Prolactin and Murine Mammary Tumorigenesis: A Review

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Summary

It is unequivocally shown that prolactin is an influential hormone in murine mammary tumorigenesis. The Berenblum hypothesis (7), a well-known theoretical model of tumorigenesis that depicts this oncogenic process as a two-step mechanism, i.e., initiation and promotion, is a conceptual scheme in which the action of prolactin in mammary tumorigenesis may be understood. According to this conceptual model, prolactin would participate in both the initiation and promotion steps of mammary tumorigenesis. In the initiation phase, variations in prolactin secretion appear to influence the metabolism of the mammary epithelium, so that the epithelium would be either more receptive to or refractory to an initiating agent (e.g., chemical carcinogens, physical carcinogens, oncogenic viruses, etc.), i.e., a permissive action. In the promotion phase, prolactin may act as either a promoter or an antipromoter of the "transformed" mammary epithelium. In promotion, the hormone may either directly or indirectly (via the ovary) stimulate mitotic activity of the "transformed" epithelium. In antipromotion the hormone, in the presence of requisite hormones (e.g., glucocorticoids), may synergistically induce differentiation (e.g., lactation) in the "transformed" epithelium. A tumor would result in the former (promotion) but not in the latter (antipromotion) case.

Whether or not prolactin is significantly influential in human breast tumorigenesis remains to be determined. This is an extremely important area of research which is justifiably receiving increased attention. For if prolactin can be shown to influence human breast epithelium in a manner similar to its effect on rodent mammary tissue, then prophylactic and/or chemotherapeutic control of human breast tumorigenesis may be feasible by appropriate drug-mediated prolactin suppression.

Introduction

In the past few years, there has been an unparalleled surge into the investigation of prolactin as a potentially important hormone in human breast tumorigenesis. The impetus for this line of investigation is derived, at least in part, from the results of a number of studies demonstrating rather convincingly that prolactin is an important hormonal factor in both the development and the growth of murine mammary tumors. This report is a literature review focusing on the role of prolactin in tumorigenesis of the rodent mammary gland. We feel that such a review is timely and that hopefully it will provide a basis for future experimentation. We will discuss: (a) the promotion by prolactin of murine mammary tumorigenesis; (b) the inhibition by prolactin of murine mammary tumorigenesis; (c) the relationship between prolactin and ovarian steroids in murine mammary tumorigenesis; and (d) the possible role of prolactin in human breast tumorigenesis. Neuroendocrine mechanisms controlling the secretion of this hormone (33, 61, 88, 142, 143, 265) and the diverse etiological factors that may influence mammary tumorigenesis (17, 35, 64, 78, 113, 124, 179, 183) have been discussed in a number of recent reviews and therefore will not be discussed here.

Promotion by Prolactin of Mammary Tumorigenesis

Mice. The hormonal control of mammary tumorigenesis in the mouse has been the subject of numerous inquiries. As early as 1916, it was shown by Lathrop and Loeb (111) that the hormonal changes of pregnancy were conducive to the development of mammary neoplasias. Subsequently, in 1939, Loeb and Kirtz (121) and, in 1959, Mühlbock and Boot (154) demonstrated that the grafting of multiple pituitary isografts to mice markedly increased the incidence of mammary tumors. The drug utilized was CB-154, a potent suppressor of prolactin secretion in situ and/or grafted pituitary isografts. This drug, at least in mice, does not markedly interfere with the secretion of other anterior pituitary hormones.

A recent study by Yanai and Nagasawa (259) has further substantiated that prolactin is the key hormone secreted by these grafts in murine mammary tumorigenesis. They showed that drug-induced suppression of prolactin secretion in pituitary isograft-bearing mice resulted in a sharp reduction in the incidence of mammary tumors. The drug utilized was CB-154, a potent suppressor of prolactin secretion in situ and/or grafted pituitaries. Additional support for the concept that prolactin is the key pituitary hormone in mouse...
mammary tumorigenesis comes from the report of Boot et al. (12), who showed that daily administration of ovine prolactin to mice increased the incidence of mammary tumors; from the study of Lacassagne and Duplan (110), who showed that the administration of the tranquilizer reserpine to mice resulted in a higher incidence of mammary tumors; and from the report of Bruni and Montemurro (21), who showed that induced hypothalamic lesions increased mammary tumor incidence. Reserpine treatment and hypothalamic lesions (median eminence) sharply increase the secretion of prolactin (9, 122, 143, 228, 252). Although it is certain that the pituitary isograft secretes large amounts of prolactin, growth hormone is also secreted by these grafts (141, 260). Since the quantity of growth hormone secreted by these grafts is considerably less than that by the in situ pituitary, it is probable that the amount of growth hormone released from these grafts is inconsequential to murine mammary tumorigenesis. This is particularly true when only a single pituitary is grafted, a procedure that sharply increased mammary tumorigenesis (119).

