

Hormonal Modulation of the Rat Mammary Gland γ -Glutamyltranspeptidase

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1. The *in vivo* effect of estradiol and domperidone (a hypophysis stimulator of prolactin secretion) in immature ovariectomized-adrenalectomized and hypophysectomized rat mammary gland was studied.

2. γ -Glutamyltranspeptidase activity was used to evaluate the role of estradiol in the specific response of the gland to prolactin.

3. Our results suggest that the activity of γ -glutamyltranspeptidase, like casein and lactose synthetase, is part of the specific response of the gland to prolactin.

Introduction

The complex process of lactation is regulated by multiple hormonal interactions, being prolactin one of the most important hormones [1, 2]. Prolactin action, however, depends on the coordinated action of other hormones, *e.g.*, insulin, corticosterone, thyroid hormones and estrogens [2]. Synthesis of milk specific products, caseins and lactose, and the activities of many enzymes provide a good biochemical index to study the biosynthetic activity of the mammary gland along the lactogenic cycle (pregnancy-lactation-involution).

There are many reports on the participation of estradiol (17 β -estradiol) in prolactin action [3, 5]. This steroid hormone has a role in the growth and differentiation of mammary gland and can act synergically with prolactin *in vivo* and *in vitro* [6, 7]. The most important relationship between estradiol and prolactin was found in an *in vitro* study of the gland's response to insulin, prolactin and corticosterone [5]. Estrogen depletion in the immature mice, through adrenalectomy and ovariectomy, causes a severe change in the actions mediated by prolactin in the gland, but this change could be reverted by the administration of estradiol immediately after endocrinectomy [3–5]. It should be emphasized that the loss of specific response to prolactin, *i.e.* synthesis of caseins and α -lactalbumin, is the only change observed following estrogen depletion; the response to

insulin, corticosterone and to the general action of prolactin remains unchanged.

Our laboratory has been studying the hormonal control of the enzyme γ -glutamyltranspeptidase (γ -GT) from rat mammary gland [8, 9]. This enzyme is regulated by prolactin *in vivo* and *in vitro* and presents a high activity during the lactating period [9, 10]. In this study, we investigated whether this enzyme is part of the specific response of the gland to prolactin *in vivo* or whether its activity is irreversibly decreased by estrogen depletion. We used immature adrenalectomized-ovariectomized and adrenalectomized-ovariectomized-hypophysectomized rats under different treatments with estradiol and/or domperidone (a hypophysis stimulator of prolactin secretion). Lactose synthetase activity was measured as a control of the specific response of the gland to prolactin.

Materials and Methods

Chemicals

L- γ -glutamyl-*p*-nitroanilide, UDP-galactose, glycyglycine and 17- β -estradiol were from Sigma Chemical Co. UDP-¹⁴C galactose, 337 mCi/mol, was from New England Nuclear and domperidone was a gift from Laboratorio SAVAL S.A. (Chile).

Animals and treatments

Four week old Sprague-Dawley rats (85–100 g), sham operated, adrenalectomized-ovariectomized

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and adrenalectomized-ovariectomized-hypophysectomized were used.

The animals were kept at a controlled temperature (25 °C) with free access to food and water 0.9% (w/v) NaCl for the endocrinectomized rats.

In the first type of experiments adrenalectomized-ovariectomized and adrenalectomized-ovariectomized-hypophysectomized rats (eight rats each) were treated with different doses of estradiol for eight days, treatments starting the same day of the operation. In the second kind of experiments, nine groups of adrenalectomized-ovariectomized rats and sham endocrinectomized rats (3,4 rats each, see below) received different treatments. Treatment I was administered daily and for four weeks, starting the same day of the operation, and treatment II was also administered daily for one week, starting immediately after treatment I. In both treatments domperidone and estradiol were dissolved in NaCl 0.9%–25% absolute ethanol and administered *via* subcutaneous injection. Estradiol (1 μ g/day), domperidone (1 mg/day), and NaCl 0.9%–25% absolute ethanol (vehicle) were administered to the controls in treatment I (see Table I for details); domperidone (2 mg/day) and the same doses of estradiol and the vehicle were administered to the controls in treatment II.

Enzymatic and DNA determinations

After the above described treatments the rats were sacrificed by decapitation and inguinal mammary glands were removed and excess fat and connective tissue were trimmed off. The glandular tissue was homogenized for 90 sec in an Ultra Turrax homogenizer (Janke and Kunkel, Staufen, F.R.G.) in a 4 vol of ice cold 20 mM Tris-HCl, 10 mM MgCl₂ and 1.0 mM mercaptoethanol, pH 7.4. The homogenate was centrifuged for 5 min at 8000 \times g, and the supernatant was used for γ -GT determinations by monitoring the *p*-nitroaniline release from the synthetic γ -glutamyl donor L-glutamyl-*p*-nitroanilide [11]. Lactose synthetase was measured with UDP-¹⁴C galactose and glucose as substrates [12]. The DNA was determined by the method of Burton [13]. Each value corresponds to the mean of at least 3–4 observations \pm SEM. The activity of γ -GT (nmol *p*-nitroaniline/mg DNA) and lactose synthetase (nmol lactose/mg DNA) is expressed as a percentage of the control groups. Data were analyzed using the Student's "t" test.

