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Effect of Pituitary Isografts on the Concentration of Estrogen and Glucocorticoid Receptors in C3H Mice Mammary Tumors*

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Abstract—*We have investigated the effects of pituitary isografts in C3H mice on the induction and steroid receptor levels of mammary tumors. The latent period of tumorigenesis was shortened but the final incidence of the tumors was unaffected. Pituitary grafting resulted in a three-fold increase in the concentration of cytosol estrogen receptor sites in the tumors when measured* 1 day *after castration; however, the affinity of the receptor for estradiol was not modified. In contrast, glucocorticoid receptor levels were not increased and the progesterone receptor concentration was negligible with or without pituitary grafts. The sustained high levels of prolactin in the mice grafted with pituitaries are probably responsible for the increase in the concentration of estrogen receptor sites. We propose that the stimulating effect of prolactin on mammary tumor induction might not only be due to the activation of the prolactin receptors but also to the accumulation of the estrogen receptor resulting in a local hypersensitivity to estrogens.*

INTRODUCTION

THE CONSTITUTED mammary tumors of C3H mice are considered to be hormone independent since neither ovariectomy nor adrenalectomy inhibit their growth [1]. Conversely, hormones and mainly prolactin and estrogens are required for the induction of these tumors [2]. Pituitary isografts have been used to increase the rate of induction of mammary tumors [3–5] probably by increasing plasma prolactin levels. Actually, prolactin is the main hormone secreted by pituitary grafts [6] and CB 154, which inhibits prolactin secretion, prevents the effect of these isografts [7].

It is not known how prolactin increases tumorigenesis. It may act directly on mammary tissue; however it is not excluded that it acts indirectly either by affecting metabolism [8] or modulating the effects of other hormones. In this paper, we consider the possibility that the synergism between prolactin and steroid hormones on the induction and growth of mammary tumors could be related to an effect of prolactin on the steroid receptor content of mammary cells.

MATERIALS AND METHODS

The C3H mice were from our breeding-colony which was initiated in 1976 from C3H/He mice kindly provided by Dr. G. Rudali (Paris). Fifteen, 6 week-old nulliparous female C3H mice, received 2 isologous pituitary grafts from two males of the same litter under the right kidney capsule [9]. They were put in forced breeding at 7 weeks of age. Fifteen control C3H mice were put in forced breeding at 6 weeks. Starting at the 4th month, the mice were palpated every week to determine the time required for the mammary tumor induction. When the tumors were detected ($\simeq 0.25\,cm^2$ surface area), female and male mice were separated and the tumors allowed to grow for $\simeq 1$ month until they reached a surface area $> 2\,cm^2$. They were then collected for receptor assays. In tumor growth

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experiments the tumors were allowed to grow for ≃40 days, growth being monitored by measuring their average diameters with calipers.

The estrogen receptors (ER) of mammary tumors were assayed *in vitro*, generally 1 day after bilateral ovariectomy as described previously [10]. The cytosol (Rc) and the unoccupied nuclear (Rn) receptors were analysed at 2°C in the cytosol and the KCl nuclear extract respectively. The estrogen receptors were progressively saturated *in vitro* with increasing concentrations of ^{3}H estradiol (CEA, SA = 50 Ci/mmole) and measured by dextran coated charcoal assay [10].

The glucocorticoid receptors (GR) were assayed by DCC [11] one day after bilateral adrenalectomy using ^{3}H dexamethasone (CEA, France, SA = 15 Ci/mmole). The protein concentration in both extracts were assayed by OD absorption at 260 and 280 nm [10].

RESULTS

1. *Induction of mammary tumors*

Thirteen out of fifteen mice survived the pituitary grafts and developed mammary tumors. A 100% incidence of mammary tumors was found both for the grafted and the control mice. As shown in Fig. A tumors appeared

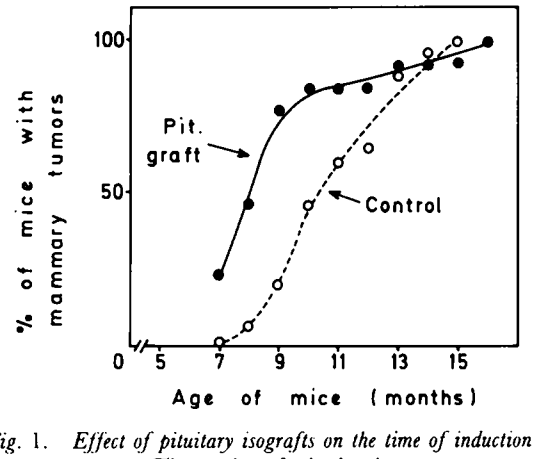

Fig. 1. Effect of pituitary isografts on the time of induction of mammary tumors. The number of mice bearing mammary tumors in the pituitary grafted (●) and control (○) groups was evaluated every month and expressed as a percentage of the total tumoral mice. The mammary tumor incidence was 100% since all mice developed tumors at 16 months.

significantly earlier in the grafted mice than in the control mice. At 9 months of age, the tumor incidence was 70% in the grafted mice and only 20% in the control mice. In the grafted mouse which developed a tumor only after 15 months, histological examination revealed that the pituitary graft had been rejected.