From the foregoing, it is clear that, in many strains of mice, if prolactin secretion is chronically raised above normal levels then invariably there will be an increase in the incidence of mammary tumors. Is the inverse true, i.e., will a secretory level of prolactin that is below normal influence the development of mammary tumors in mice? The answer to this question was recently provided by Welsch and Gribler (241), who showed that chronic treatment of young nulliparous C3H/HeJ mice with CB-154 virtually prevented the development of mammary tumors. Over one-fourth of the controls (24 of 90) developed mammary tumors, whereas only 1 of 90 of the ergot-treated mice developed a mammary tumor. In a subsequent study, using another prolactin-suppressing drug, the ergoline derivative 6-methyl-8-β-ergoline-acetonitrile, mammary tumorigenesis was also sharply reduced (238, 242). It appears, therefore, that not only may prolactin be an important hormonal stimulant of mammary tumorigenesis but it may also be an essential hormone for neoplastic transformation of the mouse mammary gland.

Although the above-cited studies demonstrated a relationship between prolactin secretion and mammary tumorigenesis in mice, the measurement of pituitary and/or blood levels of this hormone in mice as a predictive test for mammary tumor susceptibility has not yielded a significant correlation. For example, there were few consistent patterns observed in pituitary or blood levels of prolactin in either virgin, pregnant or lactating, high-, or low-mammary-tumor strains of mice (178, 212, 215, 258), although some specificity in the pituitary secretion of prolactin and growth hormone was observed in mice of different inbred strains (213). Furthermore, no difference in serum placental lactogen levels during pregnancy was noted in the high-mammary-cancer C3H/HeJ and low-mammary-cancer C57BL/6 mice, although mammary growth was much more conspicuous in the former than in the latter mouse strain (257). These results do not negate the importance of prolactin in mammary tumorigenesis but suggest that a moderate rate of prolactin secretion may be sufficient to permit tumor development. The results also imply that the sensitivity of the mammary gland to prolactin is another important factor in mammary tumorigenesis.

Mammary sensitivity to prolactin as a potentially important factor in mammary tumorigenesis was recently investigated by Nagasawa et al. (176). Prolactin-induced mammary growth was compared in virgin females of a high-mammary-cancer strain of mouse (SHN) and a low-mammary-cancer strain (SLN). After 20 days of prolactin stimulation via pituitary isografts, the degree of mammary growth was much more marked in the high-mammary-cancer mice than in the low-cancer strain. Blood prolactin levels were similar in both groups. A similar difference in mammary susceptibility to prolactin was also observed in C3H/HeJ and C57BL/6 mice (172). In accord, membrane preparations from C3H mice have been reported to bind more human placental lactogen, a hormone chemically and physiologically very similar to prolactin, than did similar preparations obtained from C57BL mice (203). Although the factors that influence the sensitivity of mammary epithelium to prolactin remain obscure, it is apparent from the foregoing that the sensitivity of the mammary gland to prolactin is an additional etiological factor in mouse mammary oncogenesis.

HAN of the mammary gland have been described by a number of investigators and established as the precursors of many of the mammary tumors that occur in the mouse (8, 47, 135–138, 216). Morphologically similar lesions have also been described in the human breast (95, 233). In the mouse (e.g., C3H, BALB/c), HAN are considered precancerous because, when transplanted to gland-free fat pads of syngeneic hosts, tumors arise from them much more frequently, and in less time, than from normal mammary tissue. The hormonal responsiveness of these hyperplasias is shown in studies that demonstrated that hypophysectomy or ovariectomy-adrenalectomy of mammary tumor-susceptible mice suppressed their development and growth (8). Furthermore, their transformation to a carcinoma generally required hormones from the pituitary (54, 259). Recently, it was reported that prolactin appeared to be the principal pituitary hormone in the development, maintenance, and transformation of HAN (241, 255, 259). For example, chronic treatment of nulliparous or multiparous C3H mice with CB-154 resulted in a reduction in incidence of HAN (nulliparous mice), a reduction in number of HAN (multiparous mice), and a sharp reduction in mammary tumor incidence (nulliparous or multiparous mice) (241). These results underscore the striking sensitivity of the earlier developmental stages of spontaneous mouse mammary tumorigenesis to prolactin.

