PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 763–765 (1975)

Guanylate Cyclase and Cyclic-GMP Phosphodiesterase Activities in Mammary Glands of Mice during Pregnancy and Lactation[1] (39121)

R. DAVID SMITH AND JAMES A. RILLEMA[2]

Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201

Introduction. It has recently been reported from this laboratory that cyclic-3′:5′-guanosine monophosphate (cyclic-GMP) mimics certain of the metabolic actions of prolactin in the mouse mammary gland *in vitro* (1). These observations make tenable the idea that the stimulation of lactational processes by prolactin following parturition may be mediated via cyclic-GMP. The fact that cyclic-GMP concentrations are elevated in lactating rat mammary tissue vs tissues from pregnant rats was recently documented by Sapag-Hagar and Greenbaum (2). Since the intracellular concentration of cyclic-GMP is determined, at least in part, by the relative activities of guanylate cyclase and cyclic-GMP phosphodiesterase, the activities of these enzymes in mammary tissues from virgin, pregnant, and lactating mice were measured.

Materials and methods. Timed-pregnant, Swiss Webster mice were purchased from Spartan Research Animals Inc., Haslett, Mich. The animals were killed during specific days of pregnancy and lacation. If the animals to be studied were lactating for more than 1 day, the number of pups was adjusted to 10 on Day 1 of lactation.

Mammary tissues were prepared for enzyme assays by the following methods. Abdominal and inguinal mammary glands were excised, placed in an appropriate buffered media at 0–4°C and finely minced. Some tissues were then immediately assayed for enzyme activities while others were frozen in liquid nitrogen and stored at −70°C until assayed. Freezing and storage at −70°C for periods up to 1 week did not change the tissue content of guanylate cyclase or cyclic-GMP phosphodiesterase.

When guanylate cyclase activity was to be measured, the tissues were homogenized at 0–4°C in a ground glass homogenizer in 1:10 (w/v) 0.04 *M* Tris–HCl buffer, pH 7.4, containing 0.25 *M* sucrose, 0.01 *M* MnCl and 0.1 *M* theophylline. Guanylate cyclase activity was assessed by the rate of conversion of α-labeled [^{32}P]GTP to [^{32}P]cyclic-GMP as is described in an adjoining publication (3).

Cyclic-GMP phosphodiesterase activity was determined in tissues homogenized at 0–4°C in a ground glass homogenizer in 1:10 (w/v) 0.04 *M* Tris–HCl buffer, pH 7.4, containing 0.25 *M* sucrose. Enzyme activity in 0.1 ml of this homogenate was measured using the method of Beavo *et al.* (4). This method is based on the rate of conversion of ^{3}H-cyclic-GMP to ^{3}H-5′-GMP. Since two K_m values have been reported for cyclic-GMP phosphodiesterase, enzyme activity was measured using two substrate concentrations (10 μM and 100 μM cyclic-GMP). The enzyme reaction was carried out in the following manner. Mammary gland homogenate (0.1 ml) was mixed with 0.15 ml of a buffered solution such that the reaction mixture contained the following: 0.04 *M* Tris–HCl, pH 7.4, 0.002 *M* $MgCl_2$, 10 or 100 μM cyclic-GMP (ICN Nutritional Biochemicals) and 0.5–1.0 μCi/ml cyclic-^{3}H-GMP (New England Nuclear Corp.). The reaction was initiated and carried out by placing the reaction tubes in a water bath maintained at 30°C. Incubations were carried out for 3 or 6 min and the reaction was terminated by immersing the reaction tubes in a boiling water bath for 2 min. Immediately prior to terminating the incubations, 0.05 ml of a solution containing 1.5 m*M* cyclic-GMP and 5 m*M* 5′-GMP (Sigma Chemical Co.) was added to the reaction mixtures. The ^{3}H-guanosine formed during the incubation period was converted to ^{3}H-guanine by a subsequent incubation with crotalus adamanteous venom

[1] This investigation was supported by NIH Grant Number HD 06571 from the National Institutes of Child Health and Human Development, U.S.A.

[2] To whom all correspondence should be addressed.

TABLE I. GUANYLATE CYCLASE AND CYCLIC-GMP PHOSPHODIESTERASE ACTIVITIES IN MAMMARY GLANDS FROM VIRGIN, PREGNANT, AND LACTATING MICE[a]

Physiological state	Guanylate cyclase (p*M*/min/mg tissue)	Cyclic-GMP phosphodiesterase (pmoles/min/mg tissue)	
		10 μM cyclic-GMP	100 μM cyclic-GMP
Virgin	0.304 ± 0.018 (11)[a]	49.9 ± 4.4 (6)[a]	121 ± 9 (6)[a]
10–15 days pregnant	0.408 ± 0.021 (11)[b]	87.0 ± 11.5 (5)[b]	163 ± 11 (5)[b]
18 days pregnant	0.403 ± 0.038 (9)[b]	82.4 ± 9.9 (8)[b]	162 ± 15 (8)[b]
1–3 days lactating	0.523 ± 0.019 (14)[c]	87.2 ± 7.4 (10)[b]	181 ± 11 (10)[b]

