Disordered Nocturnal Prolactin Regulation in Women with Breast Cancer

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SUMMARY

Mean 24-hr prolactin concentrations were determined in 25 female control subjects, 16 women with benign breast masses, and 23 subjects with breast cancer. This evaluation performed before breast surgery revealed significantly decreased (p < 0.02) nocturnal (12 a.m. to 7 a.m.) prolactin concentrations in 12 postmenopausal breast cancer subjects that contrasted with significantly increased (p < 0.05) nocturnal prolactin levels in five luteal-phase premenopausal women with breast cancers. Prolactin concentrations in patients with benign breast disease were not significantly different from control subjects. Two of the premenopausal breast cancer patients had marked preoperative elevations in their mean 24-hr prolactin levels, and they were two of the three subjects who have since expired. Nocturnal prolactin secretion was significantly decreased (p < 0.03) in four premenopausal breast cancer patients when they were studied 1 year after surgery; however, it remained the same in the eight postmenopausal breast cancer patients similarly evaluated. Although disordered prolactin regulation has been found in these women with breast cancer, its role in the etiology and progression of human cancer is still uncertain.

INTRODUCTION

PRL produces a variety of effects on breast tissue including influences on milk protein synthesis (27), uridine conversion and incorporation into DNA (19), mammary cell sodium transport (5), and breast fatty acid synthetase activity (25). Since several of these processes resemble events that occur in growing tissues, the role of PRL in the production of and/or the promotion of human breast cancer is an issue that is being actively pursued (24). Initial reports concerning the role of PRL in breast cancer were generated from animal work. PRL in concert with insulin was mitogenic for mammary alveoli in vitro (4) and in vivo (17). Medina et al. (14) showed that pituitary isografts (which secrete large amounts of PRL), when transplanted into the inguinal fat pad of host mice, reduced the time for mammary nodule-inducing viruses to become visible in nodule outgrowths. There is also firm evidence that PRL suppressants can influence the appearance (30) and growth (22, 26) of mammary tumors in animals.

For definition of the role of PRL in human breast tumors, PRL secretion has recently been evaluated in women with breast cancer (6, 8, 16, 21, 29). Several reports suggest that PRL levels are normal in women with mammary cancer (6, 16, 21, 29) but elevated in daughters of women with breast cancer (8). Often the reports do not state at what hr the blood was sampled, which is important since serum PRL levels exhibit marked diurnal variation. In addition, since approximately 50% of serum PRL secretion occurs from 12 a.m. to 7 a.m. (11), an attempt to evaluate the contribution of PRL to a disease process would require study of its nocturnal secretion. Therefore, for further definition of PRL secretion in women with breast cancer, we have determined preoperatively hourly serum PRL levels over a 24-hr period in women with breast masses. In this study we have found nocturnal PRL concentrations to be significantly decreased in postmenopausal breast cancer patients and significantly elevated in premenopausal women with breast cancer when compared to age- and weight-matched control subjects.

MATERIALS AND METHODS

Protocol. For definition of the mean 24-hr PRL secretion in patients with breast cancer, 34 women with breast masses were evaluated on the clinical research unit several days prior to breast surgery. Initial study included a history, a physical examination, and a variety of routine clinical and endocrine laboratory tests. Patients ate and slept at their usual times, but their activity was restricted to the hospital room. No patient was on any form of endocrine medication, and drugs were not given during the study. Blood samples were obtained through an indwelling heparinized scalp vein needle placed in a forearm vein to permit hourly sampling without repeated venipuncture. The patients were able to sleep throughout the night, and a sleep record was maintained on each patient during the 24-hr study period. Serum samples were stored at −20° until assayed for PRL.