Results

The first step in this study was to establish the effect of estradiol on the activities of γ -GT and lactose synthetase in adrenalectomized-ovariectomized and adrenalectomized-ovariectomized-hypophysectomized rats (Fig. 1). Estradiol caused a dose-dependent stimulation on both enzymatic activities in the adrenalectomized-ovariectomized group, and the maximal effect, 4–6-fold stimulation, was observed with an estradiol dose of 100 μ g per day. Neither of these enzymatic activities changed in the adrenalectomized-ovariectomized-hypophysectomized group.

The activities of γ -GT and lactose synthetase obtained with the different treatments used (see Table I for details), are shown in Fig. 2. According to our experimental approach, two control groups, SVV and OVV, were used. SVV or sham adrenalectomized-ovariectomized and OVV endocrinectomized rats, only received the vehicle during both treatments.

A comparison of the SVV and SVD groups, shows that the administration of the vehicle in treatment I and domperidone in treatment II (SVD group), caused a significant increase in both enzymatic activities. However, the treatment with domperidone in both groups of endocrinectomized rats (groups ODD and OVD) produced a very small increase in the activities and only in relation to the OVV control group.

The daily administration of 1.0 μ g of estradiol during both treatments to the endocrinectomized group

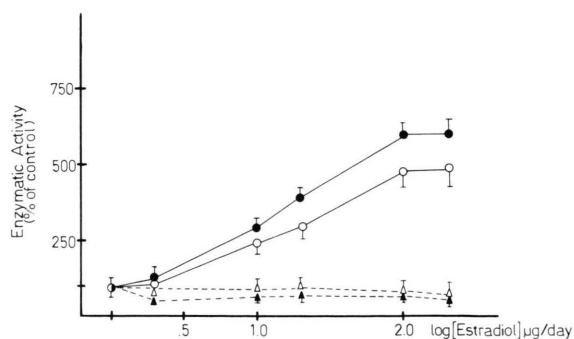


Fig. 1. Estradiol effect on γ -GT and lactose synthetase activities. ● γ -GT, ○ lactose synthetase in the adrenalectomized-ovariectomized group. ▲ γ -GT, △ lactose synthetase in the adrenalectomized-ovariectomized-hypophysectomized group. ● Control groups, endocrinectomized rats without estradiol treatment. Enzymatic activities expressed as percentages \pm SEM.

Table I. Treatment schedule.

Group*	n	Characteristics Surgical condition	Treatment**	
			I	II
SVV	3	sham operation	vehicle	vehicle
SVD	4	sham operation	vehicle	domperidone
OVV	3	adrenalectomy-ovariectomy	vehicle	vehicle
OVD	3	adrenalectomy-ovariectomy	vehicle	domperidone
OEE	3	adrenalectomy-ovariectomy	estradiol	estradiol
OE-ED	4	adrenalectomy-ovariectomy	estradiol	estradiol + domperidone
ODD	3	adrenalectomy-ovariectomy	domperidone	domperidone
OV-ED	3	adrenalectomy-ovariectomy	vehicle	estradiol + domperidone
OED	4	adrenalectomy-ovariectomy	estradiol	domperidone

* The first letter indicates the surgical conditions; S: sham operation, O: adrenalectomy-ovariectomy. The second letter indicates the first treatment (I); V: vehicle only; E: estradiol; D: domperidone; and the third letter indicates the second treatment (II); V, D, E already described and ED: estradiol plus domperidone.

** Treatment I was administered during 4 weeks and Treatment II during one week.

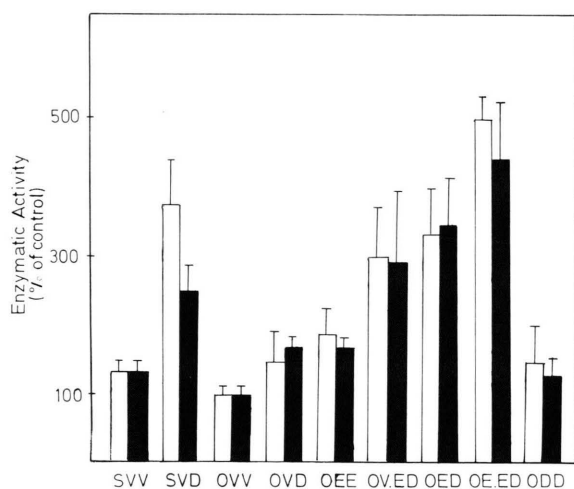


Fig. 2. *In vivo* effect on the different treatments with estradiol and domperidone on the activities of γ -GT (\square) and lactose synthetase (\blacksquare). For details see Table I (Treatment Schedule). The groups SVD, OE-ED, OV-ED and OED are significantly higher than all other groups examined ($p < 0.025$).

