A DIRECT EFFECT OF PROLACTIN AND PLACENTAL LACTOGEN ON MAMMARY EPITHELIAL NUCLEI

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SUMMARY: Placental lactogen and prolactin stimulate the rate of RNA synthesis by nuclei isolated from mammary epithelial cells. The effect is tissue specific. It is suggested that these protein hormones may perform some of their functions within the mammary cell.

INTRODUCTION: Non-secretory mammary epithelial cells can be converted into secretory cells *in vitro* in the presence of insulin, glucocorticoid and either prolactin or human placental lactogen (1-3). Some of the contributions of insulin (4,5), glucocorticoid (6-8) and prolactin (6,9) to this complex conversion have been delineated. Progression of the cells from an intermediate stage of development to the terminal stage requires the addition of either prolactin or placental lactogen (6,10). A manifestation of this terminal differentiation is the emergence of the synthesis of casein and α-lactalbumin. RNA synthesis is necessary for the formation of these milk proteins (11), and prolactin stimulates the formation of several molecular species of RNA (12,13).

It is commonly thought that protein hormones exert their characteristic effects on target cells by impinging on the outer cell membrane. The effects are presumably transmitted from membrane-sites to the appropriate intra-cellular site. In some instances (14) it appears that the adenylyl-cyclase system is part of the membrane-site, and that cyclic 3',5'-adenosine monophosphate is the transmitter. With other protein hormones, however, neither the membrane-site nor the transmitter has been identified. Prolactin and human placental lactogen are cases in point. Although it has been reported (15,16) that the outer cell membranes derived from mammary epithelial cells possess binding sites which are specific for prolactin, the relevancy of such binding to the biological

effect produced by the hormone has not yet been established. The mode of transmission has also not been determined.

In initiating the present study we made the unorthodox assumption that prolactin and human placental lactogen actually enter the mammary epithelial cell, and therein elicit some of their characteristic responses. Evidence bearing on the possibility that these hormones act directly on the nuclei is presented.

MATERIALS AND METHODS: Materials - ATP and GTP were purchased from Sigma Chemical Co. UTP, CTP and bovine pancreatic ribonuclease (5X recrystallized) were CalBiochem products. [5,6-^{3}H]UTP (36 Ci per mmole), purchased from New England Nuclear, was diluted with non-isotopic UTP such that the final specific radioactivity was 100 Ci per mole. Actinomycin D was purchased from Merck and Co. Human placental lactogen was purchased from Nutritional Biochemicals Corp. Ovine prolactin (NIH-P-S-9) was a gift from NIAMDD. Crystalline pork insulin was a gift from the Eli Lilly Co.

Animals - Tissues from Sprague-Dawley rats and C3H/HeN mice were used. The animals were either 15-17 days into their first pregnancy, or 5-7 days into their first lactation.

Isolation of Nuclei - Nuclei were isolated by a method adapted from Chauveau et al. (17). All operations were conducted at 4°. Abdominal mammary glands (1-3 gm) were minced with scissors, and homogenized with a Teflon pestle-glass homogenizer in 2 volumes of buffer (0.25 M sucrose, 20 mM Tris-HCl, pH 7.8, 2 mM $MgCl_2$, 1 mM dithiothreitol). The homogenate was filtered through nylon net, and centrifuged for 10 min at 700 x g. The pellet was suspended in 1-2 ml of 1.8 M sucrose solution containing 20 mM Tris-HCl, pH 7.8, 2 mM $MgCl_2$ and 1 mM dithiothreitol. This suspension was layered upon 3 ml of the same sucrose-buffer solution and centrifuged in a SW 50.1 rotor for 1 hr at 100,000 x g. The nuclear pellet was resuspended in the homogenization buffer (1 ml per gm tissue equivalent) and assayed immediately for RNA synthetic ability. These nuclear preparations were free from intact cells and relatively

free from other particulate matter, as judged from light microscopy. Nuclei from liver and kidney were prepared in the same way.

RNA Synthesis - Assays for Mg^{++}- or Mn^{++}-dependent RNA synthesis were performed by incubating nuclei (30 µl of nuclear suspension containing 20-40 µg DNA) in a final volume of 0.25 ml of reaction medium containing 15% glycerol, 40 mM Tris-HCl, pH 8.0, 0.4 mM ATP, 0.4 mM GTP, 0.4 mM CTP, 0.05 mM [^{3}H]UTP (1.25 µCi), 1 mM dithiothreitol, and 4 mM $MgCl_2$ or 3 mM $MnCl_2$ plus 0.1 M $(NH_4)_2\ SO_4$, according to the method of Chomczynski et al. (18). Under the conditions used the nuclei comprise the limiting component of the system. The reaction was started by addition of the nuclei to the other components. Incubation, with shaking, was at 37° for 15 min, unless stated otherwise. The reaction was terminated by placing the tubes in ice, and adding 0.2 ml of a 0.1% solution of bovine serum albumin and 2 volumes of 10% trichloroacetic acid. The precipitates were collected on Whatman GF/A glass filters, and washed with 1 ml of cold trichloroacetic acid 6 times.