Patients. Eight of 12 postmenopausal breast cancer patients had infiltrating ductal carcinomas, and 4 subjects had infiltrating lobular carcinomas. Only 1 subject, a patient with an intraductal carcinoma, had more than 4 nodes that contained metastases. The 6 premenopausal patients with breast cancer had the following pathological diagnoses: 2 subjects had infiltrating ductal carcinomas; 3 patients had...
infiltrating lobular carcinomas; and 1 subject had a comedocarcinoma. None of these patients had more than 4 axillary nodes with metastases. Five of these 6 patients were evaluated in the luteal phases of their menstrual cycles. Fifty % of the patients with benign disease of the breast had pathologically confirmed benign adenomas, and the other half had cystic disease of the breast. Several of the patients were readmitted 12 months after their surgeries for repeat endocrine evaluations.

We also evaluated in the Clinical Research Center 16 normal premenopausal and 9 normal postmenopausal women whose ages, heights, and weights were similar to those of breast mass subjects (13). Many of these women were nonpaid volunteers who were recruited through the efforts of the Columbus Cancer Clinic. A control subject or premenopausal breast mass patient was assigned to the luteal-phase group if she was in the second half of her menstrual cycle and if her serum progesterone level was compatible with ovulation. Conversely, follicular-phase individuals were, by history and serum progesterone level, in the first half of their cycles. In addition, we evaluated hourly PRL concentrations in 5 patients with breast cancer whose ovaries had been removed 1 to 7 years before study (2 operations were prophylactic, and 3 were for metastatic breast cancer).

**Assay and Statistical Methods.** PRL was determined by a homologous radioimmunoassay (23) with the use of the NIH standard VLS No. 2 in which the intraassay, and interassay coefficients of variation were less than 11%. All samples from a single patient were run in the same assay.

The PRL-secretory patterns and concentrations over a 24-hr period in normal women and women with breast cancer, before and after surgery, were evaluated by analysis of variance. Two slightly different statistical models were used in the course of this investigation. In the comparison of 24-hr PRL concentrations among the follicular, luteal, and postmenopausal control groups a 3 x 24 analysis of variance with repeated measures on the time factor was used. This model was also used for the examination of the PRL concentrations of postmenopausal cancer and control women in a 2 x 24 and 2 x 12 design. The same design was used in the study of luteal cancer and control women. For the studies of the effects of breast surgery in women with cancer, we used another repeated measures analysis-of-variance model. All of the above analyses were carried out twice, once for the examination of the effects during the daytime and evening periods, and again for the study of nocturnal differences from 12 a.m. to 7 a.m.

By inclusion of hourly data in the analysis of variance, it was possible to study differences in the shape of the PRL response pattern (profile) as well as differences between groups averaged over time. Differences in the profile were determined by the F statistic testing the group-hr interaction.

In order to make the PRL variances more nearly equal from group to group, we applied a log transformation to the original values. Analyses were carried out for both the original values and the log-transformed values. The results obtained from the raw data and the log-transformed data were similar. Decisions in all instances were unchanged except for the significant difference of nocturnal means for the luteal cancer and control groups, for which a \( p < 0.05 \) was obtained by the use of transformed values.

**RESULTS**

**Pre- and Postoperative PRL Concentrations in Women with Breast Masses.** Mean ± S.E. 24-hr and daytime PRL concentrations were similar in normal premenopausal controls (follicular and luteal phase of menstrual cycle) and postmenopausal control women (Tables 1 and 2). Mean nocturnal PRL concentrations, however, were significantly different (\( p < 0.02 \)) between the luteal-phase control subjects (19.1 ± 0.9 ng/ml) and the postmenopausal control patients [13.4 ± 1.3 ng/ml (Chart 1; Tables 1 and 2)].

Twelve postmenopausal women with breast cancer had a significantly diminished (\( p < 0.02 \)) mean nocturnal PRL concentration of 9.3 ± 0.9 ng/ml as compared to the control concentration of 13.4 ± 1.3 ng/ml (Tables 1 and 2; Chart 2). In addition, their nocturnal patterns of secretion were significantly different (\( p < 0.02 \)) (Table 2; Chart 2), since the postmenopausal breast cancer women had blunted nocturnal PRL secretion. The daytime and 24-hr mean PRL levels and patterns of secretion, however, were not different between the control and postmenopausal cancer subjects (Tables 1 and 2). The postmenopausal women with benign breast masses had mean serum PRL levels that fell between the serum PRL concentrations of the control subjects and the breast cancer patients (Table 1). The benign breast mass serum PRL levels, however, were not significantly different from either the malignant or the control serum PRL concentrations.

