γ-GLUTAMYLTRANSPEPTIDASE ACTIVITY AND CYCLIC AMP LEVELS IN RAT LIVER AND MAMMARY GLAND DURING THE LACTOGENIC CYCLE AND IN THE OESTRADIOL–PROGESTERONE PSEUDO-INDUCED PREGNANCY

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1. Introduction

The mammary gland is a tissue subject to close hormonal control over the whole course of the lactation cycle with the most striking changes in hormonal balance occurring at parturition and the onset of lactation [1].

Studies of enzyme changes in the tissue at this transition have shown dramatic increases in the activity of those enzymes involved in the biosynthesis of the milk constituents and, in some cases, a hormonal regulation of their activity has been implied [2,3].

Although the membrane-bound enzyme γ-glutamyltranspeptidase has been found in most secretory tissues its activity has not yet been fully studied in mammary gland where, as the enzyme initiating the γ-glutamyl cycle [4], it may be expected to fill an important role in regulating the entry of amino acids into the cell. The enzyme has been reported to be regulated by cyclic AMP in liver [5] while, in rat seminal vesicles, its activity appears to be testosterone-dependent [6].

This investigation is a study of γ-glutamyltranspeptidase in the liver and mammary glands taken from pregnant and lactating rats and from rats in which mammary involution (caused by weaning of the pups) had been allowed to proceed for 5 days. Parallel studies were also carried out on the same tissues from hormonally induced pseudopregnant animals.

The results show that the enzyme increases slowly in the pregnant mammary gland and then sharply in lactation. The activity in liver declines during pregnancy, as does the cAMP content, both reaching a minimum at the time of parturition. The results also indicate that in the gland from the pseudopregnant rat the enzyme shows an absolute dependence on the presence of oestradiol and progesterone to attain values approximating to those found in tissue of a normal pregnant animal.

2. Materials and methods

2.1. *Animals*

Primiparous Sprague-Dawley rats (180–230 g body wt) were taken at different stages of pregnancy and lactation and on day 5 of mammary involution. For the production of pseudopregnant animals, virgin rats were bilaterally ovariectomized [7] and then given daily subcutaneous injections of 2 μg 17-β-oestradiol benzoate and 6 mg progesterone in 0.1 ml vegetable oil for 23 days. Control rats were injected with saline for the same period. The ovariectomized animals were used 10–12 days after the operation.

2.2. *Materials*

L-γ-glutamyl-*p*-nitranilide and glycylglycine were obtained from the Sigma Chemical Co. β-oestradiol and progesterone were from Merck, Darmstadt. Cyclic [8-^{3}H]AMP was obtained from the Radiochemical Centre, Amersham.

2.3. *Enzyme assays*

Animals were killed by decapitation and the liver and mammary gland rapidly removed and homogenized in 4 vol. 0.15 M Tris (pH 8.0)/0.01 M KCl in an Ultra-Turrax blendor (Janke and Kunkel, Staufen). The γ-glutamyltranspeptidase activity of these extracts was assayed by the method in [8] using L-γ-glutamyl-*p*-nitranilide as donor and glycylglycine as acceptor. A unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmol product per 30 min at 25°C. Cyclic AMP was assayed by the isotope dilution technique using the assay conditions previously described [9]. Protein was determined by the Lowry method [10].

3. Results

The γ-glutamyltranspeptidase of rat mammary gland resembles the enzyme from other tissues, e.g., seminal vesicles and kidney [6,11] in that it is inhibited by a mixture of 5 mM serine and 5 mM borate, but not by either of the two components separately. Further, the mammary gland enzyme exhibited a glutaminase activity in the presence of 0.1 M maleate.

Figure 1 shows the changes of γ-glutamyltranspeptidase activity in mammary gland and liver, as well as the cAMP levels in the liver, at different stages of pregnancy and lactation. The mammary gland enzyme increases slowly through pregnancy to a value some 4–5-times the value in the virgin gland

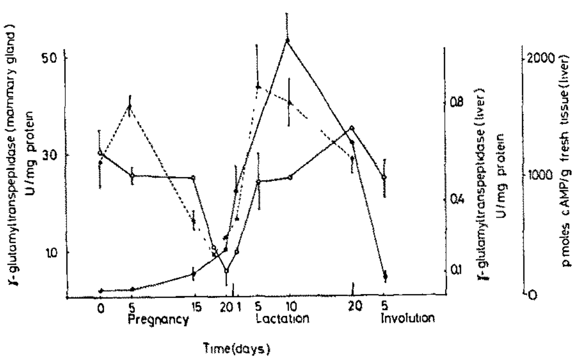

Fig.1. cAMP levels in liver and γ-glutamyltranspeptidase activities in liver and mammary gland during the lactogenic cycle. Results are given as the mean values ± SEM; each value represents the mean of 5 animals. (△–△) pmol cAMP; (–○–○–) liver γ-glutamyltranspeptidase; (–●–●–) mammary gland γ-glutamyltranspeptidase.