Although the developmental stages of mouse mammary tumorigenesis appear to be markedly influenced by secretory levels of prolactin, the advanced spontaneous mammary tumors in most strains of mice appear to be prolactin independent. Treatment of C3H/HeJ female mice bearing advanced spontaneous mammary tumors with the prolactin suppressor CB-154 did not significantly influence the growth of these tumors (241). Furthermore, the growth of these tumors was not significantly influenced by the chronic administration of prolactin (161) or pituitary isografts (173). It appears, therefore, that spontaneous C3H mouse mammary neoplasms gradually but fairly consistently evolve from a stage of prolactin responsiveness to a stage of pro-
Prolactin and Mammary Tumorigenesis

The role of prolactin in the development and growth of carcino-gen-induced mammary tumors in mice has not been as extensively investigated and consequently is less clearly understood. It has been known for a number of years that the hormonal conditions of pseudopregnancy and pregnancy generally enhance chemical carcinogenesis in many strains of mice (126, 127). The secretion of prolactin is periodically increased during these physiological states (1, 109, 157, 192). Since the endocrinic status of a mouse during pseudopregnancy or pregnancy is very complex, it remains to be determined whether or not prolactin is the key oncogenic hormone during this process. Transplantation of prolactin-secreting pituitary tumors to mice has been another method used to evaluate the role of prolactin in chemical carcinogenesis of the mouse mammary gland. Haran-Ghera (75) transplanted prolactin-secreting pituitary tumors to female mice (C57L x A/He F) preceding, during, and/or following treatment with MCA. The secretions from the transplanted pituitary tumors markedly increased the incidence of mammary tumors to 35% when given prior to and during carcinogen treatment. Treatment with the carcino-gen alone resulted in a 0% mammary tumor incidence, whereas treatment with the pituitary tumor alone yielded a mammary tumor incidence of 9%. It was apparent that prior stimulation of the mouse mammary gland with pituitary hormones was conducive to chemical carcinogenesis, an event very much unlike that which occurs in the rat, as will be described later. Since the pituitary tumor used in these studies secretes some growth hormone as well, it is impossible to evaluate the singular participation of prolactin in this process. However, the recent studies of Lin et al. (120) suggested that prolactin might be the key pituitary hormone in this process. They demonstrated that prior stimulation of the mouse (BALB/c) mammary gland with only prolactin, insulin, aldosterone, and cortisol in vitro and subsequent treatment with DMBA in vitro was sufficient to elicit hyperplastic changes in the mammae of these organ cultures that were indistinguishable from the precancerous changes (HAN) seen in the mouse mammary gland in vivo. It appears, therefore, that prior or concurrent stimulation of the mouse mammary gland with mammaryotropic hormones, particularly prolactin, increases the sensitivity of the gland to the action of the carcinogen.

Rats. It has been recognized for a number of years that multiparous rats have a higher incidence of spontaneous mammary tumors than do nulliparous rats (180), suggesting that prolactin may be a stimulatory hormone in spontaneous mammary tumorigenesis in this species. As in the mouse, but differing in certain subtle respects, prolactin secretion does increase during parity in the rat (1). To determine whether or not a sustained increase in the secretion of prolactin would increase mammary tumor incidence in rats, Welsch et al. (244) grafted multiple pituitaries to both nulliparous and multiparous Sprague-Dawley rats. Rats bearing the pituitary homografts, regardless of parity status, had a much higher mammary tumor incidence than did the nongrafted control rats. The results of these studies strongly implicated prolactin as an important hormone in spontaneous mammary tumorigenesis in the rat and confirmed and extended the earlier observations by Kwa et al. (108), who reported a positive correlation between elevated blood prolactin levels, pituitary hypertrophy, and the presence of mammary tumors in aged R-Amsterdam female rats. That prolactin is the principal pituitary hormone in this process is supported by the results of a study showing that placement of median-eminence hypothalamic lesions into female rats resulted in a striking increase in spontaneous mammary tumor incidence (250). These lesions in the rat cause an increased secretion of prolactin and decreased secretion of other anterior pituitary hormones (9, 252). This latter study emphasizes the key role of prolactin in rat mammary tumorigenesis and is particularly significant, inasmuch as previous studies have involved elevating endogenous prolactin levels in animals with a normal functional pituitary gland, which does not preclude the participation of other anterior pituitary hormones in this process. It appears, therefore, that an endocrine imbalance characterized by an increased secretion of prolactin and reduced secretion of most if not all of the other anterior pituitary hormones can be mammary tumorigenic, at least in the rat.

Not only is the genesis of spontaneous rat mammary tumors profoundly enhanced by increased secretory levels of prolactin, but the growth of the established spontaneous rat mammary tumor also appears to be significantly influenced by changes in the secretion of this hormone. Treatment of Sprague-Dawley rats bearing large palpable spontaneous mammary tumors with certain prolactin-suppressing ergot alkaloids resulted in a prompt and prolonged regression of the tumors (191). After termination of drug treatment, prompt resumption of mammary tumor growth was observed. This is very much unlike what occurs in the mouse, as previously described, because advanced spontaneous mouse mammary tumors are generally prolactin independent.