2. *Concentration of estrogen receptor*

The ER levels of 9 mammary tumors developed under pituitary graft stimulation were compared to those of the control tumors. The same affinity for ^{3}H estradiol was found in both tissues as shown by saturation analysis (Fig. 2). However, the mean concentration of

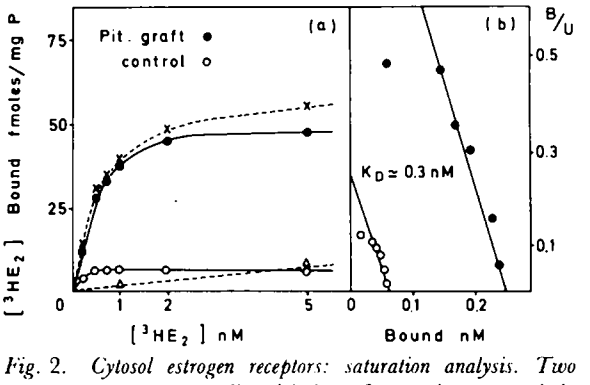

Fig. 2. Cytosol estrogen receptors: saturation analysis. Two mammary tumors were collected 1 day *after ovariectomy, and the cytosol* (≃3.5 mg *protein*/ml) *was incubated for* 2 hr *at* 4°C *with various concentrations of* (^{3}H) *estradiol without* (+) *or with* (△) *non radioactive diethylstilboestrol. The specific binding in grafted* (●) *and control* (○) *mice was evaluated by DCC assay* [10].

(*a*) *Direct representation of the total* (X), *non saturable* (△) *and specific* (○) *binding for the grafted mice and of the specific binding of the control mice* (○) *in function of the concentration of incubated estradiol.*

(*b*) *Scatchard plot,* B = *Specifically bound estradiol;* U = *Concentration of unbound estradiol.*

cytosol receptor sites was increased three-fold in the grafted mice as compared to the control mice. From 18 days after castration, this difference was not visible in the two tumors analysed (Table 1). This observation suggests that ovariectomy decreases the ER concentration in the grafted mice whereas it has no effect in the control mice [12]. The increase of ER concentration by pituitary grafts was more dramatic in the earlier tumors since the two tumors which developed in mice older than 10 months did not have a significantly increased number of sites (Fig. 3). The number of accessible nuclear receptors was increased by pituitary graft to the same extent as the cytosol receptors (Table 1). A similar increase of ER cytosol sites has also been observed in the mammary tumors of PS mice [13] (and E. Coezy, unpublished).

The cytosol glucocorticoid receptors of C3H mammary tumors displayed the same affinity (KD ≃ 2.5 nM) and binding stereospecificity as that described previously [13, 14]. The pituitary isografts modified neither the affinity

Table 1. Effect of pituitary grafts and ovariectomy on the concentration of estradiol receptor in C3H mammary tumors

Female C3H	Days after ovx	No. exp.	ER sites fmole/m P mean ± S.D. Cytosol	Nuclear	% nuclear sites
Pituitary Isograft	1	6	58 ± 18*	17 ± 12†	23 ± 12
	4	1	28	0	
	18	1	11	3	
	60	1	12	0	
Control	1	9	15 ± 5*	6 ± 3†	27 ± 13
	4	1	8.3	0	
	15	1	15.8	0	

Of the 15 mice which were grafted, only 9 were analysed for ER content using DCC assay. The difference between grafted and control mice was evaluated by the Student's *t*-test and was found significant for the cytosol ER (*$P<0.01$) but not for the nuclear ER ($P>0.2$).

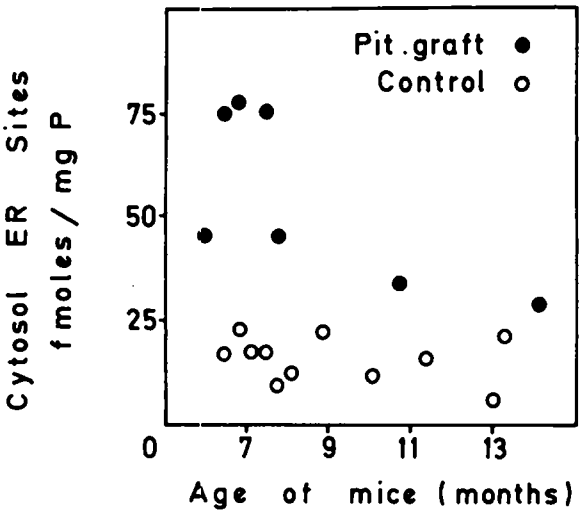

Fig. 3. Effect of pituitary grafts on the concentration of cytosol ER sites. The ER sites were assayed by DCC in the cytosol of control (○) and pituitary grafted (●) tumors and are plotted according to the age of the mice at sacrifice. Each tumor was processed for receptor assays 30 ± 6 days after its appearance and 1–4 days after ovariectomy. The two tumors which were analysed after 18 days ovariectomy (Table 1) are not represented.

nor the specificity of these receptors. In two experiments, in which the ER sites were increased by the graft, the GR concentrations were 100 and 130 fmole/mg of protein in the control mice, but only 40 and 30 fmole/mg of protein in the grafted mice. These results strongly suggested that the effect of pituitary grafts on ER and GR was dissociated.

