Regulation of mouse mammary-gland γ -glutamyltranspeptidase mRNA during pregnancy, lactation and weaning

Sylvie SIEGRIST,* Yannick LAPERCHE,* Marie Noelle CHOBERT,* Frédérique BULLE,* Hira L. NAKHASI† and Georges GUELLAËN*†

*Unité INSERM 99 Hôpital H. Mondor, 94010 Creteil, France, and †Division of Biochemistry and Biophysics, Food and Drug Administration, Bethesda, MD 20892, U.S.A.

The level of γ -glutamyltranspeptidase (GGT) activity and of its mRNA were determined in the mouse mammary gland during pregnancy, lactation and weaning. The GGT activity, which is very low in the virgin-mouse mammary gland (5 munits/mg of protein), increases progressively during pregnancy (3-fold), reaches its maximum at the onset of lactation (8-fold) and returns rapidly to basal level at weaning. Although no GGT-specific mRNA is detected in the virgin-mouse mammary gland, a single faint band of 2.2 kb in size is found during pregnancy. During lactation, an additional mRNA of 2.4 kb in size appears, and the level of both mRNAs is higher. This high level of mRNA persists during weaning as well. Southern-blot analysis of mouse mammary-gland DNA provides convincing evidence that there is only one gene which codes for the two mRNAs. The present study provides the first evidence for a physiological regulation of the two GGT mRNAs in the same tissue.

INTRODUCTION

γ -Glutamyltranspeptidase (GGT, EC 2.3.2.2) catalyses the degradation of glutathione to glutamic acid and cysteinylglycine [1]. The two GGT subunits, which are encoded by a common mRNA [2,3], are located at the outer surface of the plasma membrane and play a key role in inter-organ glutathione transport [4].

Although GGT is expressed at a high level in the kidney [1], the GGT expression in other tissues, such as liver and mammary gland, has attracted considerable attention because of its regulation under different physiopathological conditions in these tissues. In humans, liver GGT activity is increased during chronic alcoholism [5] or in hepatocellular carcinoma [6,7]. In rat-liver-derived cell lines, GGT activity is also increased by alcohol [8], glucocorticoids [9,10] and carcinogens [11]. In the last-named case the hepatic GGT activity is correlated with an increase in a specific GGT mRNA different from the one present in kidney [12].

In rat mammary gland, GGT activity increases during pregnancy and, to a greater extent, at the onset of lactation [13,14]. The activity is under the control of both placental lactogen and prolactin [13–15]. However, to date there are no data on the level and mechanism of the regulation of GGT-gene expression in mammary gland. In the present study we used a rat kidney GGT cDNA as a probe [16] to analyse the expression of GGT mRNA in the mammary gland during pregnancy, lactation and weaning.

EXPERIMENTAL

Animal and tissue preparation

Mammary glands from primiparous (C3H,HeN) mice at different stages of pregnancy, lactation and mammary involution were used for both enzymic assays and mRNA preparations. Mammary glands and kidneys from nulliparous mice were used as controls.

Enzymic assays

Mammary glands were homogenized (10 mg/ml) in 50 mM-Tris/HCl, pH 7.4, using an Ultra-Turrax blender. The GGT activity of these extracts was assayed by using L- γ -glutamic acid *p*-nitroanilide as donor and glycylglycine as acceptor [17]. Protein was determined as described by Lowry *et al.* [18], with BSA as standard. One unit of enzyme activity is defined as 1 μ mol of *p*-nitroaniline released/min at 37 °C.

Isolation of poly (A)⁺ mRNA

Total mRNA from mammary gland and kidney was isolated by the guanidine isothiocyanate method [19]. Poly(A)⁺ mRNA were purified by two cycles of oligo(dT)/Trisacryl chromatography (IBF Biotechnics) as described by Aviv & Leder [20].

