Increased Incidence of Mammary Tumors in the Female Rat Grafted with Multiple Pituitaries

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SUMMARY

The purpose of this study was to evaluate the role of pituitary homografts on mammary tumorigenesis in the female Sprague-Dawley rat. Five pituitaries were grafted unilaterally over the inguinal, abdominal, and thoracic regions of the mammary gland and two pituitaries were grafted underneath the kidney capsule of each rat. Pituitaries were obtained from donor rats of varying ages but of the same sex and strain as the recipients. The pituitaries were transplanted to mammary tumor-free 2-month-old nulliparous rats (Group I), 8-month-old nulliparous rats (Group II), and 8-month-old multiparous rats (Group III). Mammary tumor-free nongrafted rats of comparable age and breeding status served as controls for each group. Number and percentage of rats with mammary tumors 9 months after pituitary grafting were: Group I, 13/45 (30%); Group II, 9/12 (75%); and Group III, 8/13 (61%); this contrasted with 2/27 (7%), 1/12 (8%), and 3/16 (19%) in the nongrafted controls. Since pituitary homografts secrete relatively large amounts of prolactin and small amounts of all other pituitary hormones, the present results indicate that an additional source of prolactin significantly enhances mammary tumorigenesis in the female rat.

INTRODUCTION

Spontaneous mammary tumors are common in female rats allowed to live a normal life-span (16). It is well established that the incidence of mammary tumors in rats can be increased by administration of estrogens (16) and decreased by ovariectomy (5). Numerous investigations have demonstrated that estrogens can act both on the hypothalamus and directly on the pituitary to promote synthesis and release of prolactin (13). Furth (7) and others (17) have suggested that the primary role of estrogens in mammary tumorigenesis is promotion of pituitary prolactin secretion. Transplantation of grafts of pituitary glands is a well-established means of eliciting additional endogenous prolactin secretion. These grafts, free from direct hypothalamic influence, secrete increased amounts of prolactin and very little adrenocorticotropic hormone, thyroid-stimulating hormone, growth hormone, follicle-stimulating hormone, or luteinizing hormone (13). It was our purpose, therefore, to evaluate the effects of increased amounts of circulating prolactin elicited by pituitary homografts on mammary tumorigenesis in the female rat.

MATERIALS AND METHODS

All animals used in this study were female Sprague-Dawley rats (Spartan Animal Farms, Inc., Haslett, Mich.), maintained in an air-conditioned room at a temperature of 75 ± 2°F. and fed ad libitum for the duration of this investigation. Pituitary donor rats were aged breeder rats discarded from the breeding colony and weanling rats of the same sex and strain as the host animals.

The donor rats were decapitated and the whole pituitaries were excised and immediately transplanted to the recipient. Five pituitaries were grafted s.c. over the right inguinal, abdominal, and thoracic mammary glands and 2 pituitaries were grafted underneath the kidney capsule of the right kidney of each rat. Grafting of pituitaries s.c. involved a medial abdominal incision in the skin and insertion of the pituitary s.c. in the mammary region with the aid of a mall probe. Grafting of pituitaries under the kidney capsule was accomplished by a dorsal medial incision in the skin and bilateral incisions in the lumbo-dorsal muscle, exposing the kidneys. With the aid of a dissecting forceps and a mall probe, the pituitary was eased under the kidney capsule to the side opposite the capsule incision. Pituitaries were grafted to the following groups: Group IB, sixty 2-month-old nulliparous rats; Group IIB, fifteen 8-month-old nulliparous rats; and Group IIB, twenty 8-month-old multiparous rats. Thirty 2-month-old nulliparous nongrafted rats (Group IA), fifteen 8-month-old nulliparous nongrafted rats (Group IIA), and twenty 8-month-old multiparous nongrafted rats (Group IIIA) served as controls. One-half of the grafted rats of each group were recipients of pituitaries from aged breeder rats and the remaining half were recipient of pituitaries from weanling rats.

Before grafting, all rats were free of palpable mammary tumors. After grafting the animals were examined at varying intervals, as frequently as once weekly and no less than once monthly, for palpable mammary tumors. Mean latency
period of tumor appearance was based on the average number of days from pituitary grafting to detection of each palpable tumor. When a mammary tumor grew to a diameter of approximately 3 cm, about 90% of the tumor was aseptically excised, fixed in Bouin's fluid, and stained with hematoxylin and eosin for histological evaluation. The remaining tumorous tissue was left attached to the fascia and allowed to resume growth. Approximately once a month, each rat was given an injection i.m. of Bicillin Fortified (Wyeth Laboratories, Philadelphia, Pa.) to minimize respiratory complications.

