates of adult female subhuman primates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>No. positive/total</td>
</tr>
<tr>
<td>M. nemestrina</td>
<td>3/3</td>
</tr>
<tr>
<td>Papio species (baboons)</td>
<td>11/11</td>
</tr>
<tr>
<td>M. mulatta (rhesus monkeys)</td>
<td>4/5</td>
</tr>
</tbody>
</table>

Chart 1. Effect of ovine prolactin (1000 ng/ml) on α-lactalbumin production in culture medium (A) and tissue homogenates (B) of mammary tissue from 3 nulliparous subhuman primates (M. nemestrina). Results express accumulated α-lactalbumin in medium during Days 7 to 9 in culture and in tissue homogenates kept in culture for 9 days.

Chart 2. Effect of ovine prolactin on α-lactalbumin production in culture medium bathing mammary tissue from 7 adult baboons after 3 and 6 days in culture. Results are expressed as mean α-lactalbumin levels for all 7.
respectively. With a higher dose of ovine prolactin (1000 ng/ml), α-lactalbumin was significantly higher than in both the 3- and 6-day controls in all instances (mean, 596 ng/ml; range, 86.3 to 1773 ng/ml), indicating that additional milk protein production was taking place.

A further demonstration of the stimulatory effect of prolactin on α-lactalbumin production comes from studies on the comparison of the effects of human and ovine prolactin on α-lactalbumin production in tissue from 2 premenarchal rhesus monkeys (Chart 3). Culture methods were identical with those described above, but neither the ovine prolactin nor the human material (kindly provided by Dr. Henry Friesen of the University of Manitoba, Canada) was filter sterilized. Results are expressed as total production of α-lactalbumin in medium. In the absence of prolactin, α-lactalbumin was undetectable in one experiment and barely detectable in the other, the total production being 1.3 ng. Equal concentrations of ovine and human prolactin (100 ng/ml) resulted in α-lactalbumin production of 39.8 and 104 ng, respectively. Similarly, the addition of both species of prolactin (300 ng/ml) resulted in an α-lactalbumin output of 68.6 ng for ovine and 167.7 ng for human prolactin. Thus human prolactin was found to possess approximately 2.5 times the biological activity of ovine prolactin in this primate system. If allowances are made for species differences and filter sterilization, then dishes containing 100 ng of ovine prolactin per ml contain activity equivalent to approximately 27 ng of human prolactin.

Studies in Humans. α-Lactalbumin was detected in a greater number of normal breast tissue samples from patients with breast cancer than cancer tissue (Table 2). Examination of homogenates of tissue revealed that 48.5% of normal breast tissues and 23.5% of cancers contained α-lactalbumin, even though epithelial cells were usually more numerous in the cancers (4). In those 17 patients in whom both normal and cancer tissues were available, 3 patterns emerged (Chart 4). The first was represented by a group of 8 patients who had detectable α-lactalbumin in samples of histologically normal tissue but unmeasurable concentrations in the cancer; a ninth patient who was 2 months pregnant had much higher levels in her normal tissue (950 pg/mg) than in the cancer (15.1 pg/mg). Another group of 6 patients had no α-lactalbumin in either type of tissue. A third pattern was evident in 2 patients whose cancer tissue contained much greater quantities of α-lact-

Table 2
α-Lactalbumin in human breast cancer and in histologically normal breast tissue from cancer patients

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. positive/total</th>
<th>% positive</th>
<th>Mean α-lactalbumin (ng/mg)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue homogenates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16/33</td>
<td>48.5</td>
<td>3.8</td>
<td>0.002-55</td>
</tr>
<tr>
<td>Cancer</td>
<td>21/89</td>
<td>23.5</td>
<td>1.03</td>
<td>0.003-2.2</td>
</tr>
<tr>
<td>Culture medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>9/22</td>
<td>41</td>
<td>8.6</td>
<td>0.8-54</td>
</tr>
<tr>
<td>Cancer</td>
<td>7/33</td>
<td>21</td>
<td>2.3</td>
<td>0.6-7.3</td>
</tr>
</tbody>
</table>

Chart 3. Comparison of the effects of ovine and human prolactin on α-lactalbumin production in mammary tissue from 2 premenarchal rhesus monkeys.
bumin than did the normal tissue. A fuller description of these studies has been recently published (4).

