γ -Glutamyltransferase activity in mammary gland of pregnant rats and its regulation by ovarian hormones, prolactin and placental lactogen

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Ovariectomy and ovariectomy plus hysterectomy on day 18 of pregnancy increased γ -glutamyltransferase activity in the mammary gland. The withdrawal of progesterone and the subsequent release of prolactin are responsible for the rise in enzyme activity. Rat placental lactogen in the absence of prolactin and progesterone is able to induce γ -glutamyltransferase activity.

It has been reported that γ -glutamyltransferase (EC 2.3.2.2) is involved in amino acid translocation into the lactating mammary gland (Viña *et al.*, 1981a). The enzyme progressively increases in the pregnant mammary gland, but a sharp increase occurs immediately after parturition (Puente *et al.*, 1979; Pocius *et al.*, 1980). Ovarian steroids appear to affect the activity of γ -glutamyltransferase in mammary gland of ovariectomized pseudo-pregnant rats (Puente *et al.*, 1979). In previous studies, we have established that the surges of oestrogen, prolactin and other lactogenic hormones after the withdrawal of ovarian progesterone at the end of pregnancy play an important role in the induction of lactogenesis (Vermouth & Deis, 1972, 1974; Bussmann & Deis, 1979; Deis & Delouis, 1983; Bussmann *et al.*, 1983). The present study was undertaken to examine the participation of the ovarian and placental hormones in the γ -glutamyltransferase activity at the end of pregnancy.

Materials and methods

Animals

White nulliparous rats approx. 3 months old (180–200 g) were used. They were each placed with a male rat during the night after proestrus. The next morning was counted as day 0 of pregnancy if spermatozoa were found in the vaginal smear. In our colony, rats usually deliver on day 22. The rats were kept in a constant-temperature room ($24 \pm 2^\circ\text{C}$) in which the lighting schedule was 14 h light:10 h dark (lights on 06:00–20:00 h). The experimental animals were divided into seven groups and treated as follows: group 1, sham ovariectomy on day 18 of pregnancy; group 2, ovariectomy on day 18; group 3, ovariectomy on

day 18 plus progesterone treatment; group 4, ovariectomy on day 18 plus oestradiol benzoate 12 h after surgery; group 5, ovariectomy on day 18 and treatment with CB-154 on days 18 and 19; group 6, ovariectomy on day 18; group 7, ovariectomy on day 18 and treatment with CB-154.

Surgical procedures and drug administration

All surgical procedures were started between 08:00 and 10:00 h on day 18 of pregnancy. Bilateral ovariectomy was performed through dorsolateral incisions under diethyl ether anaesthesia. Ovariectomy was performed through a ventral incision. In the sham-operated groups only the dorsolateral or ventral incisions were performed. 2-Bromo- α -ergocryptine mesylate (CB-154; Sandoz, Basel, Switzerland) (1.5 mg/kg) was injected subcutaneously every 12 h, starting immediately after surgery. In the ovariectomized groups treated subcutaneously with ovarian steroids, oestradiol benzoate (1 μg) was injected 12 h after surgery and progesterone (5 mg) immediately after operation and 12 h after. All rats were killed by decapitation 28 h after surgery and the inguinal mammary gland was dissected out immediately afterwards, wrapped in aluminium foil and kept frozen until the assay of γ -glutamyltransferase activities. Mammary tissue (1 g) was cut into small pieces and homogenized in 4 ml of 10 mM-Tris/HCl/80 mM-MgCl₂ buffer, pH 8.0, with an Ultra-Turrax homogenizer. The homogenate was centrifuged at 600 g for 5 min; the supernatant, under the fat layer, was recovered with a Pasteur pipette and used for the enzyme-activity determination. γ -Glutamyltransferase activity was determined spectrophotometrically at 37°C with 4 mM- γ -glutamyl *p*-nitroanilide as donor and 50 mM-glycylgly-

cine as acceptor in 50mM-Tris/HCl/75mM-NaCl buffer, pH8.2. The appearance of *p*-nitroaniline was followed at 405nm. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. All drugs and cofactors were obtained from Sigma. Student's *t* test was used to assess significance.