DNA Determination - The DNA content of aliquots of the nuclear suspensions was determined by the diphenylamine method (19), using calf thymus DNA as a standard.

Determination of Radioactivity - The glass filters, with the washed precipitates, were placed in scintillation vials, with 1.0 ml of NCS tissue solubilizer. POP-POPOP toluene scintillation cocktail was added, and radioactivity was determined in a scintillation counter at a counting efficiency, for tritium, of 40%. Zero-time values were subtracted from all data.

RESULTS: Figure 1 shows the extent of incorporation of [^{3}H]UTP into RNA in the presence of various concentrations of human placental lactogen or prolactin, compared to controls incubated in the absence of added hormones. Maximal effects were elicited in the hormone concentration range, 12-50 nM, approximately. At higher concentrations the effect of placental lactogen is usually less than maximal.

The results presented in Figure 2 indicate that human placental lactogen

TABLE I. Characterization of Acid-Insoluble Material into Which [^{3}H]UTP is Incorporated.

System	Incorporation of [^{3}H]UTP pmoles/mg DNA	
	NH	+HPL
Mg^{++}		
Complete	97	132
+ Actinomycin D	4	5
Ribonuclease	10	9
Alkaline hydrolysis	8	11
Mn^{++}		
Complete	183	242
+ Actinomycin D	5	7
Ribonuclease	12	15
Alkaline hydrolysis	21	17

Mammary nuclei from pregnant rats were used (cf. Materials and Methods). Systems with no hormone (NH) were compared to systems with human placental lactogen (HPL, 5 x 10^{-8} M). Actinomycin D (8 µg/ml) was added at zero-time. Ribonuclease (2 µg/ml) was added at the end of the 15 min incorporation period, and incubation at 37° was continued for an additional 15 min. Alkaline hydrolysis, initiated at the end of the 15 min incorporation period by addition of an equal volume of 1 N NaOH, lasted 1 hr at 37°. Similar results were obtained in several experiments.

increases the initial rate of incorporation of [^{3}H]UTP into RNA by isolated rat mammary nuclei.

Data in Table I establish the fact that the nuclei isolated from mammary glands of pregnant rats incorporate [^{3}H]UTP into authentic RNA, since the incorporation is prevented by actinomycin D, and the product is hydrolyzed by ribonuclease and 0.5 N NaOH. Also, omission of any one of the non-isotopic nucleoside triphosphates drastically reduced the incorporation of [^{3}H]UTP (18). Similar results were obtained with mammary nuclei isolated from lactating rats.

The results shown in Table II indicate that the stimulation of RNA synthesis by isolated nuclei is specific for both hormone and cell-type. Placental lactogen and prolactin stimulate nuclei from rat and mouse mammary tissue, but do

TABLE II. Cell and Hormone Specificity.

Nuclei derived from	Per cent stimulation of incorporation of [^{3}H]UTP compared to no-hormone control					
	Mg^{++} system			Mn^{++} system		
	HPL	P	I	HPL	P	I
Rat mammary gland						
Pregnant	52 (5)	30 (4)	n.s.(2)	34 (5)	32 (3)	n.s.(2)
Lactating	24 (4)	20 (2)	n.s.(2)	43 (4)	28 (2)	n.s.(2)
Rat Liver	n.s.(3)	n.s.(2)	n.s.(2)	n.s.(2)	n.s.(2)	n.s.(2)
Mouse mammary gland						
Pregnant	41 (5)	35 (3)	n.s.(1)	24 (5)	18 (3)	-
Lactating	23 (4)	14 (3)	-	35 (4)	20 (3)	n.s.(1)
Mouse liver	n.s.(2)	n.s.(2)	-	n.s.(2)	n.s.(2)	-
Mouse kidney	n.s.(1)	n.s.(1)	-	n.s.(1)	n.s.(1)	-

The effects of human placental lactogen (HPL, 5×10^{-8} M), prolactin (P, 5×10^{-8} M) and insulin (I, 10^{-7} M) on incorporation of [^{3}H]UTP into RNA by nuclei from various cell sources were compared. Details of preparation of nuclei, and conditions of incor poration are given in Materials and Methods. Each value is the average stimulation obtained in the number of experiments recorded in parentheses. Several determinations were made in each experiment. n.s. Signifies a value not significantly different from that of the no-hormone control.

not stimulate nuclei from liver or kidney. Insulin has no stimulatory effect on RNA synthesis by nuclei from mammary tissue or liver, when used at a concentration of 10^{-7} M, or 5×10^{-7} and 10^{-8} M (not shown). Placental lactogen exerted similar effects on nuclei (not shown) from mouse mammary cells which were prepared by treating minced tissue with collagenase (20). This indicates that the hormone affects the mammary epithelial nuclei. Similar lactogen effects were also obtained when [^{3}H]GTP was used instead of [^{3}H]UTP. No lactogen effect was observed on the rate of incorporation of [^{3}H]TTP into DNA by the isolated nuclei.