Carcinogen-induced mammary tumors in the rat have been extensively studied in recent years, and their dependency upon pituitary hormones has been clearly established. Indeed, there may be a direct correlation between serum prolactin levels and the genetically determined susceptibility of different strains of rats to carcino-gen-induced mammary tumorigenesis (14). In general, most treatments that cause a hyperprolactinemia in female rats already bearing either DMBA- or MCA-induced mammary tumors cause a striking increase in growth of these neoplasms. For example, physiological conditions or treatments that markedly excite carcino-gen-induced mammary tumor growth in female rats and that also cause a hyperprolactinemia are adrenalectomy (32); pregnancy (45, 128); pseudopregnancy (45); pituitary homografts (74, 239); pituitary tumors (100, 101, 223); hypothalamic lesions (102, 209, 240); hypothalamic implants of steroids (159, 162); certain neuroendocrine-influencing drugs such as reserpine (248), perphenazine (186), and haloperidol (188); and high dietary fat (24, 28, 29, 67). In accord, a number of drugs that induce a hyperprolactinemia, e.g., certain ergot alkaloids (25, 28, 81, 163, 220, 222, 243), cyclic imide derivatives (152), l-tyrosine derivatives (222), L-dopa (189), pargyline (188), and anti-rat prolactin serum (23), also cause a marked
diminution in growth of these tumors. It is clear from these studies, therefore, that marked fluctuations in secretory levels of prolactin profoundly influence the growth of these tumors. However, there appears to be no correlation between serum prolactin levels that lie in the "normal range" and rats bearing either fast-growing or slow-growing carcinogen-induced mammary tumors (160). It is conceivable, therefore, that variations in carcinogen-induced mammary tumor growth may also result from the sensitivity of the tumor cells to prolactin; thus subtle changes in the secretion of this hormone, changes that are not detected in the serum analysis of the hormone, could markedly influence tumor cell proliferation.

Whether or not growth hormone interacts with prolactin or has a direct effect in stimulating growth of carcinogen-induced rat mammary tumors is not certain. Since median-eminence hypothalamic lesions cause rapid growth of these tumors (102, 209, 240) and such lesions suppress growth hormone secretion, this suggests that growth hormone may not be critical to this growth process. The administration of growth hormone to carcinogen-induced mammary tumor-bearing rats has been reported to have no effect (164, 186) or to have a slight but significant growth-stimulatory effect (118). A recent in vitro study showed that growth hormone may stimulate DNA synthesis in organ cultures of carcinogen-induced rat mammary tumors, although this stimulatory effect was considerably less than that shown for prolactin (91).

Inhibition by Prolactin of Mammary Tumorigenesis

There are considerably more examples experimentally of a stimulatory effect of prolactin in murine mammary tumorigenesis than one of inhibition. Nevertheless, there are certain endocrinic conditions in which an increased secretion of this hormone in rats and mice is consistently inhibitory to this neoplastic process. One of the most striking examples of this phenomenon is the carcinogen-induced rat mammary tumor system. If prolactin secretion is increased by pregnancy (43), pituitary homografts (239), ether stress (66), reserpine (248), estrogens (104), oral contraceptives (232, 247), or hypothalamic lesions (102, 240), prior to carcinogen treatment, mammary tumor incidence will be sharply reduced when compared with controls. As previously described, these treatments consistently enhance the growth of existing carcinogen-induced rat mammary tumors. These treatments generally elicit an intense stimulation of the mammary gland resulting in a predominant lobulo-alveolar morphology in contrast to a predominant ductal system seen in the nontreated controls. It appears, therefore, that a predominant lobuloalveolar system in the rat is refractory to the action of the carcinogen. Carcinogen-induced mammary tumors in the rat appear to arise from the ductal or end-bud elements of the mammary gland (144, 156, 210, 211) rather than from alveoli which, at least in part, may explain this phenomenon.

Another example in which a sustained increased secretion of prolactin is inhibitory to chemical carcinogenesis of the rodent mammary gland is during lactation. Lactating rats and mice are relatively refractory to the action of chemical carcinogens when compared with nonlactating controls (43, 125, 126). It is conceivable that this inhibition may be accounted for by an increased elimination of the carcinogen by the secretory mammae (43, 126, 202). In support of this view is the demonstration that irradiation-induced tumorigenesis of the rat mammary gland is not impaired by lactation (202). Alternatively, the low rate of DNA synthesis that is characteristic of the lactating mammary gland (3, 71, 171) may be the significant influential factor in this process. It has been believed for a number of years that the frequency of cell division at the time of carcinogen treatment may be an important limiting factor in chemical carcinogenesis of the mammary gland. Chemical carcinogenesis of the rat mammary gland by polycyclic aromatic hydrocarbons is most effective at 50 to 60 days of age (42, 90) when DNA synthesis of the gland appears to reach its peak (170). Inhibition by CB-154-induced prolactin suppression of mammary gland DNA synthesis at the time of carcinogen treatment resulted in a decrease of mammary tumorigenesis in rats, further indicating the importance of the rate of DNA synthesis during chemical carcinogenesis of the rat mammary gland (177). During lactation, DNA synthesis of the mammary epithelium is quite low (3, 71, 71), despite relatively high secretory rates of prolactin (1).

