ACTINOMYCIN D AND LACTOGENIC HORMONES MODULATE CASEIN GENE EXPRESSION IN BOVINE ACINAR CULTURE

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Introduction

The purposes of this research were 1) to study the relationship between milk protein mRNA accumulation and milk protein secretion in acinar culture as influenced by actinomycin-D (ACT-D) and 2) to examine the effects of various combinations of lactogenic hormones on milk protein gene expression in acinar cultures of lactating bovine mammary tissue.

Materials and Methods

Mammary Acinar Culture

Mammary tissue was collected at slaughter from mid-lactation Holstein cows, trimmed free of large pieces of connective and adipose tissue, and transported to the laboratory on ice (transmit time of 15 min). Isolation and culturing of acinar mammary epithelial cells were done(Park et al., 1979).

The cells were plated on plastic tissue culture dishes (approximately 10^6 cells/dish). The basic culture medium was Eagle's 1X MEM (Eagle, 1959) with 0.2% (wt/vol) glucose, 5% (vol/vol) fetal bovine serum and antibiotics.

Four culture dishes were set up for each observation with contents pooled after 18 hours incubation for protein secretion and mRNA determination. The ^{3}H-lysine (0.5 μ Ci/ml) was added to culture medium to determine in vitro secretion of milk protein.

A series of bovine acinar epithelial cell culture studies were conducted. Studies involved: 1) increasing concentration of ACT-D (0,1,2,4,10 μg/ml) on presence of lactogenic hormones (insulin, 10 μg/ml + hydrocortisone, 10 μg/ml + prolactin, 5 μg/ml) and 2) various combination of lactogenic hormones (control, insulin, hydrocortisone, prolactin, insulin + hydrocortisone, insulin + prolactin, prolactin + hydrocortisone, insulin + prolactin + hydrocortisone).

Quantification of mRNA

Bovine β-casein complementary DNA (cDNA) clone (Choi, 1987) was used to determine the relative specific activities of total cytoplasmic mRNA of casein genes.

Total cytoplasmic RNA was extracted (White and Bancroft, 1982) from cells in acinar culture. After ethanol precipitation, RNA was quantified and dotted directly onto a nitrocellulose sheet employing a 96-well filteration manifold (Schleicher and Schuell) (Choi, 1987). The sheet was baked in vacuo at 80°C for 2 h (Wiens et al., 1987), sealed in a plastic bag, and stored at 4°C until hybridization. Nick translation (nick translation kit, Amersham) and hybridization were essentially as described by Maniatis et al. (1982). Nick translated ^{32}P cDNA was recovered by column chromatography separation on Sephadex G-50.

RNA dotted nitrocellulose sheets were prehybridized and hybridized with the specific nick translated cDNA (Maniatis et al., 1982). After hybridization to the appropriate probe and subsequent washing, the nitrocellulose sheets were dried. An autoradiogram was prepared by exposing the dried nitrocellulose sheet to XAR-5 X-ray film (Eastman Kodak Co.) at -70°C for 36 h. The extent of hybridization was determined by a scanning densitometry (Hoeffer Scanning Densitometer GS 300, Hoefer Sci. Ins.).

Results and Discussion

The table 1 and autoradiogram in figure 1 show the effect of increasing concentrations of ACT-D on the induction of β-casein mRNA of mammary acinar epithelial cells in culture. ACT-D concentration ranging from 0 to 10 μg/ml of culture

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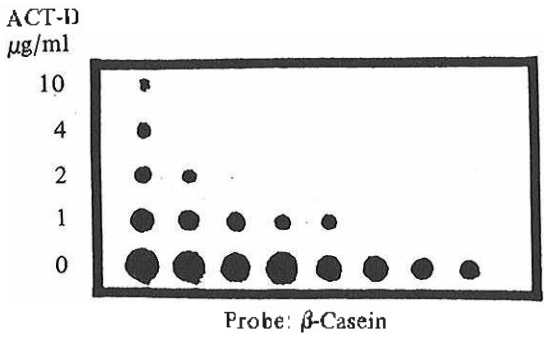

Figure 1. Effect of increasing concentrations of ACT D on the induction of bovine β-casein mRNA level.

medium significantly decreased accumulation of β-casein mRNA for an 18 h incubation in the presence of all three lactogenic hormones.

Addition of ACT-D to culture media at a level of 1 μg/ml inhibited protein secretion by 52% over control levels. At 10 μg/ml ACT-D this inhibitory effect was much more pronounced, amounting to 87% of control value (table 1).