One mammary tumor which originated in a rat in Group 1B and was considered typical with regard to growth and histological characteristics was removed in toto and transplanted to six 3-month-old nulliparous intact rats bearing 4 pituitary transplants, five 3-month-old nulliparous ovariectomized rats bearing 4 pituitary transplants, six 3-month-old intact nulliparous nongrafted rats, and five 3-month-old ovariectomized nulliparous nongrafted rats. The transplantation procedure involved aseptic removal of the tumor, subsequent mincing of the tumor in Medium 199 (Difco Laboratories, Detroit, Mich.) with iris scissors, and injection of 0.2 ml of the tumor mince s.c. in the dorsum of each rat. After tumor transplantation each rat was palpated weekly for appearance of the transplanted mammary tumor.

Animals of Group I and Groups II and III were sacrificed 13 and 11 months, respectively, after pituitary grafting. Anterior pituitaries of animals of Group I were excised and weighed. Ovaries from the animals of Group I were excised and fixed in Bouin's fluid for histological evaluation. Inguinal mammary glands of animals of all groups were excised, spread flat on cork, fixed in Bouin's fluid, and stained by a standard procedure for whole-mount evaluation. Each mammary gland was rated for development as follows: 1, few ducts, few or no end buds; 2, moderate duct growth, moderate number of end buds; 3, numerous ducts and branches, many end buds; 4, numerous ducts and branches, minimum lobuloalveolar growth; 5, numerous ducts and branches, moderate lobuloalveolar growth; and 6, numerous ducts and branches, dense lobuloalveolar growth as in advanced pregnancy. To avoid bias, evaluation was done without knowledge of previous treatments. Daily vaginal smears were performed on animals of Group I for 30 days approximately 4 weeks after pituitary grafting to ascertain the effectiveness of the graft.

Significance of differences between mean body weights, pituitary weights, and mammary gland ratings were calculated by Student's t test.

RESULTS

Transplantation of pituitaries to 2-month-old nulliparous rats, 8-month-old nulliparous rats, and 8-month-old multiparous rats resulted in a marked increase in incidence of mammary tumors 3, 6, and 9 months after pituitary grafting (Chart 1). Mammary tumor incidence 9 months after pituitary grafting of these groups was 30, 75, and 61%, respectively, in contrast to an incidence of 7, 8, and 19% in the nongrafted controls. The tumorigenic effects of the grafts were most apparent in the 8-month-old nulliparous (Group II) and multiparous (Group III) rats.

Chart 1. Effects of pituitary homografts on mammary tumor incidence 3, 6, and 9 months after grafting. Rats of Group 1B (nulliparous) were grafted with pituitaries at 2 months of age. Rats of Groups IIB (nulliparous) and IIIB (multiparous) were grafted with pituitaries at 8 months of age. Cont., control.
Mean latency period of mammary tumor appearance was considerably shortened in rats bearing pituitary grafts (Table 1). Mammary tumors appeared earlier in 2-month-old grafted nulliparous rats (Group IB) than in the 2-month-old non-grafted controls (Group IA). In addition, mammary tumors developed sooner in grafted 8-month-old nulliparous rats (Group IIB) than in 2-month-old grafted nulliparous rats (Group IB). Several of the graft-induced mammary tumors were allowed to grow continuously and reached a large size (6 cm). Despite the fact that the pituitary grafts were transplanted unilaterally, mammary tumor development was bilateral. No metastases of tumors were observed. Rarely did more than 1 tumor develop per rat. No difference was noted in tumor incidence between rats grafted with pituitaries from aged breeder rats and those grafted with pituitaries from weanling rats.

Histological evaluation of the mammary tumors revealed 4 basic types (Figs. 1 to 4). The most common tumor type in the grafted rats was a well-differentiated adenoma (Fig. 1), followed by a moderate frequency of adenomas comprised of varying amounts of connective tissue (Fig. 2). A lesser frequency was found of predominantly connective tissue tumors containing varying amounts of glandular tissue, and an occasional tumor consisted almost entirely of connective tissue (Fig. 3). A mammary adenocarcinoma, similar histologically to the type induced with hydrocarbon carcinogens (9), was observed only once in a portion of an adenomatous type tumor (Fig. 4). In the nongrafted controls, the same general spectrum of tumors was observed, but these tumors showed a slightly increased accumulation of connective tissue elements. When the bulk (approximately 90%) of the tumor was excised, the histological characteristics of the remaining vital tumorous tissue was not appreciably altered. Large portions of several graft-induced tumors were excised 4 times during this study. A few of these tumors showed a slight shift toward accumulation of connective tissue elements.