Increased secretion of prolactin during lactation not only inhibited the induction of chemical carcinogenesis of the rat mammary gland but also caused regression of existing carcinogen-induced rat mammary tumors (45, 128-130). Development and growth of spontaneous and carcinogen-induced mammary tumors in mice have also been reported to be suppressed during lactation (125, 126, 153, 266). The mechanism by which lactation is inhibitory to mammary tumor growth is unknown, but it may reflect a deficiency of certain endocrine secretions (e.g., ovarian) (130) and/or a hormonal induction of differentiation of the neoplastic mammary epithelium. There are 2 transplantable rat mammary tumors (R3230AC and 35-MT) the growth of which appears to be inhibited by increasing prolactin secretion (11, 82, 83). Elevation of blood prolactin by grafting of pituitary tumors (83) or by the injection of perphenazine and its derivatives (11, 82) to these tumor-bearing rats inhibited growth of the neoplasms. Histological analysis of the mammary glands of these rats showed marked epithelial stimulation and some secretion. Whether or not the tumor per se was secretory was not reported. It is possible that under certain endocrinic conditions an increased secretion of prolactin might induce in mammary tumor cells a degree of differentiation, a physiological phenomenon that could oppose tumor cell proliferation.

Relationship between Prolactin and Ovarian Hormones in Mammary Tumorigenesis

One of the most intriguing problems pertaining to the hormonal control of murine mammary tumorigenesis is the nature of the interaction of peptide and steroid hormones in this neoplastic process. Although this interaction under most conditions is no doubt crucial, this very complicated
interrelationship remains to be elucidated. It has been known for many years that hypophysectomy of rodents sharply reduces and in most cases totally prevents the development of spontaneous mammary tumors (151). This is particularly true if this ablative procedure is performed at a very young age. Similarly, mice or rats that are ovariectomized at a young age develop fewer mammary tumors and, if ovariectomized-adrenalectomized, rarely develop these neoplasms (8, 10, 53, 194, 205). The age at which the ovaries are removed is important, i.e., the younger the animal at the time of surgery, the fewer are the number of mammary tumors that will develop (194).

The administration of estrogens to certain strains of mice and rats sharply increases mammary tumor incidence (8, 22, 40, 68, 79, 182, 235). This treatment increases prolactin secretion and ovariectomy reduces the secretion of this hormone (31, 143), an observation that has led to the well-known concept proposed originally by Furth (64) and colleagues (99-101) that estrogens are mammary oncogenic primarily because of the stimulatory effect of the steroids on prolactin secretion. Treatment of hypophysectomized animals with estrogens, in an effort to determine whether or not these steroids are capable of inducing spontaneous mammary tumors in animals free of pituitary hormones, has not been effective because hypophysectomized rodents are generally intolerant of prolonged steroid treatment. However, prolactin secretion can be suppressed in rodents chronically treated with estrogens by concurrent treatment with a number of ergot alkaloids (19, 65), despite the stimulatory effect of these steroids on the secretion of this peptide. Recently, it was reported (18, 235) that chronic treatment of female C3H mice with estrogens or the oral contraceptive Enovid (norethynodrel plus mestranol) increased the incidence of mammary hyperplasias and mammary tumors; however, upon concurrent prolactin suppression with the ergot drug CB-154, the hyperplasias and mammary tumor incidence were sharply reduced to a level comparable to the non-steroid-treated control animals. Thus, in this particular study it appeared that estrogens were mammary tumorigenic primarily because of their stimulatory effect on prolactin secretion, an observation that is in accord with the concept developed by Furth (64). We do not wish to infer that the mammary oncogenic activities of estrogens are mediated solely via the pituitary gland; instead we wish to emphasize that the indirect activity of the steroid is also a significant factor in estrogen-induced murine mammary tumorigenesis. Although the induction of murine mammary tumors by estrogen in all probability requires a functional pituitary gland, steroid-induced growth of established mammary tumors may not always require pituitary hormones. A recent report indicates that a transplantable mouse (GR/A) mammary tumor regressed upon hypophysectomy, but its growth was reactivated by acute administration of estrogen and progesterone (217). To our knowledge, this is the sole report showing that ovarian steroids can stimulate growth of existing mammary tumors in rodents lacking a functional pituitary gland.