Another approach used was the measurement of α-lactalbumin in medium bathing organ cultures of normal and cancer tissues. The results of these studies were similar to those found with tissue homogenates. α-Lactalbumin was released into medium bathing more normal tissues (41%) than cancer (21%). The addition of prolactin (100 ng/ml) resulted in increases in α-lactalbumin production of greater than 100% in 3 breast cancers, as shown graphically in Chart 5. These may be examples of prolactin-responsive human breast tumors.

Discussion

The synthesis of milk proteins during pregnancy and lactation is under the control of prolactin. Studies on mammary tissue from midpregnant or lactating rodents indicate that both casein production (15) and α-lactalbumin (10) production are increased by prolactin in organ culture. Until now, it has not been clear whether prolactin has any effect on the breast at times of life other than during pregnancy or lactation. The fact that α-lactalbumin was found in mammary tissue from nulliparous, multiparous, and premenarchal primates indicates that pregnancy is not a necessary prerequisite for the initiation of milk protein synthesis and that mammary epithelial cells of primates produce milk proteins at other times of life. That prolactin strongly influences the production of α-lactalbumin under these physiological conditions is evident from our data showing that prolactin is capable of maintaining or stimulating α-lactalbumin production in primate tissue in organ culture (6). Concentrations of prolactin required to produce these results have been lower than those previously used in the rodent studies referred to above, and in some cases prolactin doses were very close to physiological. It is not yet clear that these findings are applicable to the human situation, even though similar observations have been noted in at least 3 other primate species. α-Lactalbumin was found in from 40 to 50% of histologically normal tissues from breast cancer patients, most of whom were menopausal. This observation confirms the fact that human breast tissue is capable of producing α-lactalbumin in women who are not pregnant or lactating. Studies on breast tissue from normally cycling women will be required to determine whether the behavior of human tissue in culture is similar to that of other primates.

These and previous studies (4) provide evidence that α-lactalbumin is less frequently found in breast cancers than in histologically normal mammary tissues from cancer patients. Considering the fact that milk production is a major function of normal breast tissue, these findings are not surprising. If one considers the presence of milk proteins in tissues to be indicative of an effect of prolactin, it might be logical to assume that when α-lactalbumin is found in normal but not cancer tissue the cancer has either lost the ability to produce α-lactalbumin or is less sensitive to prolactin. Greater production of α-lactalbumin in tumors than in normal tissue, on the other hand, might suggest that the tumor is more sensitive to prolactin or is producing this protein autonomously.

Although the significance of the presence of milk proteins in breast cancer has not been established, it is clear that some tumors do contain these substances. In our studies α-lactalbumin was found in slightly more than 20% of tumors, and Schultz and Ebner (14) noted that 55 of 72 tumors studied contained this milk protein. An explanation for this discrepancy is not immediately apparent. The facts that Monaco et al. (8) detected casein in 17% of breast tumors and that specific prolactin binding of greater than 1% was found by Holdaway and Friesen (2) in 20% of tumors may provide some support for the lower figure. There is no definitive clinical evidence that growth or maintenance of milk protein or prolactin receptor-containing tumors is dependent on prolactin. That 3 tumors maintained in organ culture responded to prolactin with increased α-lactalbumin production provides relatively strong evidence that a small number of human tumors are prolactin responsive. Clinical trials with prolactin-lowering ergot drugs in patients with metastatic breast cancer have been disappointing (1, 12). However, the patients studied were selected randomly. The determination of the presence of α-lactalbumin in breast cancers, especially if response to prolactin can be demonstrated in vitro, might provide a useful tool for the selection of patients with prolactin-sensitive tumors. Only objective remissions in such patients treated with prolactin- or growth hormone-inhibiting measures can confirm this hypothesis.

References

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