Lactose Synthetase: Progesterone Inhibition of the Induction of α-Lactalbumin

Abstract. *Lactose synthesis in the mammary gland is dependent on the hormonally controlled synthesis of the two protein components of lactose synthetase, α-lactalbumin and a galactosyltransferase. Prolactin induces the synthesis of both proteins in mammary gland explants treated with insulin and hydrocortisone, but the induction kinetics cannot account for the asynchronous synthesis of the two proteins that are observed in vivo. Progesterone appears to take part in the control of lactose synthesis and acts to repress the formation of α-lactalbumin throughout pregnancy. At parturition, when the concentration of progesterone in the plasma decreases, the rate of α-lactalbumin synthesis increases.*

In the lactating mammary gland the final step in lactose synthesis is accomplished by the interaction of two proteins, the A and B proteins of lactose synthetase. The A protein is a particulate galactosyltransferase (*1*); the B protein is the secretory whey protein α-lactalbumin (*2*). α-Lactalbumin can modify the substrate specificity of the galactosyltransferase to include glucose, thus specifying the synthesis of lactose (*1*). During the latter half of pregnancy, galactosyltransferase activity in the mouse mammary gland rises to nearly maximum values, whereas the α-lactalbumin concentration remains low, rising rapidly to maximum values only after parturition (*3*). Since lactose synthetase appears to be rate limiting for lactose synthesis at parturition (*4*), the onset of lactose formation at lactogenesis appears to be regulated primarily by controlling the rate of α-lactalbumin synthesis. Both the galactosyltransferase and α-lactalbumin can be induced by prolactin and insulin in mouse mammary epithelial cells which have been formed in organ culture in the presence of insulin and hydrocortisone (*3*). However, the induction kinetics observed in this system did not account for the differential time course of the two proteins observed during the development in vivo, suggesting that additional hormonal factors may regulate this enzyme system. We now report evidence that progesterone interacts with mammary epithelial cells to regulate the activity of lactose synthetase.

Mammary gland explants from pregnant C3H/HeJ mice were prepared and cultured on medium 199 (Microbiological Associates) (*3*). Crystalline beef insulin (Lilly), ovine prolactin (Endocrinology Study Section, NIH) or human placental lactogen (Lederle), and hydrocortisone were each present in the medium at a concentration of 5 μg/ml. 17β-Estradiol and progesterone (Sigma and Mann Research) were added to the medium in a small amount of absolute ethanol. Control media without ovarian steroids contained a corresponding amount of ethanol.

Galactosyltransferase reactions of the A protein and α-lactalbumin were assayed by a radioactivity method (*1*, *3*). The rate of casein synthesis was measured by allowing explants to incorporate ^{32}P-phosphate into protein precipitated by rennin. The methods used for isolation of the ^{32}P-casein from tissue homogenates and for polyacrylamide electrophoresis of casein have been described (*5*).

The components of lactose synthetase, the galactosyltransferase and α-lactalbumin, are induced by prolactin and insulin in mammary epithelial cells which have been formed in vitro in the presence of insulin and hydrocortisone. The typical kinetics of induction in response to the addition of prolactin to the medium are shown in Fig. 1. Addition of progesterone at a final concentration of 2×10^{-6} mole/liter simultaneously with prolactin allows full induction of the galactosyltransferase activity but prevents an increase in the activity of α-lactalbumin. Addition of progesterone at a similar concentration to tissue homogenates did not change significantly the enzymatic activities observed (*6*), a result consistent with the concept that this action of the hormone may require an intact cell. Extracts of tissues incubated with or without progesterone were also mixed and assayed. The activities observed were approximately additive, indicating that the decreased α-lactalbumin activity in progesterone-treated tissue does not result from the presence of an enzyme inhibitor which is functional under the assay conditions.

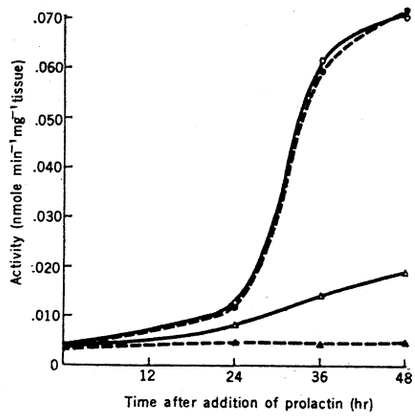

Fig. 1. Effect of progesterone on the induction of lactose synthetase in vitro. Mammary explants from mice midway through pregnancy were incubated in media with insulin and hydrocortisone for 72 hours; then the medium was changed to include prolactin with progesterone (2×10^{-6} mole/liter) (closed symbols) or without progesterone (open symbols). Circles, galactosyltransferase activity; triangles, α-lactalbumin activity.