DISCUSSION: In the present study, which is an extension of an earlier report (18), it has been shown that prolactin and human placental lactogen

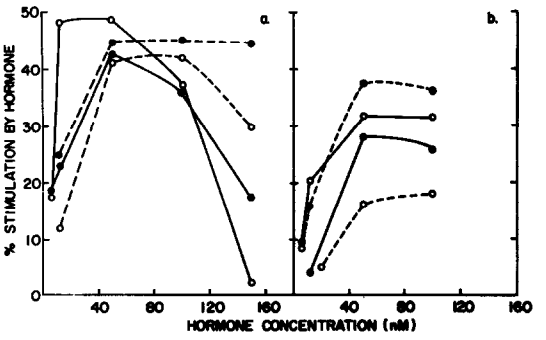

Fig. 1. Effect of various concentrations of human placental lactogen (a), and prolactin (b) on incorporation of [^{3}H]UTP into RNA by mammary nuclei. Nuclei were isolated, and RNA synthesis was determined as described in Materials and Methods. Solid lines: nuclei from pregnant rats. Dotted lines: nuclei from lactating rats. ●, Mg^{++} system; O, Mn^{++} system.

stimulate promptly the rate of RNA synthesis by isolated nuclei derived from mammary epithelial cells. These may be the first demonstrations of such an effect on isolated target-cell nuclei by protein hormones. Stimulation of both the Mg^{++}- and Mn^{++}-dependent polymerase systems is consistent with the Observation (13) that prolactin stimulates the synthesis of various molecular species of RNA in mammary cells. The effect is specific in terms of both the origin of the nuclei and the hormones. Neither prolactin nor the placental lactogen stimulates RNA synthesis by nuclei from either liver or kidney, and insulin does not stimulate nuclei isolated from mammary epithelial cells. Insulin does, however, increase the rate of RNA synthesis by mammary epithelial cells *in vitro* (12). This is consistent with the conclusion (21) that insulin initiates all of its biological effects at the level of the outer cell membrane. In this respect prolactin and human placental lactogen may be members of a different class of protein hormones.

The concentration range of prolactin required for stimulation of RNA synthesis by the isolated nuclei is similar to that required for enhancement of casein synthesis by mammary explants (11). Earlier studies showed that stimulation of RNA synthesis by explants under the influence of prolactin or placental lactogen appears to be closely related to the production of milk-

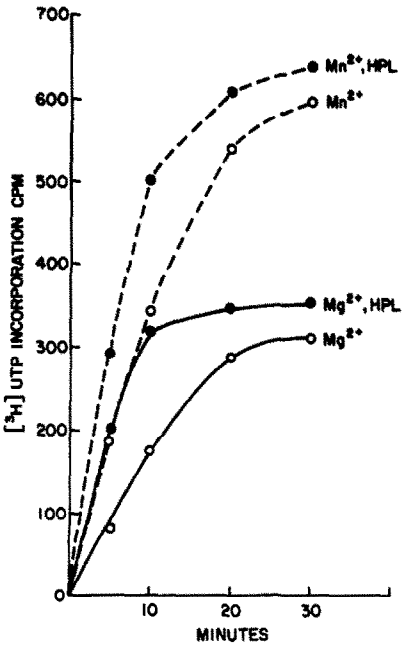

Fig. 2. Time-course of incorporation of [^{3}H]UTP into RNA by isolated mammary nuclei from pregnant rats. Nuclei were isolated, and RNA synthesis was determined as described in Materials and Methods. Human placental lactogen (HPL) was used at a concentration of 5 x 10^{-8} M.

proteins. Thus, prolactin-associated RNA synthesis precedes casein synthesis (7), and inhibition of RNA synthesis prevents the emergence of casein formation in the presence of prolactin (11). Such considerations suggest that the effects observed with isolated nuclei may, in part, reflect events which occur in the intact cell.

However, the relevancy of one event to another in biological systems is often difficult to establish. For example, steroid hormone-receptor complexes have been shown to stimulate RNA synthesis by isolated target-cell nuclei (22,23), and prolactin and placental lactogen, as we have seen, exert similar effects on isolated nuclei from their target cells. In each case the response is specific for hormone and cell-type. Yet, in spite of this and other positive circumstantial evidence described above, determination of the relationship between the observed hormone effects on isolated nuclei and the hormone effects on cellular development will require further investigation.

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