Transplants of a mammary tumor excised from a pituitary grafted rat grew and took (5/6) exceptionally well when transplanted to intact rats bearing pituitary grafts. The rate of growth of the transplanted tumor appeared to be only slightly less than its growth in the donor animal. In contrast, the number of takes of the transplanted tumor in intact controls, ovariectomized controls, and pituitary-grafted ovariectomized rats was 3/6, 0/6, and 1/5, respectively. In addition, the rate of growth of the transplantable tumor in these groups was extremely slow, as the developing tumors were barely palpable 4 months after transplantation.

The effects of pituitary homografts on mammary gland development are illustrated in Table 1 and Figs. 5 and 6. Marked mammary gland stimulation was observed in all rats bearing pituitary homografts in contrast to the nongrafted controls. Many of the rats bearing the pituitary grafts showed extensive lobuloalveolar growth characteristic of rats in advanced pregnancy (Fig. 6). Numerous hyperplastic alveolar nodules were observed in the mammary glands of all the rats, but particularly in the older rats (Figs. 7 and 8). The pituitary-grafted rats appeared to have more hyperplastic alveolar nodules than the respective controls, but, due to the extensive lobuloalveolar development observed in the grafted rats, it was occasionally difficult to differentiate between normal and hyperplastic alveolar structures.

The pituitary homografts grafted beneath the kidney capsule were all recovered, whereas only about one-half of the pituitaries grafted s.c. could be located. Incomplete recovery of the grafted pituitaries may be related, at least in part, to the relative lack of homogeneity among the rats of

Table 1

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Average mammary gland ratingsa,b</th>
<th>Mean latency period of mammary tumor appearance (days)(a)</th>
</tr>
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<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
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<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Controls</td>
<td>3.6 ± 0.1 (a)</td>
<td>260–384</td>
</tr>
<tr>
<td>B. Pituitary homografts</td>
<td>4.9 ± 0.2 (b)</td>
<td>78–398</td>
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<td></td>
<td></td>
<td>247 ± 23</td>
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<tr>
<td>Group II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Controls</td>
<td>3.7 ± 0.1 (a)</td>
<td>295</td>
</tr>
<tr>
<td>B. Pituitary homografts</td>
<td>5.1 ± 0.1 (b)</td>
<td>50–295</td>
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<tr>
<td></td>
<td></td>
<td>156 ± 31</td>
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<tr>
<td>Group III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Controls</td>
<td>3.8 ± 0.1 (a)</td>
<td>119–331</td>
</tr>
<tr>
<td>B. Pituitary homografts</td>
<td>5.0 ± 0.2 (b)</td>
<td>79–274</td>
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<td>202 ± 23</td>
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\(a\)Mean ± S.E.  
\(b\)a/b, \(p < 0.01.\)
this strain. All pituitary homografts, whether obtained from aged breeder or weanling rats and whether transplanted s.c. or beneath the kidney capsule, were considerably smaller than the in situ pituitary. Vaginal smears of the pituitary-grafted rats indicated a prolonged diestrus, interrupted by only an occasional period of estrus. Ovaries from these rats contained large and numerous corpora lutea.

The in situ pituitaries of rats bearing pituitary homografts were significantly reduced in weight (14.1 ± 0.8 mg) when compared with the pituitaries of the nongrafted controls (21.0 ± 1.3 mg). Average body weights of the grafted rats (425 ± 15 g) were significantly greater than the nongrafted controls (369 ± 9 g). Increase in body weight and reduced in situ pituitary weight of female rats grafted with multiple pituitaries has previously been reported (22).

**DISCUSSION**

It is clear from the results of this study that intact female rats grafted with multiple pituitaries show a marked increase in incidence of mammary tumors in contrast to nongrafted controls, female rats grafted at 8 months of age develop mammary tumors sooner and in greater numbers than rats grafted at 2 months of age, and pituitary grafts produce a marked concurrent stimulation of normal mammary development and growth. Thus, these observations strengthen the concept proposed by Furth (7) that prolactin is the principal hormone involved in development of mammary tumors in the rat. It was reported previously that prolactin is a major hormone in promoting growth of carcinogen-induced rat mammary tumors. Transplantable pituitary tumors (10), pituitary homografts (19), reserpine (21), and hypothalamic (median eminence) lesions (20), all of which increase circulating prolactin levels, enhance carcinogen-induced mammary tumor growth in rats.

