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ALPHA-LACTALBUMIN ACTS AS A BIMODAL REGULATOR OF RAT PAROTID ACINAR CELL GROWTH

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Isoproterenol, a ß-adrenergic receptor agonist, causes hypertrophy and hyperplasia of the rat parotid gland. The stimulation of parotid acinar cells to a growth phase is accompanied by a cell surface localization of the enzyme 4ß-galactosyltransferase. Alpha-lactalbumin, a specific modifier protein for 4ß-galactosyltransferase, when given subsequent to the initiation of isoproterenol treatment and the commencement of parotid enlargement, resulted in a termination of gland hypertrophy and DNA synthesis. Gland size did not, however, return to control levels with the continued injection of isoproterenol and alpha- lactalbumin. In contrast, the injection of alpha-lactalbumin in neonatal rats (7-14 days post-partum) stimulated parotid gland hypertrophy and DNA synthesis. This treatment also lead to the precocious expression of the major parotid gland salivary enzyme, amylase.

Membrane-associated glycosyltransferases classically have been localized to the Golgi apparatus [17,18,28,37]. A portion of the cellular activity has also been found at the cell surface [23-25,35] where it has been suggested to be involved in a number of biological processes. Numerous reports have shown that cell surface galactosyltransferase activity may be involved in mediating such developmental events as cell adhesion [25,26] recognition [23], differentiation [41] and embryogenesis [36].

Isoproterenol, a ß-adrenergic receptor agonist causes parotid gland hypertrophy and hyperplasia in the adult rat [3,7,27,30,31]. The physiological change in the gland recently has been reported to be accompanied by a dramatic increase in the synthesis and accumulation at the cell surface of the enzyme

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Abbreviations used: uridine diphosphate galactose, UDP-Gal; isoproterenol IPR; trichloroacetic acid, TCA; alpha-lactalbumin, α-LA; N-acetylglucosamine, GlcNAc; glucose, glc.

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4ß-galactosyltransferase (E.C. # 2.4.1.38) [14-16,20]. Furthermore, the incorporation of the specific modifier protein for 4ß-galactosyltransferase, α-lactalbumin [6,9-17,28,37], along with the isoproterenol drug regimen, can effectively block parotid gland hypertrophy and hyperplasia [16,20].

The rat parotid gland has been studied extensively as a developmental model for tissue maturation [for review see 31]. Histological observations have established that the neonatal parotid gland is immature with regard to terminally differentiated acinar cells at the time of birth [2,32,33]. Since α-lactalbumin demonstrated a dramatic retarding effect on adult acinar cell growth when administered at the time of isoproterenol treatment it was of interest to follow the effect of α-lactalbumin on neonatal parotid gland development. Contrary to the inhibitory effect observed on isoproterenol hypertrophy of parotid gland in the adult rat, α-lactalbumin had a growth enhancing effect on neonatal rat parotid gland. The precocious effects of α-lactalbumin resulted in changes in function and morphology of the gland.

Materials and Methods

Chemicals d,l-Isoproterenol, trichloracetic acid, α-lactalbumin, and UDP-galactose were purchased from Sigma Chemical Co. UDP-[^{14}C]galactose (sp. act. 300 Ci/mmole) and [6-^{3}H]thymidine (sp. act. 6.7 Ci/mmole) were purchased from New England Nuclear. All other chemicals were of reagent quality and purchased through commercial sources.

Drug Treatment Adult Long-Evans rats (Charles River Breeding Farms) weighing 200 g received twice daily, intraperitoneal injections of 5 mg isoproterenol in 0.5 ml sterile saline for 5 days. After 5 days of such treatment one group of rats was additionally given intraperitoneal injections of 10 mM å-lactalbumin in 0.5ml volume alone with continued isoproterenol treatment. Following a total of 3 days or 8 days of the combined drug regimen, animals were given 100 uCi of [^{3}H]thymidine and sacrificed 5 h later to estimate the rates of DNA synthesis. As controls, one group of animals did not receive any drugs while another group of animals received only isoproterenol for the 13 day duration of the drug regimen.