The inhibition of mRNA synthesis by ACT-D coincides with the decreasing of milk protein secretion (table 1). It is then possible to correlate β-casein mRNA level with the amount of milk protein secreted.

To determine the possibility that lactogenic hormones induce the accumulation of specific

TABLE 1. INFLUENCE OF ACTINOMYCIN-D ON β-CASEIN mRNA ACCUMULATION AND MILK PROTEIN SECRETION IN BOVINE MAMMARY ACINAR CULTURE[a]

	Actinomycin-D (μg/ml)				
Parameter	0	1	2	4	10
β-casein mRNA[b] (Specific activity)	734.2	313.4	155.6	52.8	24.3
Protein secretion[c] (dpm/mg protein x 10^{-2})	54.3	26.7	15.4	10.5	7.1

[a] Acinar cells isolated from lactating bovine mammary tissue were plated at a density of 1 x 10^6 cells/dish and incubated for 18 h in the culture medium containing concentrations of ACT-D in the presence of lactogenic hormones [insulin (10 μg/ml) + hydrocortisone (10 μg/ml) + prolactin (5 μg/ml)].

[b] Specific activity = relative intensity/total cytoplasmic RNA extracted from pooled cells of four dishes. Relative intensity was determined by scanning densitometry of individual dots on autoradiograms. Data were calculated from the midpoint of linear regression equations; Each value is the mean of 2 observations with 4 culture dishes/observation.

[c] Each value is the mean of 3 observations with 4 culture dishes/observation.

milk protein mRNA and subsequent gene expression of milk protein, cells were cultured in media containing various combinations of hormones (table 2). Data clearly indicate that lactating mammary acinar epithelial cells in culture maintain the hormone-inducible milk protein gene expression as seen in the value of milk protein secretion as well as messages for β-casein.

The amount of secreted protein was increased as much as 1.1 fold in cultures with hydrocortisone alone; 1.8 fold with prolactin alone; and 3.8 fold with prolactin plus hydrocortisone over that of cultures without added hormones. The combination of three lactogenic hormones augmented the amount of secreted protein more than 1.7 fold relative to that found in acinar cultures with insulin plus prolactin, but only 1.1 fold compared to cultures with prolactin plus hydrocortisone (table 2). This suggests that hydrocortisone in the presence of prolactin amplifies the lactogenic action of mammary epithelium.

All parameters in this study support that hypothesis that maximum induction of β-casein mRNA and milk protein secretion in acinar culture from the lactating mammary tissue can occur in the presence of three hormones, in accord with the results of mammary explant studies from intact mice and rats (Guyette et al., 1979; Hobbs et al., 1982; Taketani and Oka, 1986).

It was observed that the potentiation of milk

TABLE 2. THE EFFECT OF INSULIN, HYDROCORTISONE AND PROLACTIN ON THE AMOUNT OF SECRETED PROTEIN AND CASEIN mRNA IN BOVINE ACINAR CULTURE[a]

Treatment[d]	Secreted protein[c]	β-casein mRNA
	dpm/mg x 10^{-2}	Specific activity[b]
Control	16.9	155.3
I	18.6	170.8
H	18.8	174.2
P	30.4	403.8
I + H	22.0	198.7
I + P	41.7	434.8
P + H	64.2	729.9
I + P + H	69.3	869.7

[a,b,c]See table 1.

[d]Abbreviations: I = insulin (10 μg/ml); H = hydrocortisone (10 μg/ml); P = prolactin (5 μg/ml).

protein synthesis by lactogenic hormones is accompanied by an increase in β-casein mRNA concentration (table 2). The accumulation of β-casein mRNA in acinar culture was increased more than 2.6 fold by prolactin alone, 2.8 fold by prolactin plus insulin and about 4.7 fold by prolactin plus hydrocortisone. The combination of three lactogenic hormones increased the accumulation of β-casein mRNA more than 1.2- and 2.0- fold, respectively, compared to cultures with prolactin plus hydrocortisone or prolactin plus insulin

Results of the present work also indicate that prolactin, even in the absence of insulin and/or hydrocortisone, is capable of inducing the secretion of a copious amount of milk protein. This effect was correlated with an increase of β-casein mRNA concentration. Prolactin has been shown to be mammogenic in vitro and in vivo, but its primary role may actually be in lactogenesis.

Our results demonstrate that lactating mammary epithelial cells in acinar culture require all three lactogenic hormones.

(Key Words: Casein Messenger RNA, Milk Protein Gene Expression, Acinar Culture, Actinomycin-D, Lactogenic Hormone, Protein Secretion)

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