Preparation of single-stranded RNA probe

A 1.6-kbp endonuclease-*Pst*I–*Pvu*II fragment from rat kidney cDNA cloned in plasmid pSP 64/39-1 [21] and containing 92 % of the GGT coding sequence was subcloned into plasmid PGEM-4 (PGEM-4/1-39). A RNA probe, complementary to the mRNA strand, was transcribed from the phage-T₇-polymerase-specific promoter of this plasmid linearized by *Sac*I. Transcription was performed in the presence of [α -³²P]UTP (sp. radioactivity 3000 Ci/mmol) (Amersham International) under the conditions recommended by the supplier [(Riboprobe System) Promega Biotechnology, Madison, WI, U.S.A.

Northern-blot hybridization

Poly(A)⁺ mRNA was denatured in 20 mM-Mops (pH 7.8)/8 mM-sodium acetate/1 mM-EDTA/50 % (v/v) formamide/2.2 M-formaldehyde, fractionated on a 1.5 % (w/v) agarose/2.2 M-formaldehyde denaturing gel and transferred on to a nylon membrane (Hybond-N, Amersham International) [22]. The RNA was fixed on to the membrane by u.v. irradiation for 5 min and by baking for 2 h at 80 °C. The blot was prehybridized overnight at 60 °C in 50 % formamide/5 % (w/v) SDS/5 × SSPE containing 200 μ g of denatured salmon sperm DNA/ml [23]. The GGT-specific RNA probe (10⁹ c.p.m./ μ g of template) was then added

Abbreviations used: GGT, γ -glutamyltranspeptidase; SSPE, 750 mM-NaCl/50 mM-NaH₂PO₄/5 mM-EDTA, pH 7.4; poly(A)⁺, polyadenylated.

† To whom correspondence and reprint requests should be sent.

in the same medium. Hybridization was carried out at 60 °C for 16–20 h and the blot was washed twice in 0.1 % SDS/0.1 × SSPE for 1 h at 68–70 °C and exposed to Amersham X-ray film at –80 °C with an intensifying screen. A 0.8-kb *SmaI*–*KpnI* nick-translated fragment of a mouse cDNA β -actin (pAL 41) clone [24] was used in control experiments.

Quantification of the relative amounts of specific RNA transcripts was performed by densitometric analysis of the hybridization signals by using an Ultrosan laser densitometer associated with microcomputer software (GENOFIT).

Southern-blot hybridization

High-molecular-mass DNA was extracted from Wistar-rat or C57-Black-mouse kidney as previously described [25]. Human DNA was extracted from human lymphocytes by the same protocol, except that the sample was dissolved directly in the urea buffer. The Southern blot was hybridized with the nick-translated *EcoRI*–*Pst* fragment from clone 39 [25] specific for rat kidney GGT.

RESULTS AND DISCUSSION

GGT activity in mammary gland

The activity of GGT in mouse mammary gland at different stages of pregnancy and lactation is shown in Fig. 1. Activity is very low in virgin mammary tissue (4 munits/mg of protein) and gradually increases during pregnancy and lactation, reaching 30–40 units/mg of protein. At weaning, the activity returns to a value comparable with that observed in virgin-mouse mammary gland. Similar changes in rat mammary GGT activity during pregnancy and lactation have been described [13–14]. The changes in GGT activity during pregnancy, lactation and involution are similar to those observed with proteins that participate in milk synthesis, such as α -lactalbumin and caseins [26].

Fig. 1. GGT activity of mouse mammary tissue during pregnancy, lactation and involution

Mice were killed at the times indicated. Mammary tissue homogenate was assayed for GGT activity, which is expressed in munits/mg of protein. The data presented are representative of two independent experiments.

Fig. 2. Northern-blot analysis of mouse mammary gland mRNA (mMG) in different physiological states

A portion (1 μ g) of poly(A)⁺ RNA taken at the virgin (V), pregnancy (P), lactation (L) or weaning (W) stage was used, except that for 10-day-pregnant-mouse mammary gland a 3 μ g portion was also used (*); 50 ng of mouse kidney (mK) and 50 ng of rat kidney (rK) poly(A)⁺ RNA were used as controls. The blot was hybridized with a single-strand RNA probe specific for rat GGT as described in the Experimental section. The nylon membrane was autoradiographed at –80 °C for 3 h (*) or for 24 h with an intensifying screen.