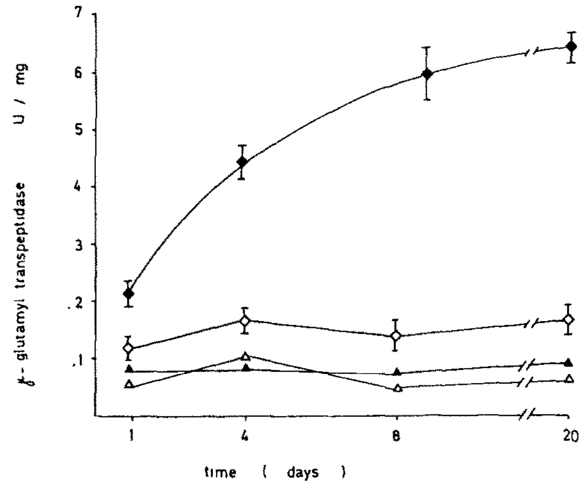

Fig.2. Effect of daily injections of estradiol–progesterone and saline on γ-glutamyltranspeptidase activities in liver and mammary gland of ovariectomized rats. For details see the text. Each value represents the mean ± SEM of 3 animals. (●–●) mammary gland; (▲–▲) control mammary gland; (○–○) liver; (△–△) control liver.

but, following parturition, the activity increases sharply to reach a maximum on day 10 of lactation some 5-times that at the end of pregnancy. The value falls thereafter and, by day 5 of mammary involution, has returned to the virgin gland level. The liver activity changes far less and, apart from the fall around the end of pregnancy and the beginning of lactation, remains sensibly constant over the entire lactation cycle. The changes of the cyclic AMP content of the liver follow closely the same pattern as the liver enzyme.

Figure 2 shows the effect of continued treatment of ovariectomized animals with β-oestradiol and progesterone on the γ-glutamyltranspeptidase activity of liver and mammary gland. The mammary gland enzyme showed a marked dependence on the presence of the hormones and, in the 20 days of treatment, responded with an increase almost identical to that observed in the gland in a natural pregnancy. The hepatic enzyme showed no change after hormone treatment. The results (table 1) also show that the mammary gland enzyme responds to β-oestradiol (X3) or β-oestradiol + progesterone (X4) but not to progesterone.

Table 1
Effect of ovariectomy and hormone administration on γ-glutamyltranspeptidase activity in liver and mammary gland

Treatment	γ-Glutamyltranspeptidase activity (mU/mg)	
	Mammary gland	Liver
Ovariectomized	140 ± 13[d]	50 ± 4
Ovariectomized + progesterone[a]	130 ± 11	60 ± 17
Ovariectomized + estradiol[b]	410 ± 57[e]	45 ± 5
Ovariectomized + estradiol–progesterone[c]	613 ± 79[f]	37 ± 8
Control (non ovariectomized)	200 ± 20	62 ± 16

[a] 6 mg in 0.1 ml vegetable oil for 8 days
[b] 2 μg in 0.1 ml vegetable oil for 8 days
[c] 2 μg estradiol–6 mg progesterone in 0.1 ml vegetable oil for 8 days
[d] SEM
[e] Significantly larger than ovariectomized, $P < 0.05$
[f] Significantly larger than ovariectomized, $P < 0.05$

4. Discussion

The increase in the activity of rat mammary gland γ-glutamyltranspeptidase activity reported here may reflect a stage in the differentiation of the secretory epithelial cells, the number of which increase as a tissue response to the adjustments of the hormonal bombardment during the lactogenic cycle. On this view, the enhanced transpeptidase activity may represent an anticipatory adaptation in preparation for the greater demand for transport of amino acids which would be needed for the synthesis of milk protein. This view assumes a role for γ-glutamyltranspeptidase in such transport although recent enzymatic data on the mode of action, localization and relative activity of renal γ-glutamyltranspeptidase raise some doubts on the actual role of the enzyme in such transport [12]. The contrast between the observed increase in the activity of γ-glutamyltranspeptidase of the mammary gland during pregnancy and the behaviour of many other enzymes associated with milk formation, which do not begin to increase until the onset of lactation [2], is especially noteworthy. In contrast to the mammary gland enzyme, the liver enzyme activity falls, to 20% of its initial level, by the end of the pregnancy and it is interesting to note the coincidence, in time and magnitude, of the fall in the cAMP content of the same tissue, an observation consistent with previous suggestions [5] proposing that cAMP acts as inducer of γ-glutamyltranspeptidase. On the other hand, the lack of any such parallelism between the enzyme and cAMP in mammary tissue, would appear to indicate that, in this organ, cAMP has neither a direct nor immediate effect on the enzyme.

The fact that the response of the γ-glutamyltranspeptidase in the mammary gland of ovariectomized rats to treatment of the animals with β-oestradiol/progesterone reaches a maximum (fig.2), coupled with the observation that the hormonal fluctuation attendant on the normal oestrogenic cycle, fail to influence its activity, both point to the conclusion that the changes found after treatment with β-oestradiol/progesterone may well arise as a secondary consequence of the changes in the amount of secretory epithelium which results from such hormonal treatment rather than as a direct effect of the hormone on the enzyme. No changes were observed in the liver enzyme following hormone injections, either in ovariectomized or intact rats. The data in table 1 appear to suggest that while β-oestradiol alone will augment the activity of γ-glutamyltranspeptidase in mammary gland and progesterone will not, in combination the two hormones act synergistically.

It may be concluded that while the changes in the activity of γ-glutamyltranspeptidase activity in the mammary gland which occur during a normal pregnancy can be simulated in the gland of pseudopregnant rats by the combined action of β-oestradiol and progesterone, the liver enzyme appears to be independent of such control but may, instead, be responsive to changes in the tissue level of cAMP.

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