Neonatal rats were given twice daily intraperitoneal injections of 10 mM α-lactalbumin in proportion to their body weights, based on 10 mM α-lactalbumin (in a physiological saline solution) in 0.5 ml/175g rat. Control litter mates received an injeaction of equal volume of saline alone. The injection series was initiated 24 h after birth.

Tissue Preparation. The parotid glands were removed following anesthetization with 1% pentabarbital and exsanguination. The tissue was dispersed by blending in a polytron (setting # 6) for 30 sec in 2 ml of 10mM Tris buffer, pH 8.0, and cell breakage completed by a Dounce apparatus. The slurry was then centrifuged at 100,000 xg for 1 h to obtain the cell lysate soluble and insoluble membrane fractions. Protein assays were performed by a modification of the Lowry method with bovine serum albumin as a standard [29].

Plasma Membrane Isolation. Plasma membrane was isolated by the procedure of Arvan and Castle [1], as previously described by Marchase et al [20]. The final plasma membrane pellet was resuspended in 10 mM Tris buffer pH 8.0 for

subsequent assays for protein and 4ß-galactosyltransferase activity. The acinar cell plasma membrane marker enzyme gamma-Glutamyl Transpeptidase [16] was assayed as a control for membrane enrichment.

Glycosyltransferase Assay. The activity of 4ß-galactosyltransferase was measured as described previously [14]. The assay mixture contained 0.1 M MES (pH 6.3) 2.5 mM $MnCl_2$, 0.5% Triton X-100, 1 mM UDP-[1-^{14}C]galactose (10^6 cpm/µmol), 10 mg ovalbumin as exogenous acceptor and 0-0.5 mg membrane protein. For assays of the sialyltransferase CMP-[1-^{3}H]-sialic acid (10-15 Ci/mmol) and asialofetuin were substituted for the radiolabeled sugar donor and acceptor respectively.

Amylase assay. Amylase activity was assayed by the method of Bernfeld [4]. The substrate solution consisted of 1 g of soluble starch and 40 mg of NaCl in 100 ml 0.02 M potassium phosphate buffer (pH 7.0) and the solution for stopping the reaction consisted of 3,5 dinitrosaliayclic acid and 30 g of sodium potassium tartrate in 100 ml of 0.4 N NaOH. The reaction were carried out at 37°C in a volume of 0.5 ml using 100 µl of enzyme containing cell lysate. The reaction was terminated after a 30 min incubation by adding 0.5 ml stop buffer, boiled to allow color development and absorbance measured at a wavelength of 540 nm after the addition of 5 ml water. Amylase activity was expressed as units/mg protein. One unit of enzyme activity will liberate 1.0 µmole of maltose from starch per min.

Incorporation of thymidine into DNA. In vivo DNA synthesis was followed in neonatal and isoproterenol-treated adult parotid glands by monitoring the incorporation of [^{3}H]thymidine into TCA precipitable counts. The animals were sacrificed 5 h following this injection, their glands were removed and the tissue was homogenized at 4°C. 100 µl were removed for TCA precipitation on glass fiber filters, followed by scintillation counting for ^{3}H incorporation. Untreated animals were used to determine basal rates of DNA synthesis.

Results and Discussion

The introduction of α-lactalbumin, a specific modifier protein for 4ß-galactosyltransferase, as well as other agents specific for 4ß-galactosyltransferase, at the time of isoproterenol treatment, resulted in a nearly complete block in hypertrophy and hyperplasia of acinar cells of the rat parotid gland [16,20]. The ability of α-lactalbumin to reverse parotid gland hypertrophy was therefore investigated in rats which were given prior injections of isoproterenol. As shown in Table 1, the introduction of α-lactalbumin for 3 days, (5 days after the initiation of parotid gland hypertrophy) caused a cessation of a further increase in gland weight. The incorporation of ^{3}H thymidine into DNA declined to nearly basal levels of DNA synthesis observed in control, untreated animals. This drug regimen was continued for an additional 5 days (8 days total of α-lactalbumin) with the same result as that observed after only 3 days with the α-lactalbumin regimen. Parotid gland weight was the same as that obtained after 5 days of isoproterenol-treatment but did not decline. Animals receiving only