Fig. 3. GGT mRNA levels in mouse mammary gland during pregnancy, lactation and weaning

The intensities of the bands at 2.2 kb (Δ) or 2.4 kb (∇) were determined by densitometric analysis (a) of the autoradiogram (Fig. 2). The relative ratio of the intensities of both bands (2.4 kb/2.2 kb) is represented in the upper panel (b).

Fig. 4. Southern blot of rat (a), mouse (b) and human (c) genomic DNA

Genomic DNA (15 μ g) was digested with restriction enzymes (B, *Bam*H1; K, *Kpn*I) and electrophoresed on a 0.8 % agarose gel. After transfer to nylon membrane the DNA fragments were hybridized to the nick-translated probe specific for rat GGT as described in the Experimental section. The nylon membrane was autoradiographed at -80°C for 48 h with an intensifying screen.

The changes in the levels of these proteins reflect the changes in their mRNA levels [26]. It has been shown that the production of both α - and κ -casein mRNA is modulated by prolactin and epidermal growth factor [27,28]. As for GGT, according to the work of Viña & Viña [29], the increase in GGT protein in the lactating mammary gland would contribute to the amino acid translocation that takes place during lactation.

Analysis of GGT mRNA in mammary gland

GGT poly(A)⁺ mRNA from mouse mammary gland during pregnancy, lactation and weaning were analysed by Northern-blot hybridization using a GGT single-stranded RNA probe and compared with kidney mRNA as control (Fig. 2). No mRNA was detected in the virgin-mouse mammary gland. A single mRNA of size 2.2 kb was detected in pregnant-mouse mammary-gland RNA, whereas, during lactation and weaning, two mRNA species of 2.2 and 2.4 kb respectively were observed. The changes in the level of GGT mRNAs during lactation were analysed by densitometric scanning of the autoradiograms (Fig. 3a). Compared with the situation at 10 days of pregnancy, GGT mRNAs rise 5-fold at 2 days *post partum*, reaching a peak (10–15-fold) at 8 days, followed by a decline at 15 days *post*

partum. They remain elevated at weaning. The rapid increase in the hybridization intensity correlates with the increased level of GGT activity in the mammary gland (Fig. 1). Although both GGT mRNAs are induced during lactation, the analysis of the ratio of the two mRNA intensities as a function of the physiological state (Fig. 3b) exhibits a variation in the relative amount of the two species. The ratio of the amount of 2.4 kb mRNA to 2.2 kb mRNA increases gradually throughout lactation, i.e. from 0 to 0.8 at 15 days *post partum* (Fig. 3b), and it parallels the increase in the GGT activity (Fig. 1). This suggests that the regulation of GGT during lactation would involve more specifically the 2.4 kb mRNA than the 2.2 kb mRNA species.

Analysis of mouse genomic DNA

Recently we have demonstrated that there is only one gene encoding GGT in rat, but multiple genes in humans [25]. The complexity of the GGT sequences in the mouse genome was assessed on a Southern blot by hybridization of mouse genomic DNA digested by restriction enzymes to a rat cDNA probe (Fig. 4). The hybridization pattern on the mouse DNA is very similar to that of the rat and is less complex than the human situation. This suggests that there is also a single GGT gene in mouse coding for the two mRNA species.

In the present study: (i) we have the first evidence for the co-expression of two GGT mRNAs in the same tissue whose concentrations vary under physiological conditions; (ii) we have observed a similar hybridization pattern of the GGT probe on mouse and rat genomic DNA. Recently, we have cloned and sequenced two full-length cDNAs from rat kidney which differ only in their 5' non-coding region [16]. Since these GGT mRNAs are transcribed from a single gene, they are likely to be generated by start of transcription at two different promoters [30]. Thus the synthesis of two GGT mRNAs in mouse mammary gland is due either to the initiation of transcription from two different promoters of the single GGT gene, as in rat, and/or to an alternate splicing mechanism.