3. *Ovarian dependency of the tumors*

The influence of ovarian hormones on the tumors stimulated by pituitary isografts was evaluated by measuring both the tumor area (before and after ovariectomy) and the progesterone receptor which is known to be specifically induced by estrogens in hormone-dependent mammary tumors [15].

Since the ER concentration was increased by pituitary grafts, it was expected that the tumors might still respond to estrogens in the same way as their precursors in the hyperplastic alveolar nodules [16].

We confirmed that the growth of mammary tumors in the control C3H mice was not modified by castration (Fig. 4). A slight and transitory decrease in the rate of growth of the tumors stimulated by pituitary graft was observed within the 12 days following castration as compared to the non-castrated control

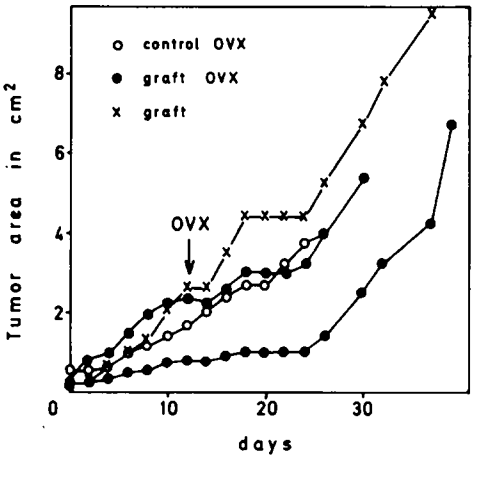

Fig. 4. Effect of pituitary graft on the growth of mammary tumor before and after ovariectomy. The area of mammary tumors was measured every 2 days starting from time 0 which is defined as the time of appearance of the tumor (≃0.25 cm² area). Bilateral ovariectomy was performed on the 12th day in 2 grafted mice (●) and in one control mouse (○). Results from a non-ovariectomized animal are also shown (X).

(Fig. 4). However no regression could be observed and the progression toward total ovarian insensitivity then occurred after 24 days. Preliminary experiment indicated that the concentration of the progesterone receptor, which is negligible in control C3H mammary tumours [12], was not increased in the tumors of the grafted mice when assayed at tumor age of 41 weeks.

We, therefore, concluded that, at the time of ovariectomy, pituitary grafts do not prevent the progression toward hormone independency.

DISCUSSION

We have studied the effect of pituitary isograft on the induction of mammary tumors in the C3H mice. As shown previously [5] the final tumor incidence was not modified but the tumors appeared earlier after pituitary grafts and were quite different in their steroid receptor content. We found a significant increase in the concentration of ER in the mammary tumors of C3H mice bearing isologous pituitary grafts, while the glucocorticoid receptors were not stimulated. The specific and sustained increase of endogenous prolactin in the plasma of the grafted mice [4, 6] is likely to be responsible for this effect. In fact, prolactin has already been shown to increase the concentration of ER sites in DMBA rat mammary tumors [10, 17] which contain prolactin receptors. Conversely, in tissues which do not contain prolactin receptors, such as the rat uterus or the constituted mammary tumors of C3H mice, exogenous prolactin was without effect on the ER concentration [10] and unpublished observations.

The mechanism by which prolactin increases the ER concentration is unknown. The time at which the grafts were applied suggests that they could favor the genesis and the growth of the hyperplastic alveolar nodules (HAN) which are the precursors of the final tumor [5]. This treatment appears to favor lobulo alveolar development of the tumor at the expense of duct growth. However, it is not known whether the specific increase of ER in the whole tumor is due to selection of cells containing more ER than GR, or whether there is specific ER accumulation without cell selection.

This paper also suggests that by increasing the concentrations of the receptors which bind the hormones involved in cell growth regulation, one might hope to transform hormone-independent tumors into hormone-dependent tumors. This has been attempted with the MTW_9 transplantable rat mammary tumor [18]. In the C3H mice by sustaining a high level of prolactin secretion, we were able to increase the ER concentrations; however, the estrogen sensitivity of these tumors was not markedly modified.

The relationship beyween the increased ER and the stimulation of tumor induction in grafted animals is unclear. The stimulatory effect of endogenous pituitary prolactin on tumor growth could obviously be direct, mediated by prolactin receptors but it may also be indirect via an accumulation of estrogen receptor, thus favoring the synergism between prolactin and estrogen for tumor induction and growth. Our observations could be compared to the correlation described in human between breast cancer incidence and the intake of prolactin releasing drugs such as reserpine [19]. Therefore, we suggest that the effect of a sustained high level of prolactin on normal or hyperplastic mammary glands could favor cancer induction by locally increasing the ER concentration. It is however necessary first to demonstrate that estradiol and the ER present in mammary cells are responsible for favoring tumor induction.

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