Table 1. Effects of α-lactalbumin on isoproterenol-stimulated parotid gland hypertrophy

Treatment*	Parotid Gland Wt.+	Incorporation ^{3}H thymidine≠	Plasma Membrane 4ß-galactosyltranferase^P
Control	0.24 ± 0.09	625 ± 50	0.02 ± 0.01
5dIPR	0.83 ± 0.10	4,350 ± 100	3.37 ± 0.26
8dIPR	1.05 ± 0.12	19,600 ± 100	3.76 ± 0.30
5dIPR/3α-LA	0.80 ± 0.05	1,600 ± 200	3.35 ± 0.36
13dIPR	1.83 ± 0.22	22,500 ± 200	3.74 ± 0.29
13dIPR/8α-LA	0.82 ± 0.17	1,500 ± 100	3.52 ± 0.42

* Animals received twice daily injections of 5 mg of isoproterenol. After 5 days groups of animals were started on injections of 10 mM α-lactalbumin in addition to continuing the isoproterenol treatment. Values are expressed as the average of four animals ± standard deviation.

\+ Values are expressed as grams of wet weight of the parotid gland.

≠ Values are expressed as cpm/mg of protein.

P Values are expressed as nmol galactose incorporation/min/mg membrane protein.

isoproterenol for the 13 day period showed a nearly 9-fold increase in gland size. [^{3}H]thymidine incorporation into DNA of 13 day isoproterenol-treated animals was slightly greater than that observed at 8 days of isoproterenol treatment.

Plasma membranes were isolated to assess the continued role of surface 4ß-galactosyltransferase in mediating gland hypertrophy. As has been previously found [16,20] surface levels of 4ß-galactosyltransferase remained elevated even though gland hypertrophy had been blocked as a consequence of the presence of α-lactalbumin. The presence of possible Golgi membrane contamination was determined by assay of another trans-Golgi enzyme sialyltransferase [22]. Assay for the presence of this enzyme using desialylated fetuin as an exogeneous acceptor was low when compared to the activity present in the total membrane preparation (0.02 ± 0.01 nmol/min/mg protein versus 0.32 ± 0.07 nmol/min/mg protein specific activity found in plasma and total membranes respectively). Both control and experimental plasma membranes exhibited a 10-fold higher level of activity for gamma-glutaminyl peptidyltransferase (a specific acinar cell plasma membrane marker enzyme [1]) than the total membrane preparation, indicating that this fraction was indeed enriched for plasma membranes.

Since parotid glands of neonatal rats are morphologically and functionally immature [32] it was of interest to determine the role of cell surface 4ß-galactosyltransferase in possibly mediating gland development. 4ß-galactosyltransferase was previously been implicated in regulating embryonic development and tissue differentiation [36,41]. Neonatal animals were given injections of α-lactalbumin starting 24 h after birth and continuing until they were sacrificed at the times indicated. Contrary to the expected inhibition of gland enlargement and development when animals were given α-lactalbumin, the drug regimen resulted in a precosious stimulation of development over the first two weeks of birth. This was evident not only in the greater size of the α-lactalbumin-treated parotid gland but also in the higher level of a major developmentally regulated enzyme, amylase (see Table 2). The α-lactalbumin treated glands exhibited a higher rate of [^{3}H]thymidine incorporation into DNA than the non-treated controls, again indicating a stimulation of mitotic activity. The total membrane associated 4ß-galactosyltransferase enzyme activity was approximately two-fold greater in treated animals when compared to controls during the 14 days of α-lactalbumin treatment post-partum. By the third week however control and treated parotid gland were essentially the same with regard to the experimental parameters measured in these studies. Plasma membrane isolated on days 6 and 14 of α-lactalbumin treatment had increased levels of surface 4ß-galactosyltransferase activity (2.35 ± 0.21 and 2.57 ± 0.37 respectively) which by 21 days decreased to 50% of the activity observed at the earlier times. By 28 days, cell surface 4ß-galactosyltransferase was 35% of the peak of enzyme activity found at 14 days post partum.

