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Letter to the Editor

Correlation of Plasma Prolactin and Growth Hormone with Normal and Preneoplastic Mammary Gland Growth in Virgin SHN Mice*

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MANY studies have been carried out in mice on the relationship between circulating levels of prolactin (PRL) and the growth and function of the mammary gland, with few consistent results [1]. However, in all of these studies, the experiments were performed using mouse strains with different mammary gland characteristics and without resort to endocrine manipulations.

In this communication we report our findings on the correlation between circulating levels of PRL and normal or preneoplastic mammary gland growth, using pooled data from SHN mice subjected to various endocrine manipulations (Table 1). Similar correlations with growth hormone (GH) were also examined in order to evaluate its role in mammary gland growth.

Data from the following two experiments were used for calculation of correlations.

Experiment I. Relationship of prolactin and progesterone to normal mammary gland growth in SHN virgin mice

Endocrine manipulations applied to the mice are given in Table 1. At autopsy the left inguinal mammary fat pads were removed, defatted and dried with hot alcohol-ether. Mammary DNA content was determined by diphenylamine reaction, as detailed previously [2].

Table 1. *Endocrine manipulation given to mice from which data were used for the calculation of correlations*

Experiment No.		Group and treatment*
I.	(1)	A single pituitary isograft under the kidney capsule (1AP)
	(2)	Subcutaneous implant of 20 mg progesterone in pellet form (PROG)
	(3)	Bilateral ovariectomy (Ovx)
	(4)	Ovx + 1AP
	(5)	Ovx + 3AP
	(6)	Ovx + PROG
	(7)	Ovx + 1AP + PROG
II	(8)	4a 1.0 mg
	(9)	4a 0.6 mg
	(10)	4a 0.3 mg
	(11)	4b 2.0 mg
	(12)	4b 0.3 mg

*In experiment I all treatments were started at 40 days of age and mice were killed after 60 days. In experiment II each dose of the compounds, 1-alkyl-4-chrolo-2-(2,6-dichrolo-4-hydroxyphenyl)-6-hydroxyindoles (4a and 4b) [3], was dissolved in 0.05 ml olive oil and injected subcutaneously every day for 21 days to 5- to 6-month-old virgin mice. The mice were killed on the day after the last injection. The number of animals in each group was 5-10.

Experiment II. Effects of 2-(hydroxyphenyl)-indoles of preneoplastic mammary gland growth in SHN virgin mice

Mice received the treatments shown in Table 1. At the end of injections the number of preneoplastic mammary hyperplastic alveolar nodules (HAN) were counted in the mounted preparations of whole bilateral third thoracic

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Table 2. Simple and partial correlation coefficients between plasma levels of prolactin or growth hormone and normal or preneoplastic mammary gland growth in virgin SHN mice

Experiment No.	Simple correlation	Partial correlation
I	$r_{PRL\cdot DNA}$* = 0.47† (55)‡	$r_{PRL\cdot DNA/GH}$ = 0.69† (52)
	$r_{GH\cdot DNA}$ = 0.25§ (53)	$r_{GH\cdot DNA/PRL}$ = 0.25 (52)
II	$r_{PRL\cdot HAN}$ = -0.01 (32)	$r_{PRL\cdot HAN/GH}$ = 0.05 (29)
	$r_{GH\cdot HAN}$ = 0.24 (31)	$r_{GH\cdot HAN/PRL}$ = 0.24 (29)

*PRL: prolactin; GH: growth hormone; DNA: mammary DNA content; HAN: No. of mammary hyperplastic alveolar nodules.
†, § Statistically significant at $P < 0.01$ and 0.05, respectively.
‡No. of observations.

mammary glands. The sum of the values for the bilateral glands represents the number in the individual.

In both experiments I and II blood was collected from each mouse by orbital puncture at autopsy and plasma levels of PRL and GH were determined by radioimmunoassay.

Simple correlation coefficients [4] were calculated between plasma levels of PRL or GH and mammary DNA content or the number of HAN. Partial correlations [4] between hormone parameters and mammary gland parameters were also examined, by fixing the value of each of the hormones in order to exclude its influence.

The results are shown in Table 2. There were statistically significant correlations between plasma levels of PRL or GH and mammary DNA content ($P < 0.01$ or 0.05). The partial correlation between PRL and mammary DNA was also significant ($P < 0.01$); however, that between GH and mammary DNA was not. The number of HAN had no significant correlations, simple or partial, with plasma hormone levels.

The significant simple as well as partial correlations between plasma PRL and mammary DNA content indicated that mammary parenchymal growth was responsive to circulating PRL levels in mice if they received various endocrine manipulations. This is supported by results obtained in experiment I, where grafting of three pituitaries restored to normal the regression of mammary glands induced by ovariectomy, whereas the single pituitary grafting had little effect [unpublished data].

Lack of correlation between plasma PRL and the number of HAN suggests that preneoplastic mammary gland growth is not affected quantitatively by circulating PRL. However, this does not negate the significance of PRL in this process; rather, it indicates that normal PRL levels are sufficient for the genesis and progression of HAN, since the susceptibility of HAN to PRL is very high [1].

The simple correlation between GH and mammary DNA content was statistically significant, but not the partial correlation when PRL level was kept fixed. Furthermore, GH levels showed no significant correlation with the number of HAN. We have recently found evidence for the participation of GH in normal and preneoplastic mammary gland growth in mice in the presence of PRL [5]. Therefore the present results suggest that the effect of GH on mammary gland is masked by PRL under conditions in which its secretion is greatly modulated.

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