Predominant Role of Prolactin in Stimulating the Growth of 7,12-Dimethylbenz(a)anthracene-induced Rat Mammary Tumor

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SUMMARY

The effect of prolactin in supporting the growth of 7,12-dimethylbenz(a)anthracene-induced mammary tumor in adult female Sprague-Dawley rats was investigated when estrogen receptors were blocked by the nonsteroidal antiestrogen, Tamoxifen, ICI 46,474.

Following an oophorectomy-induced remission, perphenazine, which stimulates endogenous prolactin release, was able to restore tumor growth whether or not Tamoxifen was added. A second course of perphenazine treatment, instituted after the tumors were allowed to shrink, was again effective in stimulating tumor growth.

After a regression in tumor size induced by oophorectomy and daily administration of Tamoxifen, perphenazine was able to restore original tumor size despite continued treatment with Tamoxifen. In intact rats, after regression was obtained by daily administration of Tamoxifen and the prolactin inhibitor, lergotriile mesylate, perphenazine induced tumor growth when the latter was discontinued, even though Tamoxifen was continued for 50 days. Estrogen receptors measured at the time of maximum stimulation by perphenazine were undetectable. On the other hand, estradiol did not stimulate tumor growth when serum prolactin was depressed to undetectable levels by lergotriile. These results indicate that prolactin supports the growth of 7,12-dimethylbenz(a)anthracene-induced rat mammary tumor and that estrogen receptors are not required under these conditions.

INTRODUCTION

The majority of rat mammary tumors induced by DMBA3 are hormone dependent, as shown by their regression after ovariectomy, adrenalectomy, and hypophysectomy. Estrogens and prolactin have been shown to influence the growth of such tumors (11, 12); however, the exact mechanism of action at the cellular level has not been completely clarified. It has been postulated that estrogens exert their action through a positive feedback mechanism on pituitary production and release of prolactin (11). However, the demonstration of a specific binding protein for estrogens in the cytosol and nuclei of DMBA-induced rat mammary tumors (9) has raised the possibility of a direct estrogen action at the tumor level. It has recently been reported that prolactin stimulates estrogen receptors at the tumor level, with the implication that prolactin sensitizes the action of estrogens at the target tissue level (13). It has also been suggested that the presence of estrogen receptors is a prerequisite for prolactin action (5).

The availability of potent, nonsteroidal antiestrogens like Nafoxidine and Tamoxifen, ICI 46,474, which exert their action through a competitive binding at the receptor level (3), offers a useful tool for studying the role of prolactin in stimulating tumor growth when the action of circulating estrogens is blocked. Tamoxifen was used in this investigation to study the effect of prolactin on tumor growth in DMBA-induced rat mammary cancer. In view of recent evidence that prolactin seems to be indispensable for estrogen action in stimulating tumor growth in this experimental model (10), we have investigated the effect of estrogens on DMBA-induced rat mammary tumor growth when prolactin was completely suppressed with the ergot derivative, lergotriile mesylate.

MATERIALS AND METHODS

Material. 17ß-[2,4,6,7-3H]Estradiol was obtained from New England Nuclear, Boston, Mass. (specific activity, 100 Ci/mmol). Tamoxifen, ICI 46,474, pure base powder was a gift from Imperial Chemical Industries, United States, Inc., Wilmington, Del. Lergotriile mesylate powder was a gift from Dr. Harold C. Halvorson, Lilly Research Laboratories, Indianapolis, Ind. Triafior® (aqueous solution of perphenazine) was obtained from Schering Corp., Bloomfield, N. J. DMBA was obtained from Eastman Organic Chemicals, Rochester, N. Y. Estradiol benzoate was obtained from Sigma Chemical Co., St. Louis, Mo.

Methods. Tumors were induced by i.g. feeding of 20 mg of DMBA to 50-day-old female Sprague-Dawley rats. Tumor sizes were derived from the product of the lengths of the 2 major axes measured with a caliper. Bilateral oophorectomy was performed via the dorsal route under light ether anesthesia. Blood was obtained by cutting the tip of the tail in the unanesthetized, caged rat, and serum prolactin was measured by radioimmunoassay as previously described (10).

