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γ-Glutamyl Transpeptidase in Rat Mammary Tissue

ACTIVITY DURING LACTOGENESIS AND REGULATION BY PROLACTIN

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The increased activity of γ-glutamyl transpeptidase (EC 2.3.2.2) in rat mammary tissue at the onset of lactation was shown to involve prolactin. The timing and magnitude of these changes and its prolactin requirement suggest an important role in mammary function.

γ-Glutamyl transpeptidase (EC 2.3.2.2) is a membrane-bound enzyme that is widely distributed in mammalian tissues (Meister & Tate, 1976). This enzyme catalyses the transfer of the γ-glutamyl moiety of glutathione (or other γ-glutamyl donor) to an amino acid (peptide) acceptor as shown below:

Glutathione + L-amino acid ⇌
L-γ-glutamyl-L-amino acid + L-cysteinylglycine

Meister (1973) first suggested this enzyme to be a component of a γ-glutamyl cycle for the transport of amino acids and peptides across cell membranes.

We have shown that lactating mammary tissue from cow and rat have relatively high γ-glutamyl transpeptidase activity (Baumrucker & Pocius, 1978). If mammary gland γ-glutamyl transpeptidase functions as an amino-acid-transport mechanism, then it is conceivable that the activity of this enzyme (like other specific enzymes involved in milk biosynthesis) would increase at the initiation of lactation. Our first objective was to characterize the activity of rat mammary γ-glutamyl transpeptidase during pregnancy, lactation, and involution. While this manuscript was in preparation, Puente *et al.* (1979) reported results from a similar study. Because prolactin is a component of the lactogenic complex necessary for the onset of lactation and milk biosynthesis in the rat (Tucker, 1974; Kuhn, 1977), our second objective was to examine the role of prolactin in regulating the activity of mammary γ-glutamyl transpeptidase during lactogenesis.

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Experimental

Primiparous rats (200–250 g body wt.; Holtzman/Sprague–Dawley strain) were fed *ad libitum* a diet of Purina rat chow (Ralston Purina, St. Louis, MO, U.S.A.). For lactating animals, litters were adjusted to eight pups immediately *post-partum*. Rats were killed by decapitation, and mammary glands were removed and trimmed of excess fat and connective tissue. Tissue was minced and homogenized for 20–40 s at medium speed with a Polytron (Brinkman) in 5 vol. of ice-cold 10 mM-Tris/HCl buffer, pH 8.0, containing 80 mM-$MgCl_2$. The homogenate was filtered through cheesecloth, and the filtrate was used for determination of γ-glutamyl transpeptidase activity. Although a portion of the activity detected in mammary gland homogenates is due to enzyme activity associated with milk membranes (Baumrucker, 1979; Baumrucker & Pocius, 1978; Majumder & Ganguli, 1972), no attempt has been made here to correct for the milk content of homogenates. Correction for milk in mammary gland homogenates results in only slightly greater estimates of γ-glutamyl transpeptidase activity (Baumrucker & Pocius, 1978). γ-Glutamyl transpeptidase activity was measured spectrophotometrically at 37°C by monitoring the release of *p*-nitroaniline ($\varepsilon = 8800$ $M^{-1} \cdot cm^{-1}$) from the artificial γ-glutamyl donor, γ-glutamyl-*p*-nitroanilide (Tate & Meister, 1974). The assay system (pH 8.0) contained 50 mM-Tris/HCl, 4.0 mM-γ-glutamyl-*p*-nitroanilide, 75 mM-NaCl, and 50 mM-glycylglycine. All assays were saturated with γ-glutamyl donor and acceptor. Release of *p*-nitroanilide in the absence of enzyme (control), was subtracted from all values. Rates represent apparent maximal velocities and were linear with regard to time and amount of enzyme. One unit of enzyme activity is defined as 1 μmol of *p*-nitroaniline released/min. Protein determinations for mammary-

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gland filtrate were by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Cofactors for the enzyme analysis and protein determinations were obtained from Sigma.

A second study examined the effects of prolactin on mammary γ-glutamyl transpeptidase activity. Prolactin deficiency was induced with bromocryptine (CB-154; donated by Sandoz, East Hanover, NJ, U.S.A.), and enhancement was by administration of bovine prolactin (PRL; NIH-PB4 donated by National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, MD, U.S.A.). Treatments were: (1) bromocryptine (0.1 mg/day); (2) bromocryptine (0.1 mg/day) plus prolactin (25 i.u./day); (3) control (placebo injections). Treatment was initiated on day 17 of pregnancy (day 1 is the sperm-positive day) and continued until the animals were killed. The daily subcutaneous injection (0.1 ml of solution) comprised 0.9% NaCl/ethanol/1 M-NaOH (8.0:9.0:0.1, by vol.) When included, bromocryptine was first dissolved in the ethanol component, and prolactin was first dissolved in the 0.9%-NaCl/NaOH components. Cumulative pup weight gain was used as an estimate of milk production as described by Ota & Yokoyama (1967).