Continuous stimulation of mammary growth in mice by pituitary grafting also results in a high incidence of spontaneous mammary tumors. This was demonstrated by Liebelt and Liebelt (11), using single pituitary isografts, and by Muhlbock and Boot (15), using multiple pituitary isografts in intact mice free of the mammary tumor virus. Subsequent studies, further emphasizing the key role of prolactin in mammary tumorigenesis, showed that pituitary isografts significantly increased mammary tumor incidence in male (8) and ovariectomized mice (1).

In the present study, there was a marked increase in number of mammary tumors in rats grafted at 8 months of age in contrast to those grafted at 2 months of age. It has been reported (6) that aged rats, in contrast to younger rats, have increased numbers of mammary gland anomalies morphologically similar to the hyperplastic alveolar nodules previously described in the mouse mammary gland (3). It is conceivable that the increased mammary tumor incidence observed in the older grafted rats is a result of an increase in number of hyperplastic alveolar nodules receptive to the tumorigenic effects of the grafts. The appearance of more than 1 mammary tumor in each grafted rat was rare, despite the presence of numerous hyperplastic nodules, particularly in the older rats. Whether or not prolactin has a significant role in the etiology of these nodules or whether these nodules are involved in mammary tumorigenesis in the rat remains to be determined.

Pituitary grafts were previously reported to induce mammary stimulation in intact, ovariectomized, and hypophysectomized-ovariectomized female rats (2). Prolactin and, perhaps to a lesser extent, growth hormone appear to be the principal hormones involved in this process, since injections of these hormones into hypophysectomized-ovariectomized-adrenalecetomized rats result in marked mammary stimulation (18). Injections of prolactin alone into such rats also significantly enhanced mammary development, whereas injections of growth hormone alone had little or no significant effect. Recently, an in vitro study indicated that only insulin and prolactin were necessary to induce lobulo-alveolar development of rat mammary tissue (4). Although the role of growth hormone in normal mammary development appears to be secondary to prolactin, we cannot rule out the possibility that secretion of this hormone by the multiple pituitary grafts was an important factor in mammary tumorigenesis. Moon et al. (14) reported that injections of growth hormone in female rats for periods up to 485 days resulted in increased incidence of mammary tumors.

The precise role of estrogens in mammary tumorigenesis is at present uncertain. It is probable that administered estrogens promote growth of mammary tumors primarily by enhancing pituitary prolactin secretion. The results of the present study demonstrate that increased amounts of circulating prolactin, maintained for several months, markedly increases the development of mammary tumors. Whether or not prolactin is instrumental in inducing the neoplastic transformation or only promotes growth of those cells already transformed cannot be ascertained from this study.

It is interesting that administration of estrogens to rats for extended periods of time results in a high incidence of mammary tumors which are primarily adenocarcinomas (16). The tumor type observed in this study was almost exclusively a well-differentiated adenoma with varying amounts of connective tissue. Prolactin and its luteotropic product progesterone have been reported primarily to stimulate development and growth of alveolar structures, whereas estrogen acts primarily on the ductal elements of the mammary gland of rats (12). It is conceivable that in the rat mammary gland estrogen is involved primarily in carcinomatous transformation and progesterone is involved in adenomatous transformation, while prolactin is the principal hormone stimulating growth of either of these types of transformed cells.

**REFERENCES**

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Figs. 1—4. X 100. Histological sections of mammary tumors from pituitary-grafted female rats.

Fig. 1. Adenoma, most common.

Fig. 2. Fibroadenoma, intermediate frequency.

Fig. 3. Fibroma, least common.

Fig. 4. Adenocarcinoma, observed only once with a well-differentiated adenoma.

Figs. 5—8. X 5. Whole mounts of mammary glands.

Fig. 5. A fifteen-month-old nongrafted nulliparous rat.

Fig. 6. A fifteen-month-old pituitary-grafted nulliparous rat.

Fig. 7. A nineteen-month-old nongrafted nulliparous rat (note numerous hyperplastic alveoli).

Fig. 8. A nineteen-month-old nongrafted multiparous rat.
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