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# EFFECTS OF ESTRADIOL AND PROLACTIN ON STEROID RECEPTOR LEVELS IN 7,12-DIMETHYLBENZ(A)ANTHRACENE-INDUCED MAMMARY TUMORS AND UTERUS IN THE RAT

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SUMMARY

In order to eliminate changes of pituitary hormone secretion secondary to treatment and be able to assess the direct effect of 17β-estradiol and prolactin at the level of hormone-dependent mammary tumors induced in the rat by 7,12-dimethylbenz(a)anthracene (DMBA), the effects of the two hormones on steroid receptor levels were investigated in hypophysectomized-ovariectomized animals. Treatment with prolactin alone led to tumor estrogen receptor levels higher than those found after estrogen administration. When the level of progesterone receptors was measured as parameter of estrogen action, it was found that combined treatment with prolactin and 17β-estradiol led to levels much higher than those found after individual hormone treatment. Tissue specificity of this interaction between prolactin and 17β-estradiol is indicated by the absence of effect of prolactin on the estrogen-induced stimulation of the progesterone receptor in uterine tissue. These data demonstrate the direct stimulatory effect of prolactin on estrogen receptor levels in DMBA-induced mammary carcinoma and indicate the central role of this pituitary hormone as modulator of estrogen action and growth of DMBA-induced mammary tumors.

## INTRODUCTION

It is well recognized that growth of mammary tumors induced in the rat by 7,12-dimethylbenz(a)-anthracene (DMBA), a model widely used for studies on the control of human breast cancer, is dependent upon the action of estrogens and prolactin [1–4]. However, much remains to be known about the relative importance of the two hormones and their interactions. As example of hormonal interactions, treatment with an inhibitor of prolactin release, CB-154, has recently been found to prevent the stimulatory effect of 17β-estradiol on the level of its own receptor in DMBA-induced mammary tumors [5]. This inhibition by CB-154 was reversed by prolactin administration, thus suggesting requirement of prolactin for estrogen action.

Since it was recently found that 17β-estradiol increases the level of progesterone receptors in DMBA tumors of castrated rats [6–8], we have chosen this sensitive and precise parameter as index of estrogen action in this tissue and have compared with the effects observed in uterus. Moreover, in order to eliminate changes of pituitary hormone secretion accompanying hormone treatments and be able to assess direct hormone effects at the tissue level, hypophysectomized-ovariectomized animals were used.

## EXPERIMENTAL

*Animals and treatments.* Female Sprague–Dawley rats obtained from Canadian Breeding Farms, St. Constant, Quebec, were used throughout these experiments and mammary tumors were induced as described [9, 10]. Three months after DMBA administration, animals bearing large tumors (having a diameter of 1.5 cm or more) were bilaterally ovariectomized under ether anesthesia. Tumor measurements were performed the day before ovariectomy and at 3–5 days intervals thereafter. Animals bearing tumors which regressed at least 50% within 10 days after ovariectomy were hypophysectomized by the transauricular approach and injected twice daily for two weeks with prolactin (1 mg oPRL kindly supplied by NIAMDD), 17β-estradiol (0.5 μg), the combination of prolactin and 17β-estradiol or the vehicle alone (0.2 ml of 0.9% NaCl—1% gelatin). Animals were given 5% glucose 0.9% NaCl as drinking water during the post-hypophysectomy period. At the end of the experiment, the number of animals bearing tumors were: control (vehicle alone) 5; prolactin, 7; 17β-estradiol, 11; combined treatment, 5. The distribution of tumors in different groups is indicated in Legend to Fig. 1.

*Binding assays.* Estrogen and progesterone receptor levels were measured in the tumor cytosol using [2,4,6,7-$^3$H]-17β-estradiol (91.3 Ci/mmol, New England Nuclear) and [$^3$H]-R5020 [6,7-$^3$H]-17,21-dimethyl-19-nor-pregna-4,9-diene-3, 20-dione, (51.4 Ci/mmol), respectively, as described [6] except that $5 \times 10^{-7}$ M dexamethasone was added in progesterone receptor assays. Under our conditions of incubation, free estrogen receptor sites were measured while the

progesterone binding assay could measure free and occupied (if present) binding sites. However, in all our experiments, animals were killed 24 h after the last hormone injection at a time where steroids should have reached negligible levels. [$^3$H]-R5020 and the unlabelled steroid were synthesized at the Roussel Research Center and kindly provided by Dr. J. P. Raynaud.

*Calculations.* Statistical significance was measured according to the multiple-range test of Duncan-Kramer [11] after verification of the homogeneity of variances. Data are expressed as mean ± S.E.M.