The administration of pituitary hormones to ovariectomized or ovariectomized-adrenalectomized rodents has also been attempted in order to evaluate whether or not these hormones are capable of inducing mammary tumorigenesis in steroid-deficient animals. Prolactin-secreting pituitary isografts have been reported to increase mammary tumor incidence in ovariectomized mice (10) and in intact and orchidectomized male mice (72, 73), suggesting perhaps a primary role for prolactin and a secondary role for ovarian steroids in this process. The adrenal glands, however, no doubt contributed significant quantities of steroid hormones to this process (56). In a more recent study, Yanai and Nagasawa (256, 259) transplanted multiple pituitaries to ovariectomized-adrenalectomized C3H mice and observed within 8 months a 51% mammary tumor incidence. No mammary tumors were observed in the nongrafted ovariectomized-adrenalectomized control mice. Furthermore, these researchers showed that human placental lactogen stimulated mammary nodularigenesis (HAN) when administered to ovariectomized mice (261). Although the presence or absence of accessory adrenal tissue in the adrenalectomized animals is unresolved, the results of these studies suggest that a very high endogenous secretion of prolactin is capable of enhancing mammary tumorigenesis in sex hormone-lacking or at least sex hormone-deficient female mice. However, the ovariectomized-adrenalectomized or ovariectomized mice that were grafted with pituitaries or treated with human placental lactogen were 7 to 11 months old and multiparous at the time of surgery and hormonal treatment; thus at that time they already had large numbers of mammary dysplasias (HAN). Prolactin may therefore have simply promoted the growth of cells already "transformed." The successful induction by pituitary hormones of spontaneous mammary tumors in rodents ovariectomized-adrenalectomized or orchidectomized-adrenalectomized at a very young age has not, to our knowledge, been reported. It remains to be determined, therefore, whether or not pituitary hormones can initiate the early induction of spontaneous murine mammary tumors in animals free of steroid (sex) hormones.

In the induction of mammary tumors in rats by chemical carcinogens (e.g., DMBA, MCA), ovarian and pituitary hormones are also very important. Ovariectomy of rats 30 days prior to carcinogen treatment prevented the occurrence of the induced tumors (41, 239). Concurrent enhancement of prolactin secretion in these animals did not increase the incidence of these tumors (239). However, if ovariectomy was performed slightly before carcinogen treatment, concurrent treatment with prolactin and growth hormone (226) increased mammary tumor incidence considerably above the ovariectomized controls. A suppression of prolactin secretion prior to carcinogen treatment also sharply reduces mammary tumor incidence in female rats (34), whereas enhanced secretion of this hormone increases mammary tumor incidence in carcinogen-treated male rats (245). It appears, therefore, that ovarian hormones and prolactin or at least their residual effects are critical, perhaps essential, in the chemical transformation of the epithelium of the rat mammary gland but that pituitary hormones alone can promote the growth of these transformed cells even in the absence of normal ovarian function, resulting in a substantial mammary tumor incidence. Alternatively, if prolactin levels are sufficiently high, perhaps at a level even ex-
ceeding the secretory capacity of the in situ pituitary, then steroid hormones may not be essential for chemical carcinogenesis of the rat mammary gland. It has not been shown that chemical carcinogenesis in the rat mammary gland can occur in animals free of pituitary hormones, because hypophysectomized rodents are generally intolerant to carcino- 
genesis treatment. The sole exception to this is the study conducted a number of years ago by Young (264), demonstrating the successful chemical (MCA) induction of mammary tumors in hypophysectomized Sprague-Dawley rats that were chronically treated with estrogen, progesterone, and large doses of bovine growth hormone. The purity of the growth hormone was not indicated in this study.

Growth of established carcinogen-induced rat mammary tumors also appears to be regulated by steroid and pituitary hormonal interactions. Ovariectomy of rats bearing carcinogen-induced mammary tumors resulted in a prompt and prolonged regression of these tumors (45, 89, 234). Concurrent enhancement of prolactin secretion by placement of median-eminence hypothalamic lesions in these animals prevents this regression; tumor growth is actually stimulated by this experimental procedure (240). This enhanced mammary tumor growth, however, would not persist for long periods unless ovaries were reimplanted into these animals (209). In accord, the daily injection of ovine prolactin or human placental lactogen into ovariectomized-adrenalectomized or ovariectomized-adrenalectomized-hypophysectomized rats bearing carcinogen-induced mammary tumors also resulted in, at least initially, increased growth of these tumors (164, 168, 186). The highest daily doses of ovine prolactin (2.5 mg) or human placental lactogen (2.0 mg) administered to these animals actually stimulated tumor growth greater than that observed in the intact controls (164, 168). These results suggest that prolactin can stimulate growth of carcinogen-induced rat mammary tumors in animals lacking ovarian steroids, but for persistent growth of these tumors, despite a hyperprolactinemia, ovarian steroids may be essential. An exception to this concept is the study by Kim and Furth (100) showing that hormonal secretions from a transplantable pituitary tumor are capable of promoting growth of carcinogen (MCA)-induced mammary tumors in ovariectomized Fisher and Wistar rats for periods of several months. The pituitary tumors (MtT· F4 and MtT· W) used in their studies secreted extremely large amounts of prolactin and growth hormone. Adrenal gland participation in this growth process was not, however, ascertained. Further evidence for a significant prolactin and ovarian hormone interaction for growth of carcinogen-induced rat mammary carcinomas is provided by a study showing that the combination of drug-induced prolactin suppression and ovariectomy caused greater tumor regression than either treatment alone (190). It is probable that the growth dependency of carcinogen-induced rat mammary tumors on prolactin and ovarian hormones will vary from tumor to tumor, i.e., some tumors will require substantially greater or lesser amounts of prolactin and/or ovarian hormones for optimal growth processes (16, 160).