Eight of 12 postmenopausal patients with breast cancer returned for an endocrine profile approximately 12 months after a modified radical mastectomy. At this time only 1 patient had clinical evidence of metastatic disease. There was no significant difference in mean PRL concentrations for these 8 postmenopausal breast cancer patients, 8.2 ± 0.5 versus 6.8 ± 0.3 ng/ml pre- and postsurgery, and their nocturnal PRL-secretory peaks remained blunted (Table 2).

Five of the 6 premenopausal patients with breast cancer were evaluated in the luteal phase of their menstrual cycles. In contrast to the postmenopausal breast cancer patients, their nocturnal PRL concentrations were significantly higher (\( p < 0.05 \)) than those found for the control subjects (Tables 1 and 2; Chart 3). The daytime and 24-hr mean PRL levels and patterns of secretion, however, were not different between the luteal-phase cancer patients and the control subjects (Tables 1 and 2; Chart 3). In addition, no significant differences in mean serum PRL concentrations were noted between the luteal-phase benign breast disease patients and either the control subjects or the breast cancer patients.

When 4 of the luteal cancer patients were restudied approximately 12 months later, their nocturnal PRL levels were significantly lower (\( p < 0.05 \)) than their nocturnal PRL concentrations had been prior to the breast surgery (21.1 ± 1.3 versus 12.3 ± 0.8 ng/ml pre- and postsurgery, respectively) (Table 2; Chart 4). Their mean daytime and 24-hr PRL concentrations were similar pre- and postoperatively.
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Table 1

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>Fasting</th>
<th>24 hr</th>
<th>8 a.m.-11 p.m.</th>
<th>12 p.m.-7 a.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular*</td>
<td>7</td>
<td>12.2 ± 2.3</td>
<td>11.3 ± 1.0</td>
<td>9.0 ± 0.8</td>
<td>15.9 ± 1.7</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>14.3 ± 2.6</td>
<td>13.6 ± 3.2</td>
<td>10.5 ± 3.2</td>
<td>19.8 ± 3.5</td>
</tr>
<tr>
<td>Benign</td>
<td>1</td>
<td>13.8</td>
<td>15.3 ± 0.9</td>
<td>13.1 ± 0.4</td>
<td>19.8 ± 1.9</td>
</tr>
<tr>
<td>Cancer</td>
<td>5</td>
<td>10.1 ± 1.6</td>
<td>14.1 ± 1.3</td>
<td>9.9 ± 0.9</td>
<td>22.5 ± 2.8</td>
</tr>
<tr>
<td>Luteal*</td>
<td>9</td>
<td>13.4 ± 1.8</td>
<td>13.1 ± 0.7</td>
<td>10.1 ± 0.7</td>
<td>19.1 ± 0.9</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>9.6 ± 2.3</td>
<td>8.7 ± 1.6</td>
<td>7.3 ± 1.3</td>
<td>11.4 ± 2.1</td>
</tr>
<tr>
<td>Benign</td>
<td>12</td>
<td>7.4 ± 0.9</td>
<td>7.5 ± 0.7</td>
<td>6.5 ± 0.8</td>
<td>9.3 ± 0.9*</td>
</tr>
<tr>
<td>Cancer</td>
<td>5</td>
<td>8.9 ± 1.2</td>
<td>8.5 ± 0.7</td>
<td>7.8 ± 0.7</td>
<td>10.0 ± 0.6*</td>
</tr>
</tbody>
</table>

| Post* oophorectomy | 5 | 8.9 ± 1.2 | 8.5 ± 0.7 | 7.8 ± 0.7 | 10.0 ± 0.6* |

* Phase of menstrual cycle when evaluated.
* Mean ± S.E.
° See Table 2.
* p < 0.004, postoophorectomy versus postmenopausal control.