REFERENCES

1. Tate, S. & Meister, A. (1981) *Mol. Cell. Biochem.* **39**, 357–368
2. Finidori, J., Laperche, Y., Tsapis, R., Barouki, R., Guellaën, G. & Hanoune, J. (1984) *J. Biol. Chem.* **259**, 4687–4690
3. Nash, R. & Tate, S. (1984) *J. Biol. Chem.* **259**, 678–685
4. Curthoys, N. P. (1983) *Miner. Electrolyte Metab.* **9**, 236–245
5. Rosalki, S. B. & Rau, D. (1972) *Clin. Chim. Acta* **39**, 41–47
6. Gerber, M. A. & Thung, S. N. (1980) *Am. J. Pathol.* **98**, 895–900
7. Tauji, A., Matsuda, Y., & Katunuma, N. (1980) *Clin. Chim. Acta* **104**, 361–366
8. Barouki, R., Chobert, M. N., Finidori, J., Aggerbeck, M., Nalpas, B. & Hanoune, J. (1983) *Hepatology* **3**, 323–329
9. Billon, M. C., Dupre, G. & Hanoune, J. (1980) *Mol. Cell. Endocrinol.* **18**, 99–108
10. Barouki, R., Chobert, M. N., Billon, M. C., Finidori, J., Tsapis, R. & Hanoune, J. (1982) *Biochim. Biophys. Acta* **721**, 11–21
11. Cameron, R., Kellen, J., Kolin, A., Malkin, A. & Farber, E. (1978) *Cancer Res.* **38**, 823–829
12. Power, C. A., Griffiths, S. A., Simpson, J. L., Laperche, Y., Guellaën, G. & Manson, M. M. (1987) *Carcinogenesis* **68**, 737–740
13. Puente, J. A., Varas, M. A., Beckhaus, G. & Sapag-Hager, M. (1979) *FEBS Lett.* **99**, 215–218
14. Pocius, P. A., Baumrucker, C. R., McNamara, J. P. & Bauman, D. E. (1980) *Biochem. J.* **188**, 565–568
15. Bussman, L. E. & Deis, R. P. (1984) *Biochem. J.* **223**, 275–277
16. Laperche, Y., Bulle, F., Aissani, T., Chobert, M. N., Aggerbeck, M., Hanoune, J. & Guellaën, G. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **63**, 937–941
17. Tate, S. S. & Meister, A. (1974) *J. Biol. Chem.* **249**, 7593–7602
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

19. Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
20. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1408–1412
21. Bulle, F., Mattei, M. G., Siegrist, S., Pawlak, A., Passage, E., Chobert, M. N., Laperche, Y., & Guellaen, G. (1987) *Hum. Genet.* **76**, 283–286
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Rebagliati, M. R., Weeks, D. L., Harvey, R. P. & Melton, D. A. (1985) *Cell* (Cambridge, Mass.) **42**, 769–777
24. Alonso, S., Minty, A., Bourlet, Y. & Buckingham, M. (1986) *J. Mol. Evol.* **23**, 11–22
25. Pawlak, A., Lahuna, O., Bulle, F., Suzuki, A., Ferry, N., Siegrist, S., Chikki, N., Chobert, M. N., Guellaen, G. & Laperche, Y. (1988) *J. Biol. Chem.* **263**, 9913–9916
26. Nakhasi, H. L. & Qasba, P. K. (1979) *J. Biol. Chem.* **254**, 6016–6025
27. Nakhasi, H. L., Grantham, F. H. & Gullino, P. M. (1984) *J. Biol. Chem.* **259**, 14894–14898
28. Vonderaar, B. K. & Nakhasi, H. L. (1986) *Endocrinology* (Baltimore) **119**, 1178–1184
29. Viña, J. & Viña, R. (1983) in *Function of Glutathione: Biochemical, Physiological, Toxicological and Chemical Aspects* (Larsson, A., ed.), pp. 23–30, Raven Press, New York
30. Chobert, M. N., Lahuna, O., Lebargy, F., Kurauchi, O., Darbouy, M., Bernaudin, J. F., Guellaen, G., Barouki, R. & Laperche, Y. (1990) *J. Biol. Chem.* **265**, 2352–2357

Received 9 October 1989; accepted 21 November 1989