[a] Enzyme assays were carried out as described in the text. Numbers in the table represent the mean ± standard error of the number of observations indicated in parentheses. Statistical differences are as follows: a vs b ($P < 0.05$); b vs c ($P < 0.05$); a vs c ($P < 0.01$).

nucleotidase (Sigma Chemical Co.). The ^{3}H-guanine thus formed was then isolated via ion exchange chromatography through Dowex 2-X8 (100–200 mesh, chloride form) columns. These columns (0.8 × 2.5 cm) were initially washed with 4 ml of 0.04 *M* Tris–HCl buffer, pH 7.5. Samples were then added to the columns and the ^{3}H-guanine was removed from the columns by elution with 26 ml of the 0.04 *M* Tris–HCl buffer (pH 7.5). All other labeled nucleotides remained on the columns. Radioactivity in the 26 ml eluate was quantitated by liquid scintillation spectroscopy. Reaction blanks were also carried out with each assay and these values were subtracted from the values obtained with tissue homogenates. The activity of cyclic-GMP phosphodiesterase was then calculated and the results were expressed as picomoles of cyclic-GMP converted to 5′-GMP per min per mg wet tissue weight. With the incubation conditions employed in this assay, ^{3}H-guanine formation was linear with time up to 6 min. Also, dilutions of tissue homogenates produced a proportionate decrease in enzyme activity.

Data obtained in this study were subjected to an analysis of variance and the means were compared using Sheffe's procedure (5).

Results. The data in Table I show the activities of guanylate cyclase and cyclic-GMP phosphodiesterase in mammary tissues from virgin, pregnant, and lactating mice. Guanylate cyclase activity was enhanced by about 35% during mid and late pregnancy when compared to activities in virgin animals. During the first 3 days of lactation, a further 40% increase in guanylate cyclase activity was observed. Cyclic-GMP phosphodiesterase activities were also increased significantly during pregnancy when compared with mammary glands from virgin mice; this increase was found when both the low and high substrate concentrations were tested. In contrast to guanylate cyclase, however, cyclic-GMP phosphodiesterase activities during the first 3 days of lactation were not different from the levels found during pregnancy.

Discussion. The concomitant increases in activities of guanylate cyclase and cyclic-GMP phosphodiesterase during pregnancy correlates with the findings of Sapag-Hagar and Greenbaum (2) that cyclic-GMP concentrations in mammary tissues from rats do not increase, and in fact slightly decrease, as the gestation period progresses from mid to late pregnancy. The reason(s) for the increased activities of guanylate cyclase and cyclic-GMP phosphodiesterase during pregnancy is not known. It is possible, however, that the increased epithelial cell content of the mammary tissue during pregnancy (6) and/or the altered hormonal milieu may contribute to these changes.

The observation that guanylate cyclase activity increases following parturition while cyclic-GMP phosphodiesterase activity is not changed, again correlates with the observations of Sapag-Hagar and Greenbaum (2). They reported a significant rise in the concentration of cyclic-GMP in the mammary glands of lactating rats when compared to late-pregnant rats. In preliminary studies in our laboratory (data not presented), we have similarly found that cyclic-GMP levels are elevated in mammary glands of lactating

mice when compared to glands from 18-day-pregnant mice. The results of these studies, therefore, make tenable the idea that elevated levels of cyclic-GMP may contribute to the mechanism(s) whereby lactation is initiated and maintained following parturition in rodents. Furthermore, the results of this study, as well as the fact that cyclic-GMP mimics certain of the actions of prolactin in the mammary gland (1), support the hypothesis that the effects of prolactin on the stimulation of lactational processes may be mediated via cyclic-GMP.

Summary. Guanylate cyclase and cyclic-GMP phosphodiesterase activities were measured in homogenates of mammary glands from virgin, pregnant, and lactating mice. Guanylate cyclase activities increased 35% in mammary tissues during pregnancy, and a further 40% increase was observed during lactation. Cyclic-GMP phosphodiesterase activity also increased during pregnancy but activities were not different in glands from lactating mice vs glands from pregnant mice. These results are discussed with regard to a possible role of cyclic-GMP in regulating lactational processes.

1. Rillema, J. A., *Horm. Metab. Res.* **7,** 45 (1975).
2. Sapag-Hagar, M., and Greenbaum, A. L., *FEBS Lett.* **46,** 180 (1974).
3. Smith, R. D., and Rillema, J. A., *Proc. Soc. Exp. Biol. Med.* (adjoining publication).
4. Beavo, J. A., Hardman, J. G., and Sutherland, E. W., *J. Biol. Chem.* **245,** 5649 (1970).
5. Sheffe, H., *Biometrika* **40,** 87 (1953).
6. Anderson, R. R., *in* "Lactation: A Comprehensive Treatise" (B. L. Larson and V. R. Smith, eds.), Vol. 1, p. 110. Academic Press, New York (1974).

Received June 9, 1975. P.S.E.B.M. 1975, Vol. 150.