Table 2

Log transformed analysis of variance summary for 24-hr PRL concentrations in normal women and women with breast cancer

<table>
<thead>
<tr>
<th>Comparison groups</th>
<th>Mean av. (daytime)</th>
<th>Pattern (daytime)</th>
<th>Mean av. (nocturnal)</th>
<th>Pattern (nocturnal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls: postmenopausal vs. follicular</td>
<td>NS*</td>
<td>NS</td>
<td>p &lt; 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Controls: postmenopausal vs. luteal</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.02</td>
<td>p &lt; 0.02</td>
</tr>
<tr>
<td>Postmenopausal cancer vs. postmenopausal control</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Postmenopausal cancer: before vs. after surgery</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Luteal cancer vs. luteal control</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Luteal cancer: before vs. after surgery</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

* NS, not significant.

Only 1 patient with breast cancer was evaluated in the follicular phase of the menstrual cycle (Table 1). Her PRL levels were similar to those obtained in at least 1 patient with a benign breast lesion evaluated in the follicular phase of the menstrual cycle.

Serum PRL Concentrations following Oophorectomy in 5 Patients with Breast Cancer. Mean serum PRL concentrations and daytime and nocturnal patterns evaluated in 5 patients, 1 to 7 yr postoophorectomy, were similar to the PRL levels of the postmenopausal breast cancer patients (Table 1), without oophorectomy. In addition, their nocturnal PRL concentrations from 12 a.m. to 7 a.m. were significantly different (p < 0.004) from those of the postmenopausal control women (Table 1).

DISCUSSION

We have demonstrated that postmenopausal women with breast cancer have diminished nocturnal PRL concentrations and that this abnormality was present before surgery and was not altered by mastectomy (Chart 2). Blunting or absence of the nocturnal PRL peak has also been reported by our laboratory (12) and by others in patients with pituitary tumors (1) and Cushing's syndrome (9). In these subjects a hypothalamic and/or pituitary defect has been considered responsible for the disordered PRL regulation. However, one can only speculate concerning the mechanism responsible for the decreased nocturnal PRL secretion in the postmenopausal breast cancer patients until the physiology of the normal sleep-related augmentation of PRL secretion is better understood.

In contrast to the postmenopausal breast cancer subjects, the 5 premenopausal breast cancer patients evaluated in the luteal phase of their menstrual cycles had a significantly increase in their nocturnal PRL concentrations when compared to age- and weight-matched control subjects also studied during the luteal phase (Table 1, Chart 3). This difference in nocturnal PRL levels was contributed to primarily by 2 subjects who had many elevated serum PRL values during the 24-hr preoperative study. These 2 patients...
Chart 1. Twenty-four-hr mean PRL concentrations in 16 normal premenopausal and 9 normal postmenopausal women. Note increased nocturnal PRL secretion in the luteal-phase women compared to the postmenopausal women.

Chart 2. Decreased 1200 to 0700 mean ± S.E. nocturnal PRL levels in 12 postmenopausal women with breast cancer (CA).

Chart 3. Significantly increased mean ± S.E. nocturnal PRL secretion was noted in the luteal phase breast cancer (CA) patients.

Chart 4. One yr after mastectomy these 4 premenopausal women with breast cancer (CA) had significantly lower nocturnal PRL levels. ADM, admission.

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with hyperprolactinemia were 2 of 3 patients who have expired from metastatic breast cancer since this study was initiated. Whether excessive PRL secretion was involved in the accelerated growth of the breast cancer or was reflective of some nonspecific stress in these 2 patients is not clear.