Results and Discussion

The activity of γ-glutamyl transpeptidase at different stages of pregnancy and lactation is shown in Fig. 1. Activity is very low in virgin mammary tissue (<0.01 units/mg of filtrate protein) and gradually increases throughout pregnancy. At the onset of lactation, the activity of γ-glutamyl transpeptidase increases over 3-fold from day 1 to day 4 of lactation and remains elevated throughout lactation. Within 48 h after weaning, γ-glutamyl transpeptidase activity falls to a value comparable with that from tissue obtained from rats in mid-pregnancy. Although fewer time periods were examined, Puente *et al.* (1979) observed similar changes in rat mammary γ-glutamyl transpeptidase during pregnancy and lactation. The adaptations we observed in γ-glutamyl transpeptidase during pregnancy, lactation and involution are similar to those occurring in many enzymes that have important roles in mammary function and milk synthesis (see review by Baldwin & Yang, 1974).

Kuhn (1977) and Tucker (1974) have summarized the possible endocrine involvement in the regulation of mammary metabolism during lactogenesis. Studies both *in vitro* and *in vivo* have demonstrated that prolactin plays a central role in the 'lactogenic complex' required for the initiation of milk synthesis. We investigated the effect of prolactin on γ-glutamyl transpeptidase activity by using bromocryptine. As shown in Fig. 2, treatment with bromocryptine dramatically decreased milk yield as shown by pup weight gains. At day 4 of lactation, pup weight gain from bromocryptine-treated

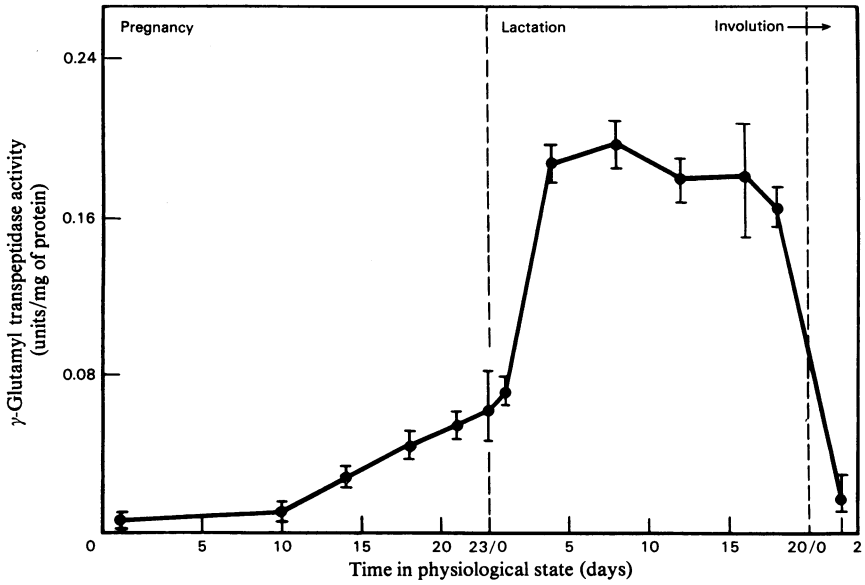

Fig. 1. *γ-Glutamyl transpeptidase activity of rat mammary tissue during pregnancy, lactation and involution*
Rats were killed at the times indicated. Mammary tissue homogenate was assayed for γ-glutamyl transpeptidase activity (see the Experimental section). Enzyme activity is expressed as units/mg of filtrate protein. Values are means ± S.E.M.

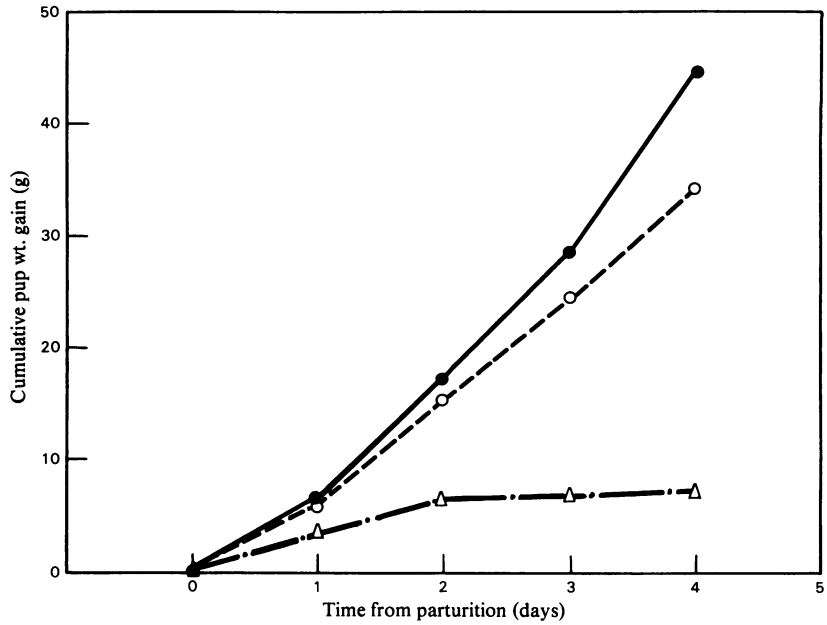

Fig. 2. *Effects of prolactin on cumulative pup weight gain*

Treatments were: △, bromocryptine (0.1 mg/day); ○, bromocryptine (0.1 mg/day) + prolactin (25 i.u./day); ●, control (placebo injections). Treatment was initiated on day 17 of pregnancy and continued until the animals were killed. Cumulative litter weight gain was monitored daily for the first 4 days of lactation (see the Experimental section). Each value is the average cumulative litter weight gain for three animals.