Progesterone at approximately 10^{-6} but not at 10^{-8} mole/liter inhibits the induction of α-lactalbumin (Table 1). At very high concentrations, such as 10^{-4} mole/liter, progesterone prevents the induction by insulin and prolactin of the galactosyltransferase and α-lactalbumin.

During pregnancy, the stimulation of galactosyltransferase and α-lactalbumin may be mediated, at least in part, by placental lactogen (*3*). The induction of α-lactalbumin by this hormone is also inhibited by progesterone (Table 1). 17β-Estradiol, present in high amounts during pregnancy, does not similarly inhibit the induction of α-lactalbumin at concentrations in the "physiological" range (2×10^{-10} to 2×10^{-8} mole/liter).

To determine whether progesterone inhibits the induction of other secretory proteins, we measured the effect of progesterone on prolactin-induced synthesis of casein. Explants were cultured as described in Fig. 1, and then exposed to medium containing ^{32}P-phosphate (carrier-free, 50 μc/ml) during the 32 to 36 hours after addition of prolactin. The electropherogram radioactivity profiles of ^{32}P-casein components formed by explants incubated with or without 2×10^{-6} mole/liter of progesterone are shown in Fig. 2. The stimulatory effect of prolactin upon the rate of synthesis of each of the casein components was unaffected by progesterone.

Results of previous studies with inhibitors of RNA and protein synthesis supported the concept that increased activities of the galactosyltransferase and α-lactalbumin depend upon the formation of RNA and protein, rather than upon activation of previously formed protein molecules (*3*). An early

Table 1. Effect of various concentrations of progesterone or 17β-estradiol on the induction by prolactin of lactose synthetase. Mammary gland explants from mice midway through pregnancy were incubated for 72 hours on medium containing insulin (I) and hydrocortisone (F) (each at 5 μg/ml), and then placed in various hormone systems as shown below. Enzyme activities were determined 36 hours later. Prolactin (P) and human placental lactogen (HPL) were each present at 5 μg/ml.

Progesterone or 17β-estradiol (mole/liter)	Activity*	
	Galactosyl-transferase	α-Lactal-bumin
	I,F	
None	0.008	0.003
	I,F,P	
None	.056	.016
	I,F,P + progesterone	
5×10^{-4}	.010	.001
4×10^{-6}	.059	.003
4×10^{-8}	.058	.015
	I,F + HPL	
None	.060	.015
	I,F + HPL + progesterone	
2×10^{-6}	.057	.005
	I,F,P + 17β-estradiol	
2×10^{-10}	.059	.017
2×10^{-8}	.061	.016
2×10^{-6}	.061	.018
2×10^{-4}	.047	.010

* Nanomoles of product per minute per milligram of tissue.

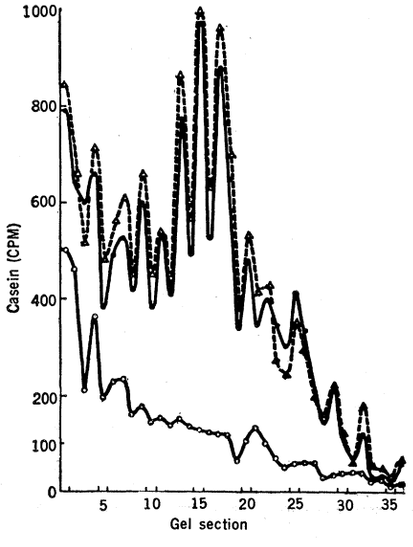

Fig. 2. Polyacrylamide electropherogram radioactivity profile of ^{32}P-casein synthesized by mammary explants from mice midway through pregnancy. Tissue was incubated for 72 hours on medium containing insulin and hydrocortisone, then the medium was changed to include prolactin with progesterone (2×10^{-6} mole/liter) (open triangles) or without progesterone (closed circles); (open circles) insulin and hydrocortisone control. Explants were allowed to incorporate $^{32}P_i$ (50 μc/ml) into rennin-calcium precipitable protein during the 32 to 36 hours after addition of prolactin. Each of the radioactive peaks corresponds to a band in the staining pattern of the authentic C3H/HeJ mouse casein carrier as previously described (*5*). Results are expressed as counts per minute (*CPM*) per milligram of tissue per 4 hours.

effect of prolactin is the stimulation of RNA synthesis in cells formed in insulin-hydrocortisone medium (Fig. 3). Progesterone reduces this stimulation of RNA synthesis by prolactin.