The excessive nocturnal PRL levels found in our premenopausal breast cancer patients stand in marked contrast to the decreased nocturnal PRL levels of our postmenopausal breast cancer women. This difference in PRL levels probably can be attributed at least in part to differences in serum estrogen and/or progesterone concentrations. Accordingly, the luteal-phase control subjects had significantly greater nocturnal PRL secretion than did the postmenopausal control patients (Tables 1 and 2; Chart 1). Exogenous estrogen administration can increase the duration of the nocturnal PRL peak (28). Finally, nocturnal PRL secretion in 5 premenopausal women who had received oophorectomies was similar to that found in the postmenopausal cancer patients.

In addition to sex steroids, other factors must be contributing to the different PRL patterns in the pre- and postmenopausal breast cancer patients. There was a significant decrease in PRL secretion in the 4 luteal-phase breast cancer women when restudied 1 year later (Chart 4). During the intervening year 1 patient had received an oophorectomy for metastatic disease, but none of the other 3 had changed clinically from their preoperative evaluations. This decrease in PRL secretion in the premenopausal cancer patients after surgery stands in contrast to the nonsignificant differences in PRL secretion found when 8 postmenopausal breast cancer patients were reevaluated 1 yr after mastectomy.

Emotional factors may have contributed to the preoperative elevation in serum PRL levels in several of the premenopausal cancer patients. Stress had been reported to increase serum PRL levels (15), and other groups have indicated that certain premenopausal women with breast cancer will have abnormally elevated fasting serum PRL concentrations (16, 21). The serum PRL levels in the benign breast patients did not support the "stress" hypothesis, however, since these samples were acquired before their diagnoses were known, and they were presumably as anxious as the cancer subjects. Also, the postmenopausal breast cancer patients were under equal stress, but their PRL levels were lower than those of the control subjects. Therefore stress may have contributed to the differences in PRL levels in the premenopausal patients between their first and second admissions (Chart 4), but one must also consider the possibility that removal of the breast mass in these patients influenced their serum PRL levels.
Several recent reports suggest that chronic disease can alter endocrine secretion and metabolism (3, 20, 31). There was no objective or subjective evidence, however, that the breast cancer patients were more ill than the patients with benign breast disease, and yet only the former group demonstrated significant serum PRL abnormalities.

Disturbed hypothalamic-pituitary function has been reported in patients with mammary cancer as p.o. glucose ingestion paradoxically stimulates rather than suppresses growth hormone secretion (2). Abnormal nocturnal PRL secretion in those patients with malignant breast lesions was probably related to direct effects of the cancer and/or the influence of chronic illness on the hypothalamic-pituitary axis.

Clinically detectable breast cancer is not required for decreased nocturnal PRL secretion, however, since similar PRL secretion was noted when 8 of the 12 postmenopausal breast cancer patients were evaluated 1 year after removal of the breast mass, at which time only 1 patient had clinically detectable metastatic disease.

An obvious issue raised by this study was the relationship of the disordered PRL secretion to the development and/or growth of the breast mass. Although most animal studies suggest that PRL promotes the development and/or growth of mammary cancer (26), there are some data to suggest that it has no influence (29), and other reports have suggested that it may protect against tumor implantation (7, 18). A recent report by Kwa et al. (10) suggests that daughters of mothers with breast cancer may have increased nocturnal PRL secretion.

Although the mean nocturnal PRL levels of the control and breast cancer patients in this study were different, the hourly PRL level of any breast cancer patient was usually within the normal range. In addition, altered nocturnal PRL secretion in these breast cancer patients may have occurred secondarily to the development of the breast cancer. Only future study will determine whether these alterations in serum PRL concentrations could influence the progression of the disease.

Therefore, on the basis of this study, postmenopausal women with breast cancer appear to have diminished nocturnal PRL secretion, and some premenopausal breast cancer patients evaluated in the luteal phase of the menstrual cycle will have elevated serum PRL concentrations. The possible contribution of these changes to the development of and/or the progression of female breast cancer requires further study.

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REFERENCES

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