OEE, increased both activities in relation to the OVV group, but this change was not significant in relation to the SVV group. With the use of estradiol during the first treatment and domperidone and domperidone-estradiol during the second treatment, OED and OE-ED groups, the increases observed

were similar to, or even higher than, the values obtained by the SVD group. On the other hand, the OV-ED group that only received domperidone and estradiol during treatment II, also shows a significant increase in relation to both controls (OVV and SVV).

Discussion

Rat mammary gland undergoes many structural changes during its development; these changes depend mainly on the age and the functional status of the endocrine glands. During the lactogenic cycle the gland experiments the most important development and attains full capacity for the synthesis and secretion of milk.

Prolactin is the main hormone in the rat for the synthesis of the milk proteins, casein and α -lactalbumin. The α -lactalbumin activity in this study, was estimated as lactose synthetase activity [14] Fig. 1, shows the stimulatory effect of estradiol, in the adrenalectomized-ovariectomized group, on both enzymatic activities. This effect, however, was abolished in the adrenalectomized-ovariectomized-hypophysectomized group and would represent a response only mediated by prolactin, because estradiol is a potent stimulator of prolactin release. The experiments analyzed below were designed to try the clarify the role of estradiol in the prolactin action, in relation to γ -GT activity.

Estrogens allow the proliferation of mammary ducts during the first weeks of life of the rats but, obviously, the most important effect of this steroid hormone is during pregnancy, together with progesterone and placental lactogen. All these hormones are responsible for the development of the lobule-alveolar structure which can finally produce and secrete milk components during lactation [1, 2]. According to its physiological state, murine mammary gland exhibits three kinds of endocrine response: complete unresponsiveness, partial unresponsiveness and complete responsiveness to hormonal action [5]. Estradiol action represents one example of the second kind of endocrine response. Estrogens depletion in virgin rats produced a great decrease in the specific response to prolactin *in vitro* in mammary gland explants stimulated to secrete; the effect is only partial because the action of cortisol and insulin were normal. There are very few reports about the *in vivo* biochemical alteration produced by estradiol depletion.

According to Fig. 2, it is apparent from the two enzymes being examined that estradiol has a direct participation in mammary gland differentiation and the activities of lactose synthetase and γ -GT are very similar in practically all the different treatments. The stimulatory action of prolactin, obtained through the action of domperidone, is not possible without estradiol (OVD and ODD groups). Other investigators have used perphenazin or pituitary transplant to increase prolactin levels [3].

Estrogen administration is the only way to prevent and reverse this lesion. Estradiol effect was clearly observed in the groups first treated with this steroid and then with domperidone (OED and OE-ED); a similar effect was observed in the SVD group (Sham) which maintained the endogenous estradiol levels. On the other hand, there is evidence that estradiol may increase the hypophisary secretion of prolactin [15]. However, this fact does not account for the results observed for lactose synthetase and γ -GT activities. The values obtained in the groups treated only with estradiol or only with domperidone, OEE and ODD respectively, were very low, indicating that estradiol or domperidone alone are not responsible for the changes observed. These results confirm that estrogens seem to be necessary for the preparation of rat mammary cells during lactation. It is possible to appreciate the very high correlation in the activities of lactose synthetase and γ -GT. Such a fact leads to conclude that γ -GT activity is part of the specific action of prolactin in the gland, this enzymatic activity being a suitable biochemical parameter for monitoring the action of prolactin in mammary gland.

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- [1] L. M. Houdebine, *Bull Cancer (Paris)* **72**, 121– (1985).
- [2] Y. J. Topper and C. S. Freeman, *Physiol. Rev.* **60**, 1049– (1980).
- [3] F. F. Bolander and Y. J. Topper, *Endocrinology* **107**, 1281– (1980).
- [4] F. F. Bolander and Y. J. Topper, *Endocrinology* **108**, 1649– (1981).
- [5] L. Sankaran, P. Qasba, and Y. J. Topper, *Biochem. Biophys. Res. Commun.* **125**, 682– (1984).
- [6] F. F. Bolander and Y. J. Topper, *Endocrinology* **106**, 490 (1980).
- [7] C. Ip and T. Dao, *Cancer Res.* **38**, 2077– (1978).
- [8] J. Puente, M. A. Varas, G. Beckhaus, and M. Sapag-Hagar, *FEBS Lett.* **99**, 215 (1979).
- [9] S. Villanueva, J. Puente, and M. Sapag-Hagar, *Res. Comm. Chem. Pathol. Pharmacol.* **57**, 99– (1987).
- [10] P. A. Pocius, C. R. Baurrucker, J. P. McNamara, and D. E. Bauman, *Biochem. J.* **188**, 565– (1980).
- [11] S. S. Tate and A. Meister, *J. Biol. Chem.* **249**, 7593– (1974).
- [12] K. Brew, T. C. Vanaman, and R. L. Hill, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 491– (1968).
- [13] K. Burton, *Biochem. J.* **62**, 315– (1956).
- [14] B. K. Vonderhaar, *Endocrinology* **100**, 1423– (1977).
- [15] J. Meites and J. A. Clemens, *Vitamins and hormones* **30**, 165 (1972).