Four experimental procedures were performed. In the 1st one, 2 groups of animals were studied. In a control group, 10 days after castration, 9 rats bearing ovarian-dependent tumors received daily i.m. injections of 1 mg of perphenazine.
zine for 8 days. This treatment was then discontinued; after 8 days of no treatment, a 2nd course of daily i.m. injections of perphenazine at the same dose was re instituted. A 2nd group of 8 rats was treated in the same way except that 200 μg Tamoxifen were added s.c. to the 1st course of perphenazine after 2 days and continued for the remaining 6 days.

In the 2nd experiment, 5 rats underwent bilateral oophorectomy and were started at the same time on 200 μg Tamoxifen s.c. daily. After 10 days, while being kept on daily injections of Tamoxifen, 1 mg perphenazine was added daily to every rat, and both treatments were continued for 20 days.

In the 3rd experiment, 5 intact rats were given daily s.c. injections of 200 μg of Tamoxifen and lergotrile mesylate by i.g. feeding daily, at 3 mg/kg. This treatment was continued for 30 days, at which time lergotrile mesylate was stopped and perphenazine, 1 mg/day, was added to Tamoxifen for an additional 20 days.

In the 4th experiment, 7 rats were oophorectomized and started on daily administration of lergotrile mesylate, 3 mg/kg p.o.; when significant tumor reduction was achieved, all rats were started on daily s.c. treatments with 5 μg of estradiol benzoate, while lergotrile mesylate was continued. In 4 of the rats serial serum prolactin determinations were done. Estrogen receptors were measured as previously described (8). Statistical analysis was conducted by the Student t test.

RESULTS

Effect of Short-Term Administration of Tamoxifen on Prolactin-induced Tumor Growth. Chart 1 shows that the endogenous release of prolactin, induced by perphenazine, did stimulate tumor growth in DMBA-induced rat mammary cancer after a regression had been induced by oophorectomy. This effect could be reproduced by a 2nd course of perphenazine after the tumors were again allowed to shrink.

As can be seen in Chart 2, the addition of Tamoxifen did not inhibit tumor growth induced by perphenazine. When perphenazine and Tamoxifen were discontinued, the tumors regressed, but administration of perphenazine restored tumor growth significantly. The fact that discontinuing perphenazine a 2nd time induced regression of the tumors indicates that we were still dealing with hormone-dependent tumors and that the 2nd growth induced by perphenazine was not just coincidental, due to the fact that the tumors had become autonomous. Thus it can be concluded that Tamoxifen did not impair the ability of perphenazine to induce tumor growth.

Effect of Long-Term Administration of Tamoxifen on Prolactin-induced Tumor Growth. Since Tamoxifen did not affect prolactin-induced tumor growth when given for 6 days, the effect of prolonged Tamoxifen administration on prolactin action was investigated. Chart 3 shows that, after a regression in tumor size induced by oophorectomy and Tamoxifen, perphenazine was able to restore the original tumor size, while the rats were still on Tamoxifen. In this experiment, daily administration of Tamoxifen for 1 month did not affect the action of endogenous prolactin in stimulating DMBA-induced rat mammary tumor. Chart 4 shows the results in the group of intact rats bearing DMBA tumors. Treatment with Tamoxifen and lergotrile mesylate reduced tumors to about one-half their original size over a period of 30 days. As can be seen, daily administration of Tamoxifen for up to 50 days did not prevent perphenazine action in restoring original tumor size. Estrogen receptors in the tumors measured at the end of the experiment at the time of stimulation by perphenazine were undetectable; this indicated that the antiestrogenic activity

Chart 1. Rats bearing DMBA-induced mammary cancer underwent oophorectomy (OVAX). After 10 days, they were started on daily i.m. treatments with 1 mg perphenazine (PERPH) for 8 days. This treatment was then discontinued. After 8 days of no treatment, a 2nd course of i.m. injections with the same daily dose of perphenazine was re instituted. Total number of tumors is given on each bar. Brackets, S.E.

