Mammary Glucocorticoid Receptor of Mice in Pregnancy and in Lactation

By PIOTR CHOMCZYNSKI and LECH ZWIERZCHOWSKI

*Department of Biochemistry, Institute of Genetics and Animal Breeding,
Polish Academy of Sciences, Jastrzębiec, 05-551 Mroków, Poland*

(Received 13 May 1976)

Activity of glucocorticoid receptor in mouse mammary cytosol changes during lactogenesis. The highest receptor activity is observed in the second half of pregnancy. The receptor from mammary glands from lactating and pregnant mice exhibits the same sedimentation pattern, as well as the same specificity and affinity for binding of steroid hormones.

It is commonly accepted that binding to receptors is essential for the transfer of steroid hormones to the nucleus and for their subsequent effect on DNA transcription (Raspe, 1971). If so, variation of the amount of receptor available might regulate tissue response to endocrine control. We have studied whether the glucocorticoid receptor concentration in the cytoplasm is a function of mammary-gland development during lactogenesis. Dexamethasone, a potent synthetic glucocorticoid, was used, and its binding to the receptor was determined in the mammary-gland cytosols isolated from virgin, pregnant and lactating mice.

Materials and Methods

Mammary tissue was obtained from CF/W mice (12–16 weeks old). Animals were either in their first pregnancy or in their first lactation.

[1(2)-³H]Dexamethasone (19.1 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Isolation of cytosol

Mice were killed by cervical dislocation. Abdominal and thoracic mammary glands were excised free of lymph nodes. The temperature throughout the preparation was kept at 0–4°C. Glands were minced with scissors and homogenized with 4 vol. (1 vol. for mammary gland from virgin mice) of 0.25 M-sucrose/10 mM-Tris/HCl (pH 7.8)/1.5 mM-EDTA in a glass homogenizer with a Teflon pestle. The homogenate was filtered through a nylon gauze. The filtrate was centrifuged for 15 min at 1000g, and the supernatant was centrifuged again for 1 h at 150 000g_{max.}. The high-speed supernatant (cytosol) was aspirated and used immediately for experiments.

Incubation of cytosol with [³H]dexamethasone and determination of specific binding

Cytosol (4–6 mg of protein/ml) was incubated with 17.5 nM-[³H]dexamethasone for 2 h at 2°C. Then 0.075 ml portions of cytosol (each sample in triplicate) were applied to DEAE-cellulose filters (DE-81; Whatman, Biochemicals, Maidstone, Kent, U.K.), and, after 1 min, filters were washed five times with the homogenization buffer (Santi *et al.*, 1973). Radioactive hormone–receptor complex retained on filters was dissolved in 0.8 ml of Hyamine hydroxide and counted for radioactivity in a liquid-scintillation spectrometer. Each series of experiments contained samples with cytosol and [³H]dexamethasone as well as samples with the same quantities of cytosol and [³H]dexamethasone plus unlabelled dexamethasone at 17.5 μM. The specific binding was determined from the difference between the two series of samples. Occasionally the charcoal method (Rousseau *et al.*, 1972) was used to test the specific binding and the results were the same as with the use of the DEAE-cellulose filter method.

Sucrose-gradient analysis

Samples of cytosol were applied to 5.2 ml gradients of 5–20% sucrose in 1.5 mM-EDTA/10 mM-Tris/HCl (pH 7.4). Gradients were centrifuged for 18 h at 2°C and 205 000g_{av.} in an MSE 3 × 6.5 ml swing-out rotor. Fractions of volume about 0.17 ml were collected directly into scintillation vials and counted for radioactivity in a dioxan scintillation fluid (Bray, 1960). The 7S region in a sucrose gradient was marked by the position of rabbit muscle aldolase.

Cytosol protein was determined as described by Lowry *et al.* (1951), and DNA in the mammary-gland homogenate was determined by the method of Burton (1956).