To determine whether progesterone may inhibit α-lactalbumin in vivo, pregnant mice were injected with progesterone daily, beginning 1 or 2 days before parturition. Untreated (control) mammary glands contained low amounts of α-lactalbumin before parturition, but after parturition the concentration of α-lactalbumin rose rapidly, nearly to a maximum (Fig. 4). The α-lactalbumin activities in mammary glands of animals treated with progesterone remained at values characteristic of late pregnancy, and galactosyltransferase activities did not differ significantly from the control values.

Our results support the concept that progesterone may regulate α-lactalbumin synthesis in vivo by selectively inhibiting the induction of this protein during pregnancy. In vitro, the galactosyltransferase and α-lactalbumin are both induced with a similar time course in response to prolactin and insulin (Fig. 1). Progesterone, at concentrations of 4×10^{-6} mole/liter, inhibits the induction of α-lactalbumin but not of the transferase. These results suggest that the α-lactalbumin concentration in mammary glands remains low during pregnancy when the concentration of progesterone in the plasma is high, but increases markedly following parturition when the concentration of progesterone in the plasma decreases (*8*). These observations are consistent with studies which show that injections of progesterone can prevent the rise in lactose content in the mammary gland induced by hysterectomy and ovariectomy of pregnant rats (*9*). Experiments in vitro indicate that progesterone can interact directly with mammary epithelial cells to mediate its inhibitory effect. An early effect of prolactin stimulation, preceding the formation of milk proteins, is a stimulation of the rate of RNA synthesis and RNA polymerase activity (*10*). Progesterone can selectively inhibit this effect on transcription because it prevents the induction of α-lactalbumin without altering the induction of the galactosyltransferase or the casein phosphoproteins. Although progesterone has been generally considered to act on the mammary gland during development, a specific biochemical effect of this hormone has not been described. The induction of avidin in the chick

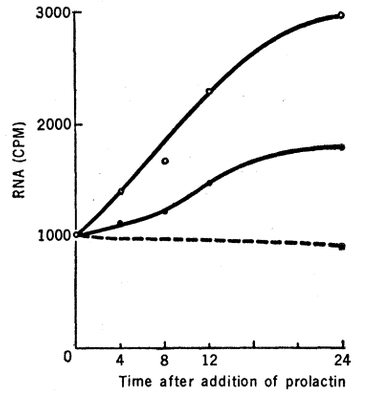

Fig. 3. Effect of progesterone upon the rate of RNA synthesis in mammary explants. Mammary explants from mice midway through pregnancy (C3H/HeJ) (free of mammary tumor virus) were incubated on medium containing insulin and hydrocortisone for 72 hours. The medium was then changed to include prolactin with progesterone 2×10^{-6} mole/liter (closed circles) or without progesterone (open circles). Closed square, insulin and hydrocortisone control. Each point represents the incorporation of tritiated uridine (0.5 μc/ml) into RNA during the preceding 4-hour period. RNA was isolated and counted as previously described (*7*). Results are expressed as counts per minute (*CPM*) per milligram of tissue per 4 hours.

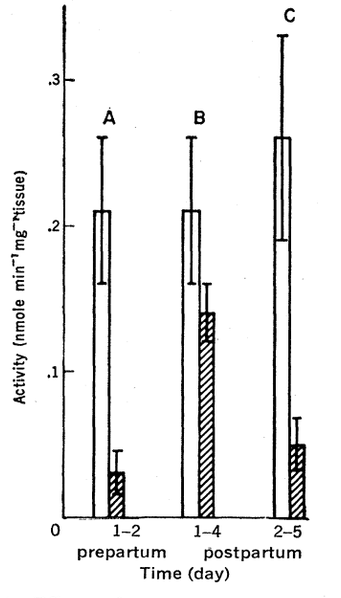

Fig. 4. Effect of progesterone treatment upon lactose synthetase activity in lactational mammary glands. Daily subcutaneous injections of 1 mg of progesterone in sesame oil or a lipid emulsion (Upjohn) were administered to mice (C3H/HeJ) from the final 1 to 2 days of pregnancy until the time of death. Control animals were injected similarly with the sesame oil or liquid emulsion only. Each group was comprised of four animals. Open bars, galactosyltransferase; hatched bars, α-lactalbumin. *A*, enzyme activities of samples 2 days prepartum; *B*, activities of control samples; *C*, activities of samples treated with progesterone.