## RESULTS

### *Effect of hormone treatment on tumor growth*

In Fig. 1, we have represented only the size variations of 5–6 representative tumors in each group. As shown in Fig. 1A, there was a rapid decrease of the size of hormone-dependent tumors during the ten days following ovariectomy and no tumor could be seen in the control group (vehicle alone) two weeks after hypophysectomy. The growth of only a few tumors was observed after 17β-estradiol treatment

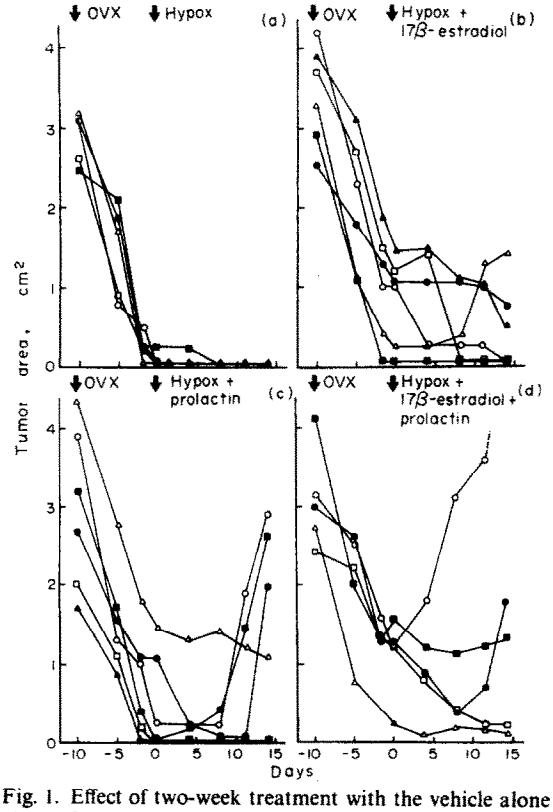


Fig. 1. Effect of two-week treatment with the vehicle alone (A), 17β-estradiol (0.5 μg/day) (B), ovine prolactin (1 mg, twice a day) (C) or combined administration of 17β-estradiol and prolactin (D) on the growth of DMBA-induced mammary tumors. Animals were castrated ten days before hypophysectomy. 5–6 representative tumors are shown in each group. The number of hormone-dependent tumors (those which regressed more than 50% within 10 days after ovariectomy) were: control (vehicle alone), 5; 17β-estradiol, 20; prolactin, 12; 17β-estradiol + prolactin, 10.

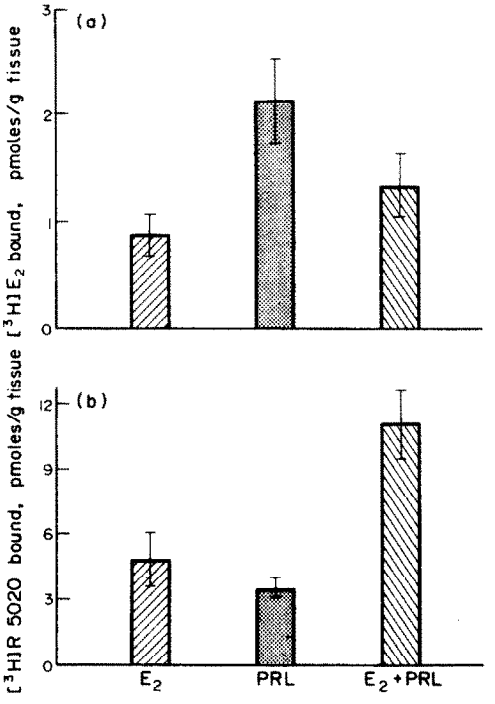


Fig. 2. Effect of treatment with 17β-estradiol, prolactin or combination of both hormones on [$^3$H]-17β-estradiol (A) and [$^3$H]-R5020 (B) binding in hormone-dependent DMBA-induced mammary tumors in hypophysectomized-ovariectomized animals. The number of tumors analyzed were: 17β-estradiol, 7; prolactin, 11; 17β-estradiol + prolactin, 7.

(Fig. 1B). In fact, 2 out of 20 tumors were stimulated with 17β-estradiol. However, after prolactin administration, the size of more tumors was increased (Fig. 1C). In fact, 6 out of 12 tumors (50%) are reactivated after prolactin administration. The combined treatment of 17β-estradiol and prolactin was not more efficient than the pituitary hormone alone, as illustrated by representative tumors in Fig. 1D. In fact, the growth of 4 out of 10 tumors (40%) was stimulated by the combined treatment.