In the present study we have found that α-lactalbumin can inhibit the growth of acinar cells already rapidly dividing as a consequence of prior stimulation with isoproterenol. The application of α-lactalbumin to neonatal animals however, resulted in a precocious stimulation of acinar cell growth and differentiation. Although the underlying basis for this stimulatory effect is unclear, we have demonstrated a bimodal response to α-lactalbumin by

Table 2. Effects of α-lactalbumin on neonatal parotid gland development

Treatment*	Gland Weight	Total membrane 4ß-galactosyltransferase activity	Incorporation of+ 3H thymidine	Amylase± activity
α-LA6 days	0.01 ± 0.005	0.03 ± 0.01	700 ± 200	7.4 ± 0.7
Control	0.005 ± 0.002	0.01 ± 0.01	200 ± 10	2.6 ± 0.5
α-LA14 days	0.03 ± 0.01	0.09 ± 0.02	2150 ± 100	78.9 ± 4.3
Control	0.01 ± 0.01	0.05 ± 0.01	450 ± 50	36.4 ± 7.6
α-LA 21 days	0.14 ± 0.03	0.09 ± 0.03	4500 ± 150	140.2 ± 5.3
Control	0.07 ± 0.02	0.09 ± 0.03	3570 ± 100	114.5 ± 6.2
α-LA 28 days	0.15 ± 0.03	0.14 ± 0.06	5000 ± 150	185.3 ± 9.1
Control	0.14 ± 0.03	0.16 ± 0.07	5500 ± 200	182.9 ± 10.3
Adult (α-LA5 days)π	0.24 ± 0.04	0.22 ± 0.03	250 ± 10	2217.0 ± 18.0
Adult Control	0.26 ± 0.03	0.26 ± 0.03	240 ± 15	2054.6 ± 201.7

* All values represent the mean from 10 experimental animals ± S.D.
\+ Incorporation of label expressed as cpm/mg protein ± S.D.
± Enzyme activity is expressed in units of activity 1 mg of protein.
π adult animal was 49 days old.

acinar cells that is dependent on their stage of development. Whether the same or a different signal transduction mechanism involving cell surface 4ß-galactosyltransferase is involved in the stimulatory and inhibitory response to α-lactalbumin remains to be determined.

The ability of the same effector molecule to demonstrate both stimulatory and inhibitory growth of cells is not without precedent. The transforming growth factor B (TGFB) is a bifunctional regulator of cell growth. This factor has been shown to inhibit the growth of a variety of tumor cells, but can also stimulate the growth of non-transformed fibroblasts [22,39]. Fisher rat 3T3 cells, transfected with the proto-oncogene *myc*, can be stimulated to divide by the synergistic action of TGFB and plaltet derived growth factor (PDGF). The substitution of epidermal growth factor for PDGF results in growth inhibition of the 3T3 cells [24,39].

The ß subunit of cholera toxin, which binds to GM1 gangliosides, also demonstrates a bimodal effect on cell proliferation. The addition of the ß subunit to quiescent mouse 3T3 cells results in a stimulation of DNA synthesis and cell division [39]. The addition of the ß subunit to *ras*-transformed 3T3 cells inhibits their growth as well as that of rapidly dividing normal 3T3 cells. The response of a cell to a growth factor may therefore depend not

only on the growth factor but to the total set of stimulatory and inhibitory agents present at the time of exposure.

The mechanism(s) by which α-lactalbumin is able to modulate the growth response of parotid acinar cells positively and negatively is unknown. However, that the only known function of α-lactalbumin is to bind to 4ß-galactosyltransferase and modulate the substrate specificity of the enzyme. α-Lactalbumin will chelate into lipid bilayers depending on pH, thereby effecting membrane fluidity and protein mobility [8,12,40]. Many membrane bound enzymes, including glycosyltransferases are sensitive to changes in membrane fluidity [5,13]. Thus the bimodal response to α-lactalbumin may be related not only to steric interactions with cell surface 4ß-galactosyltransferase but also to alterations in enzyme activity as a consequence of lipid membrane interactions of the protein as well. From our findings we propose that α-lactalbumin may be a modulator of positive and negative signals for rat acinar cell growth.

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