Whether or not estrogens can influence growth of carcinogen-induced rat mammary tumors in hypophysectomized animals was investigated by Pearson et al. (186) and Sterental et al. (221). Hypophysectomy caused a rapid regression of the mammary tumors. The administration of estrogens to the tumor-bearing, hypophysectomized rats did not cause a resumption in tumor growth, clearly demonstrating a pituitary dependency of steroids for this experimental tumor model. It is probable that there are variants of rodent mammary tumors, the growth of which can be stimulated by pituitary hormones in the absence of ovarian secretions and on rare occasions by ovarian hormones in the absence of pituitary secretions, but most hormone-responsive mammary tumors in rodents appear to require participation of both the pituitaries and ovaries for optimal growth processes.

Prolactin can influence development and growth of normal and neoplastic mammary tissue by a direct action on the mammary or by an indirect mechanism, i.e., via the ovary. It has been known for many years that this peptide is luteotropic and under certain conditions luteolytic in murine species (143, 254). Thus an increase in the secretion of this hormone in intact mice and rats may also cause an increased secretion of progesterone and some estrogen. This makes it more difficult to discern quantitatively the contribution of this hormone in murine mammary tumorigenesis. Nevertheless, it appears that the primary action of prolactin may be directly on the mammary tissue, inasmuch as a number of studies have demonstrated rather convincingly that prolactin has a direct mitogenic effect on normal and neoplastic rodent mammary tissue. For example, the administration of prolactin to ovariectomized, ovariectomized-adrenalectomized, or ovariectomized-adrenalectomized-hypophysectomized rats bearing carcinogen-induced mammary tumors resulted in an initial increase in the growth of these tumors (164, 166, 186, 240). Furthermore, the administration of this hormone to organ cultures of these tumors resulted in a marked increase in the incorporation of [3H]thymidine into DNA and [3H]leucine into protein (113, 117, 185, 224, 251) and a reduction in the conversion of testosterone to 5a-dihydrotestosterone and 5a-androsta- 
diol (145). Although these studies provide evidence indicating that prolactin alone can directly stimulate growth of rat mammary tumors, a distinct synergistic effect of ovarian hormones in the growth process does appear to occur (113, 115, 116, 190, 209). This is not unique to neoplastic mam- mae because sensitization of normal rodent mammary tis- sue with physiological levels of estrogen also resulted in an enhanced growth-promoting effect of prolactin (165).

Although physiological doses of estrogen may enhance the action of prolactin, larger doses in vitro (185, 251) and in vivo (140, 166, 167, 169) may actually inhibit the direct stimulatory effect of prolactin on normal and neoplastic rodent mammary tissues. In ovariectomized and intact rats, the injection of large doses of estrogen into rats bearing carcino- 
gen-induced mammary tumors resulted in a regression of these tumors, despite relatively high levels of blood prolac- 
in. This tumor regression was prevented by concurrent treatment with prolactin (140, 169). An inhibitory effect of large doses of estrogen, counteracted by prolactin, has also been demonstrated in vitro (30). Prolactin stimulates DNA synthesis of rat mammary tumors in vitro, an effect that can be suppressed by the addition of a relatively high level of
estrogen to the culture media (185, 251). Furthermore, an antagonistic effect of relatively high levels of estrogen on prolactin-induced growth of neoplastic and normal mammary tissue has also been demonstrated in neonatally estrogenized- or prolactinized-carcinogen-treated rats (174-176). These rats had high levels of serum prolactin and estrogen, yet mammary tumor incidence and growth was sharply reduced and the mammary glands were atrophic. Vaginal smears showed constant estrus, and the ovaries consisted only of anovulatory follicles. These results suggest, therefore, that relatively high doses of estrogen may interfere with the action of prolactin at the site of the mammary gland or mammary tumor but lower levels of the steroid may enhance the action of prolactin at these sites.