Results and discussion

Effect of ovariectomy on γ -glutamyltransferase activity and after treatment with oestrogen or progesterone (Table 1)

Ovariectomy of pregnant rats induced a significant rise in γ -glutamyltranspeptidase activity ($P < 0.001$) 28 h after surgery. When progesterone was administered to ovariectomized rats, γ -glutamyltransferase activity was decreased to control values. On the contrary, the enzymic activation was not affected by oestrogen treatment. It is well established that the withdrawal of progesterone at the end of pregnancy by ovariectomy (Vermouth & Deis, 1974; Bussmann *et al.*, 1983) or prostaglandin $F_{2\alpha}$ treatment (Vermouth & Deis, 1972; Bussmann & Deis, 1979) induced a rapid and significant increase in serum prolactin concentration. This increase is prevented by progesterone treatment (Vermouth & Deis, 1972, 1974). On the other hand, more recent studies demonstrated the involvement of prolactin in the increased activity of γ -glutamyltransferase in rat mammary gland during lactation (Pocius *et al.*, 1980). The present results give support to the proposition that γ -glutamyltransferase activity is progesterone-dependent. Since prolactin release

follows progesterone decay, it is most probable that the association of a progesterone decrease and a simultaneous prolactin release is an important step for the induction of γ -glutamyltransferase activity increase at the end of pregnancy.

We have shown that oestrogen enhanced the action of prolactin on lactose synthetase activity (Deis & Delouis, 1983; Bussmann *et al.*, 1983), without affecting galactosyltransferase activity (Deis & Delouis, 1983) in the mammary gland of ovariectomized pregnant rats. However, in the present study γ -glutamyltransferase activity was not increased by oestrogen. If we consider that, 28 h after ovariectomy, γ -glutamyltransferase had reached nearly maximum values, the oestrogen might not be able to increase this enzymic activity further. Puente *et al.* (1979) showed in mammary gland of ovariectomized virgin rats that treatment with oestradiol-17 β , alone or associated with progesterone, gave increases in γ -glutamyltransferase.

Effect of CB-154 on γ -glutamyltransferase in ovariectomized and ovari hysterectomized pregnant rats (Table 1)

A rise in γ -glutamyltransferase activity similar to that obtained in ovariectomized pregnant rats was observed when hysterectomy plus ovariectomy was performed. Treatment with CB-154 did not prevent the increase in γ -glutamyltransferase activity observed in the untreated ovariectomized rats. However, in the ovariohysterectomized groups, CB-154 inhibited enzyme activity significantly ($P < 0.01$) to below the value in untreated sham-operated rats in which placentae were present. Treatment of ovariectomized pregnant rats with CB-154 prevented the serum prolactin release (Bussmann *et al.*, 1983), and the same effect is expected to happen in the ovariohysterectomized groups. However, only in the latter group the increase of γ -glutamyltransferase activity prevented. This is clear evidence that rat placental lactogen, which is still high at day 19 of pregnancy (Bussmann & Deis, 1979), may replace the effect of prolactin on γ -glutamyltransferase activity when the pregnant rat is deprived of prolactin and progesterone. We have previously shown that rat placental lactogen induced lactose synthetase activity in pregnant rats in the absence of prolactin and progesterone (Bussmann *et al.*, 1983). It is worth considering the significant decrease in the activity of γ -glutamyltransferase in the ovariohysterectomized rats treated with CB-154, compared with the values obtained in the untreated sham-operated pregnant rats. This finding suggests that the gradual increase of γ -glutamyltransferase from day 10 of pregnancy (Puente *et*

Table 1. γ -Glutamyltransferase activity after ovariectomy and ovariohysterectomy: effect of ovarian hormones and CB-154 treatment

For full experimental details see the text. The values for each group are means $\pm$ S.E.M. for eight rats.

Treatment	γ -Glutamyltransferase activity (munits/mg of protein)
Sham operation	89.5 $\pm$ 4.9
Ovariectomy	124.2 $\pm$ 3.7
Ovariectomy + progesterone	82.8 $\pm$ 6.6
Ovariectomy + oestradiol benzoate	127.2 $\pm$ 6.6
Ovariectomy + CB-154	123.8 $\pm$ 5.5
Ovariectomy + hysterectomy	131.2 $\pm$ 5.4
Ovariectomy + hysterectomy + CB-154	59.7 $\pm$ 5.7

al., 1979; Pocius *et al.*, 1980) is mainly due to rat placental lactogen and not to the small basal concentrations of circulating prolactin in the second half of pregnancy. Previous studies (Puente *et al.*, 1979; Pocius *et al.*, 1980; Viña *et al.*, 1981*a,b*) have shown the role of γ -glutamyltransferase during lactation. The present study gives evidence of an important participation of the enzyme during lactogenesis.

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