Chart 2. Experimental condition similar to the one described in Chart 1. In this case 200 μg Tamoxifen (TAM) were added s.c. to the 1st course of perphenazine (PERPH) after 2 days and continued for the remaining 6 days. After the 2nd course of perphenazine, rats were kept untreated, allowing the tumors to shrink again. OVAX, oophorectomy.
DISCUSSION

Estrogen and prolactin have been shown to regulate the growth of DMBA-induced rat mammary cancer (5); however, the primary role of estrogens has been questioned, and their mechanism of action has been postulated to be through a positive feedback in the production and release of prolactin from the pituitary (11). Recently, however, Leung and Sasaki (5) concluded that estrogen receptors might be a prerequisite for tumor growth induced by prolactin, even though they recognized that prolactin plays an important role in activating tumors. They showed that, even though the antiestrogen Nafoxidine did not inhibit tumor growth initiated by prolactin after oophorectomy-induced remission, subsequent stimulation by prolactin was ineffective. Our studies using Tamoxifen, a nonsteroidal antiestrogen similar to Nafoxidine, are in disagreement with that of Leung and Sasaki (5) and clearly show that Tamoxifen did not impair the growth of the tumors induced by prolactin at any time, even when the length of treatment with Tamoxifen was prolonged up to 50 days. The fact that a complete antiestrogenic effect was achieved was confirmed by the undetectability of estrogen receptors measured at the time of maximum stimulation by perphenazine. These findings indicate that estrogen receptors are not indispensable for prolactin action and that prolactin can stimulate tumor growth by itself.

The presence of prolactin receptors has been documented in DMBA-induced rat mammary tumors, and after ovariectomy they decrease about 50% but do not disappear (1). Although prolactin receptors were not measured in these experiments, it seems likely that sufficient receptors were present in the tumors to permit prolactin-induced stimulation of tumor growth while estrogen receptors were undetectable. The fact that prolactin is the dominant hormone in the growth of DMBA-induced rat mammary tumor is supported by the observation that, when prolactin was suppressed to undetectable values, estrogens were unable to induce tumor growth. Furthermore, it has been shown that estrogens are ineffective in stimulating tumor growth in hypophysectomized rats (11, 12). The role of progesterone was complete even at the time of maximum prolactin stimulation which is known to stimulate estrogen receptors. That this dose of Tamoxifen is effective in blocking estrogen action is also supported by the fact that in intact rats the estrous cycle was blocked in diestrus, as determined by daily vaginal smears.

Effects of Estrogens on Tumor Growth in the Absence of Detectable Serum Prolactin. As can be seen in Chart 5, estrogens did not stimulate tumor growth when rats were given lergotrile mesylate simultaneously.

Serum prolactin was measured in 4 rats 10 days after oophorectomy and institution of lergotrile and also just before being sacrificed after 10 days of estradiol stimulation. In both cases it was found to be below the level of detectability of the radioimmunoassay (2.5 ng/ml).
receptors in tumor growth has been investigated, and these receptors were found to be under the influence of estrogens but not of prolactin (4). This indicates that progesterone receptors were not involved in the stimulation of tumor growth in our experiments.

It appears that the hormonal regulation of DMBA-induced rat mammary tumor is quite different from that of human breast cancer, where the role of prolactin seems to be much less prominent. Clinical studies have shown that effective suppression of prolactin in women with breast cancer failed to induce objective remission (2). Estrogens, on the contrary, seem to have a major role as shown by their ability to stimulate human breast cancer cells in tissue culture (6). In addition, we have been able to induce objective remissions with antiestrogens in women with proven complete hypophysectomy (7).

We have presented evidence in favor of a primary role of prolactin in stimulating DMBA-induced rat mammary tumor not requiring the presence of estrogen receptors. Still, much remains to be learned about the interaction of different hormones at the cellular level. The discovery and use of substances that selectively inhibit a specific receptor should help to clarify the independent role of different hormones. The use of tissue cultures of cancer cells also offers a controlled environment where it would be possible to study individually the action of each hormone.

REFERENCES
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