Results and Discussion

The specific binding of [3 H]dexamethasone in the mouse mammary cytosol was saturated at a hormone concentration ranging from 15 nM to 20 nM. The dissociation constant of binding of the steroid to the binding protein was estimated to be 3.9×10^{-9} M. The reaction was inhibited by glucocorticoids and progesterone, but not by oestradiol, testosterone, cortisone and androsterone. Thus the binding reaction showed properties similar to those established for the mammary-gland glucocorticoid receptor of mouse (Shyamala, 1973) and rat (Gardner & Wittliff, 1973). With respect to the specificity and the affinity of binding of steroid hormones, we found no differences between the mammary-gland glucocorticoid receptor isolated during pregnancy and the one isolated during lactation. Sedimentation coefficients of the receptors were determined by a sucrose-density-gradient analysis after binding of [3 H]dexamethasone *in vitro* (Fig. 1). The mammary-gland glucocorticoid receptor

Fig. 1. Sedimentation patterns of [3 H]dexamethasone-receptor complex from cytosol isolated from mammary gland in various physiological states

Cytosol was incubated with 17.5 nM [3 H]dexamethasone for 2 h at 2°C (—; V, virgin; P, pregnant; L, lactating; protein concentration 11.6, 5.6 and 13.3 mg/ml respectively) or with [3 H]dexamethasone plus 17.5 μ M unlabelled dexamethasone (---; sedimentation patterns of samples of mammary glands from virgin, pregnant and lactating mice were the same). After incubation, 0.2 ml of cytosol was applied to the 5–20% sucrose gradient and centrifuged as described in the Materials and Methods section.

from virgin, pregnant and lactating mice sedimented in the same region of 7S in a low-ionic-strength buffer. Binding of [3 H]dexamethasone to the 7S receptor was eliminated in the presence of unlabelled dexamethasone. The results implied that the molecule of glucocorticoid receptor did not change during the development of mammary gland. According to the sedimentation curves, the highest receptor activity was observed with the use of lactating cytosol. However, it should be noted that, under the conditions used for homogenization and centrifugation of mammary tissue, 0.2 ml of cytosol applied to a sucrose gradient contained various amounts of protein (the greatest amount in lactating cytosol). The DEAE-cellulose filter method was used to determine the changes of dexamethasone-binding capacity of cytosol during lactogenesis (Fig. 2). Amounts of dexamethasone bound to the receptor are expressed in pmol/mg of cytosol protein. In mammary gland from virgin mice, there was a considerable amount of dexamethasone bound to the receptor. From day 10 of pregnancy, binding activity in the cytosol increased, reaching its maximum value at days 16 and 17 of pregnancy. Before parturition, the amount of dexamethasone bound in cytosol/mg of protein decreased rapidly to the amount

Fig. 2. Changes in dexamethasone-binding capacity of cytosol during mammary-gland development

Vertical lines indicate the range observed when mammary-gland cytosols, isolated separately from three to five mice, were used. Preparation of cytosol and determination of binding capacity were performed as described in the Materials and Methods section.

observed during lactation. Throughout the first 5 days of lactation, the binding capacity of cytosol remained relatively constant. As calculated in pmol of [3 H]dexamethasone/mg of mammary gland, DNA-binding capacity of cytosol was 0.45 and 1.28 for days 10 and 16 of pregnancy, and 0.76 and 0.78 for days 1 and 5 of lactation. Expression of the dexamethasone-binding capacity on the basis of DNA leads to the conclusion that there is a significant increase in the amount of active receptor molecules in mammary cells by day 16 of pregnancy.

Binding assays were performed after dialysis to make certain that the observed changes in receptor activity were not due to complexing of the receptor by an endogenous hormone. Cytosols isolated on days 10 and 16 of pregnancy and day 5 of lactation were dialysed for 16 h against [3 H]dexamethasone to remove any endogenous hormone from the cytosol and from the receptor-hormone complex (Rousseau *et al.*, 1972). Cytosol from mammary gland of 16-day-pregnant mice still showed the highest dexamethasone-binding capacity (results not shown).

Thus the results indicate that the concentration of mammary-gland glucocorticoid receptor (as measured by its ability to bind dexamethasone) depends on the physiological state of the mammary gland. As has been suggested by Shyamala (1973), the receptor could regulate glucocorticoid action in the mammary gland. In view of our results, this regulation could be accomplished by means of a change in the amount of the cytoplasmic receptor within the mammary cell.

References

- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285
Burton, K. (1956) *Biochem. J.* **62**, 315–323
Gardner, D. G. & Wittliff, J. L. (1973) *Biochim. Biophys. Acta* **320**, 617–627
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Raspe, G. (ed.) (1971) *Adv. Biosci.* **7**
Rousseau, G. G., Baxter, J. D. & Tomkins, G. M. (1972) *J. Mol. Biol.* **67**, 99–115
Santi, D. V., Sibley, C. H., Perriard, E. R., Tomkins, G. M. & Baxter, J. D. (1973) *Biochemistry* **12**, 2412–2416
Shyamala, G. (1973) *Biochemistry* **12**, 3085–3090