mothers was 17% of that of controls. This response seems to be primarily related to prolactin, because it was largely overcome by the simultaneous administration of 25 i.u. of bovine prolactin/day (Fig. 2).

The effect of treatment on the activity of γ-glutamyl transpeptidase activity is shown in Fig. 3. Clearly, the activity of γ-glutamyl transpeptidase parallels the treatment differences observed in pup weight gains. At day 4 of lactation, mammary tissue activity in bromocryptine-treated rats was on average 21% of that of the untreated controls. Across the three treatments, the simple correlation coefficient between daily pup weight gain and γ-glutamyl transpeptidase activity (based on activity/mg of filtrate protein) was 0.964.

Simultaneous administration of bromocryptine and bovine prolactin did not completely restore milk yield (pup gain) or γ-glutamyl transpeptidase activity to the values observed for the controls. This could be due to other effects of bromocryptine, or that the 25 i.u./day of bovine prolactin was not sufficient to overcome the lack of endogenous prolactin secretion. Bromocryptine specifically blocks prolactin release from the anterior pituitary, and has no known effect on other hormones (Cassidy & Floss, 1977). This drug has been used to study synergisms in the pituitary release of prolactin in rats (Gala & Boss, 1975) and to investigate the role of prolactin in establishing lactation (Flückiger & Wagner, 1968; Agius *et al.*, 1979). In our study, we monitored several other parameters that could reflect other possible effects of bromocryptine that might be unrelated to prolactin. However, the three treatment groups did not differ in maternal feed intake during pregnancy and lactation, or in the number of liveborn or the total litter weight at parturition (data not shown).

Our studies demonstrate the involvement of prolactin in the adaptations of γ-glutamyl transpeptidase that occur at the onset of lactation. Puente *et al.* (1979) examined the role of oestrogen and progesterone with respect to mammary γ-glutamyl transpeptidase activity. Treatment of ovariectomized rats with oestradiol-17β and progesterone gave increases in this enzyme that corresponded to the activity observed at midpregnancy. It is possible that prolactin is also involved in the response observed by Puente *et al.* (1979). Administration of oestrogen stimulates the pituitary release of prolactin (Meites & Clemens, 1972), resulting in increased circulating prolactin and an increase in

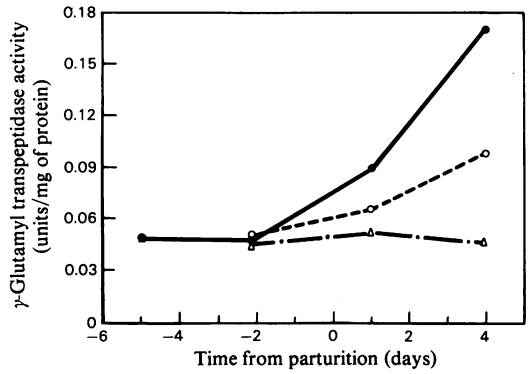

Fig. 3. *Effects of prolactin on γ-glutamyl transpeptidase activity*

Treatments were: △, bromocryptine (0.1 mg/day); ○, bromocryptine (0.1 mg/day) + prolactin (25 i.u./day); ●, control (placebo injections). Treatment was initiated on day 17 of pregnancy and continued until the animals were killed. Mammary tissue homogenate was assayed for γ-glutamyl transpeptidase activity (see the Experimental section). Enzyme activity is expressed as units/mg of filtrate protein.

the total number of mammary receptors for prolactin (Djiane *et al.*, 1977). Additional studies will be required for a definitive conclusion.

The timing and magnitude of the changes in γ-glutamyl transpeptidase activity during pregnancy, lactation and involution, as well as the involvement of prolactin in the adaptations that occur during lactogenesis, suggest this enzyme plays an important role in mammary function. The results from our studies are consistent with a role for this enzyme in amino-acid transport, as first proposed by Meister (1973). However, enzymes associated with many aspects of mammary metabolism also undergo dramatic changes in activity (Baldwin & Yang, 1974; Tucker, 1974; Kuhn, 1977) at the same periods as we observed for γ-glutamyl transpeptidase. Also, studies by Inoue *et al.* (1977) and by Griffith & Meister (1979) on the location and mode of action of γ-glutamyl transpeptidase have shifted the emphasis from a role in amino-acid transport to one of glutathione utilization (Griffith & Meister, 1979). Thus, additional studies are required to define the specific role of this enzyme in the synthesis and secretion of milk.

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