The interaction of estrogen and prolactin at the cellular level is a most intriguing biological problem. Steroid-responsive tissue, including normal and neoplastic mammary tissue, contains cytoplasmic protein receptors specific for these hormones (13, 39, 70, 94, 97, 112, 114–116, 131–134, 139, 150, 181, 193, 200, 207, 208, 217, 227, 231, 253). Prolactin-responsive tissue contains plasma membrane-bound prolactin receptors that are also specific for this peptide (20, 26, 36–39, 49, 62, 69, 86, 87, 98, 103, 187, 197, 198, 206, 218, 229). The combination of a hormone and its receptor represents the 1st step in the manifestation of intracellular events of hormone-directed cellular activities. Very recent evidence in rats has indicated that administered prolactin may increase the quantity of estrogen receptors in mammary tumors (115, 231) and that administered estrogens may increase the quantity of prolactin receptors in liver (69), suggesting that 1 mechanism of action of hormones in directing cellular activities might be, at least in part, the regulation of the quantity of hormone receptors. High levels of estrogen administered to rats bearing carcinogen-induced mammary tumors significantly reduced the prolactin membrane receptor content of the tumor tissue, thus suggesting a mechanism by which pharmacological doses of the steroid may cause mammary tumor regression (103).

Since high doses of estrogen suppressed DNA synthesis of organ cultures of rodent mammary tumors (230, 251), whether or not prolactin was added to the culture media, other mechanisms of steroid treatment appear to be operating as well. Prolactin receptors are also found in liver (20, 37, 62, 69, 187). The quantity of prolactin receptors in liver is decreased by hypophysectomy (37, 187) and increased by the administration of prolactin (37, 187) or estrogens (69). The precise changes in intracellular events after prolactin attaches to its membrane receptor are unknown but represent a very critical and viable area of future research efforts. Since a significant correlation has been shown between the number of prolactin receptors in rat mammary tumors and growth responsiveness of the tumors to prolactin (98), prolactin membrane receptor assays may yield valuable clinical information in the future, as have steroid receptor assays (93, 94, 131, 132), although not always quantitatively (13, 38, 87), in predicting hormonal responsiveness of cancerous mammary tissues.

A chronic drug-induced deficiency of prolactin secretion in mice results in animals whose mammary epithelium shows little growth and is also relatively refractory to tumor development (236, 241). This lack of epithelial growth and subsequent tumor development occurs despite normal body weight gains, cyclical ovarian activity, and the presence of the mammary tumor virus. Thus, the hypoplastic epithelium, observed in mice with suppressed prolactin secretion, appears to be strikingly refractory to the action of tumor-inducing agents. The in vitro studies of Lin et al. (120) support these earlier in vivo studies by showing that the addition of prolactin to organ cultures of mouse mammary epithelium is important for subsequent neoplastic transformation in vitro by chemical carcinogens. The mammary glands of prolactin-deficient rats are also relatively refractory to the action of chemical carcinogens (34). It is conceivable, therefore, that limited availability of prolactin during the time period in which the conversion or progression to hyperplasia-neoplasia would ordinarily occur either renders existing mammary epithelial cells metabolically refractory to the neoplastic process or interferes with the development of progenitive cells, the progeny of which would be susceptible to neoplastic transformation. An excess of prolactin, on the other hand, is often mitogenic to normal rodent mammary tissue. This has been demonstrated in vivo (44, 141, 155, 225, 263) and in vitro (50, 52). In essence, it appears that prolactin may be a critical regulatory hormone for controlling mitotic activity of rodent mammary epithelium; i.e., a deficiency of the peptide would cause a hypoplastic epithelium, whereas an excess would result in mammary hyperplasia. Prolactin-induced changes in mammary mitotic activity could influence the susceptibility of the epithelium to a number of chemical, physical, and/or viral oncogenic agents. There are far-reaching implications indicated by this hypothesis. If prolactin can be shown to be critically involved in the metabolic regulation of human breast epithelium, as it appears to be in rodents, then drug-induced prolactin suppression in the prophylactic control of breast tumorigenesis in humans may become feasible.

**A Possible Role for Prolactin in Human Breast Tumorigenesis?**

In recent years, sufficient experimental data have accumulated demonstrating a key role for prolactin in the development and growth of murine mammary tumors. These studies have provided, at least in part, the impetus to explore the hypothesis that this pituitary peptide may be similarly involved in human breast tumorigenesis (124, 146, 219). With the recent availability of a sensitive method to analyze serum quantities of prolactin in humans (63) and a means to reduce effectively the secretion of prolactin by drugs in humans (46, 92, 123, 196), a marked increase in the volume of information on this important subject has appeared in the literature.

In vivo studies designed to determine whether or not patients with breast cancer have higher mean serum prolactin levels have been conflicting. Women with metastatic breast carcinoma have been reported to have increased levels of this hormone when compared with patients without this disease (158, 195). On the other hand, Boyns et al.
human benign breast dysplasias and normal ductal tissues obtained from biopsies or mastectomy tissues has also been reported (27, 57). A very recent report (51) confirmed and extended by our laboratory (246) has shown that normal human breast tissue grown in organ culture was not stimulated by ovine prolactin but was stimulated by human prolactin or human placental lactogen. The results of these in vitro and in vivo studies, albeit somewhat conflicting and controversial, do nevertheless suggest that some human breast carcinomas may be influenced by prolactin and provide impetus further to explore the role of this hormone in human breast tumorigenesis.

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