oviduct (*11*) represents the only previously described role of progesterone in the regulation of synthesis of a specific protein. The selectivity of progesterone's action on milk protein synthesis may represent a greater sensitivity of the α-lactalbumin induction mechanism to progesterone, since at higher concentrations in vitro the induction of the galactosyltransferase is also inhibited. The inhibitory action of progesterone is manifested in vitro at concentrations which are nearly "physiological" for pregnancy (*8*), and lowering the progesterone concentration in vitro results in a release of its inhibitory effect. These observations are consistent with a proposed function of this hormone as a regulator of α-lactalbumin synthesis during pregnancy and lactation, but they do not suggest that progesterone regulates the onset or cessation of lactation itself. α-Lactalbumin acts as a "specifier" protein (*2*), interacting with the galactosyltransferase to cause inclusion of glucose as a substrate for galactosyl transfer. The hormonal regulation of product formation in this type of enzyme system may thus involve primarily a regulation of the induction of the specifier component.

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Molecular Heterogeneity of Human Lymphoid (HL-A) Alloantigens

Abstract. *Soluble preparations of HL-A alloantigens were separated by gel filtration into components having either the "LA" series or the "4" series of alloantigenic determinants. This separation may indicate that several different structural cistrons within the HL-A (locus) control the expression of these two series of determinants. The alternative possibility, in which one structural cistron with multiple mutational sites controls the synthesis of a single molecule cannot be excluded.*

We have reported the preparation of HL-A alloantigens in soluble form by papain treatment of human lymphoid cell membranes (*1*). At that time it was clear that the various products carrying alloantigenic specificities were not of the same size, because of the differences in their elution patterns from columns of Sephadex G-150. This observation has been pursued with more specific reagents detecting individual components of the "LA" and "4" series of alloantigens.

Soluble HL-A alloantigens were prepared by papain digestion of membrane extracts from human lymphoid tissue culture cells and spleen cells. Digestion mixtures were chromatographed on columns of Sephadex G-150, and the contents of individual tubes were concentrated to one-third the original volume and then tested for HL-A alloantigenic activity. A complement-dependent cytotoxicity test system was used, in which antiserums, known to detect different HL-A alloantigenic specificities, cause lysis and release of ^{51}Cr from target lymphocytes (*2*). The fractions from Sephadex G-150 were tested for their ability to inhibit the ^{51}Cr release. The reciprocal of the dilution of each fraction which gave a 50 percent inhibition of ^{51}Cr release was used to express the number of units of alloantigenic activity.

An example of a chromatogram of soluble HL-A alloantigenic material from a human lymphoid cell line (R-4265) is shown in Fig. 1. When the material from each tube was tested with various antiserums in the inhibition assay, four peaks of alloantigenic activity were found. Peak one had alloantigenic activity of the LA-2 and LA-4 determinants; peak two, the 6b and 7c determinants ("4" series); peak

Table 1. Chromatographic separation of HL-A alloantigens. Each peak listed corresponds to the region of the Sephadex chromatogram (Fig. 1) which shows HL-A alloantigenic inhibitory activity. Each region was tested for capacity to inhibit all antiserums shown, and only those serums are listed with which the alloantigens in that peak reacted. LA-1, -2, -3, -4, are alloantigenic specificities determined by the "LA" subdivision of the HL-A genetic locus, and 4a, 4d, 4c, 6b, 7c are antigenic specificities determined by the "4" subdivision.

Peak 1		Peak 2		Peak 3	
Specificities of LA	Antiserum	Specificities	Antiserum	Specificities	Antiserum
		*Tissue from R-4265**			
LA-2	Pinquette	4d, 6b	T-53		
LA-4	Jones	6b, 7c	Alvarez	LA-4	Jones
		4a	Kessler		
		4d, 6b	T-57		
		7c	Cutten		
		Tissue from spleen B			
LA-2	Pinquette	4d, 6b	T-53		
LA-3	Storm	4a	Kessler	LA-3	Storm
		7c	Cutten		
		4c	T-16		
		*Tissue from IM-1**			
LA-1	Morrison	4a	Kessler	LA-1	Morrison
LA-3	Storm	6b, 7c	Alvarez	LA-3	Storm
		4a	Buffo		
		*Tissue from RAJI**			
LA-3	Storm	4d, 6b	T-53	LA-3	Storm

* Human lymphoid tissue culture cell line.