### *Effect of hormone treatment on steroid receptor levels in tumor tissue*

Treatment with prolactin led to estrogen receptor levels 120% above those found after 17β-estradiol administration (2.1 ± 0.4 vs 0.9 ± 0.2 pmol/g tissue, $P < 0.01$, Fig. 2A). Combined treatment was accompanied by receptor levels intermediate between those following administration of 17β-estradiol or prolactin alone. It can be seen in Fig. 2B that the level of progesterone receptors in tumor tissue was high after combined 17β-estradiol and prolactin treatment while much lower levels were seen after individual hormone treatment.

### *Effect of hormone treatment on steroid receptor levels in uterus*

It was of interest to compare the effect of 17β-estradiol and prolactin treatment on steroid receptor levels

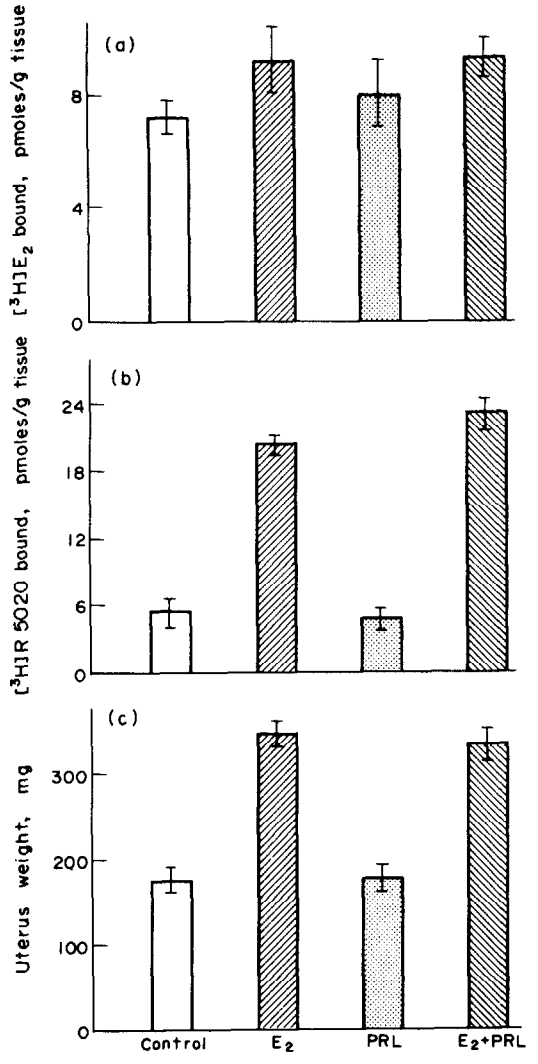


Fig. 3. Effect of treatment with 17β-estradiol, prolactin, a combination of both hormones or the vehicle alone on uterine [$^3$H]-17β-estradiol binding (A), [$^3$H]-R5020 binding (B), and uterine weight (C) in hypophysectomized-ovariectomized animals.

in hormone-dependent DMBA-induced tumors with those found in a typically estrogen-dependent tissue, the uterus. While the effect of 17β-estradiol on the level of its own receptor was not significant, it can be clearly seen that, contrary to the findings in mammary tumor cytosol, the marked stimulatory effect of 17β-estradiol on the level of the uterine progesterone receptor was not potentiated by simultaneous administration of prolactin. Figs 3B and 3C illustrate the close parallelism between changes of uterine progesterone receptor levels and uterine weight.

## DISCUSSION

The present experiments performed in hypophysectomized-ovariectomized animals permit an assessment of the direct effects of 17β-estradiol and prolactin on steroid receptor levels and growth of DMBA-induced mammary tumors without interference by changes of pituitary secretion secondary to hormone treatment. It can be clearly seen that administration of prolactin leads to a marked potentiation of the effect of 17β-estradiol on the level of progesterone receptors, a specific estrogen-dependent and precise parameter. This effect of prolactin is accompanied by higher levels of estrogen receptor in the tumor tissue. It is thus likely that under the influence of prolactin, the higher estrogen receptor levels increase the sensitivity of the tissue to the response of the circulating estrogens. This prolactin-dependent effect of 17β-estradiol clearly demonstrated in DMBA-induced tumor tissue is however not present in uterus. In fact, the stimulatory effect of 17β-estradiol on the level of uterine progesterone receptors is not potentiated by the simultaneous administration of prolactin.

The present findings stress the importance of measurements of tumor estrogen and progesterone receptor levels as sensitive parameters of the biological responses to prolactin and estrogen in the DMBA mammary tumors and human breast cancer [12].

Mammary tumors induced in the rat by DMBA administration thus offer a good model for studies of peptide and steroid hormone interactions. In fact, as can be deduced from this and previous investigations [5, 6–8], not only prolactin stimulates estrogen receptor levels and tumor growth but a third hormonal factor (progesterone), is also dependent upon a prolactin-potentiated estrogen action.

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