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Cell Surface Expression of 4β-Galactosyltransferase Accompanies Rat Parotid Gland Acinar Cell Transition to Growth

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Rat parotid gland acinar cells stimulated to divide by a chronic regimen of isoproterenol demonstrate a dramatic increase in the synthesis of the glycosyltransferase 4β-galactosyltransferase. A plasma membrane localization for much of the increase in 4β-galactosyltransferase was determined by density gradient membrane fractionation. Golgi-enriched fractions showed no increase in specific activity, while plasma membrane activity increased 40-fold. This selective increase at the cell surface was confirmed by immunofluorescence of intact, nonpermeabilized cells from treated glands, using a monospecific antibody prepared against the purified bovine milk transferase. In detergent-permeabilized cells staining of nontreated cells was seen only as groups of perinuclear vesicles, presumed to be Golgi apparatus. In isoproterenol-treated and permeabilized cells both presumptive Golgi and cell surface staining was apparent. Enzyme assays performed on intact cells established that the enzyme's active site was oriented to the exterior of the cells. The transferase could be detected as early as 3 hr after the primary challenge with isoproterenol. Pretreatment of rats with cycloheximide prevented its appearance.

Key words: galactosyltransferase, growth control, cell surface enzymes

Membrane-associated glycosyltransferases have classically been localized to the Golgi apparatus [1], but evidence continues to accumulate that a portion of the cellular activity is present at the cell surface and oriented toward the extracellular space [2–4]. It has been suggested that while the Golgi-bound forms of the enzymes function in posttranslational modification of proteins and lipids, the cell surface glycosyltransferases could be involved in intercellular recognition or cell-cell adhesion [2,3].

Galactosyltransferases at the surfaces of contacting cells have also been suggested to be possible mediators of growth control. Roth and White [5] presented

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evidence implicating these enzymes in the contact inhibition exhibited by nontransformed tissue culture cells. In addition, the sugar donor for galactosyltransferase, UDP-galactose (UDP-Gal), has been shown to inhibit the growth of cultured cells [6,7]. While Roth et al [6] suggested that this effect was due to cell surface galactosyltransferase, Klohs et al [7] presented data attributing the response to a galactosyltransferase present in the serum added to their cultures. Various differences in 4β-galactosyltransferase activity between normal and transformed cells have also been reported [8,9], and enzyme levels in human serum [10–12] have been correlated with the presence of various tumors.

In our examinations of the growth exhibited by rat parotid glands in response to isoproterenol, our attention has become focused on a role for 4β-galactosyltransferase [13]. Chronic systemic administration of isoproterenol, a β-adrenergic receptor agonist, results in physiological changes within the parotid gland that include cell hypertrophy and hyperplasia [14,15]. DNA, RNA, and protein synthesis are all increased following isoproterenol administration [16]. It has been suggested that these effects are mediated by cyclic nucleotides [17,18], since the addition of a β-adrenergic antagonist blocks these changes. One of the proteins whose synthesis is stimulated by isoproterenol is 4β-galactosyltransferase, which increases up to tenfold in activity even though a second enzyme activity, 3β-galactosyltransferase, does not change [19].

Using a subcellular fractionation procedure specifically designed for rat parotid glands [20], we here have determined that the increase in 4β-galactosyltransferase activity accompanying the transition of the parotid gland to rapid growth is at least partially due to a 40-fold increase in specific activity of the enzyme in the plasma membrane fraction. This observation has been confirmed by the demonstrations of increases in 4β-galactosyltransferase in intact cell enzyme assays and with indirect immunofluorescence using a monospecific antiserum to this transferase [21].

MATERIALS AND METHODS

Materials

d,l-isoproterenol-HCl, UDP-galactose, bovine submaxillary mucin, α-lactalbumin, ovalbumin, and N-acetylglucosamine were purchased from Sigma Chemical Co. (St. Louis, MO). Collagenase and hyaluronidase were purchased from Cooper Biomedical (Melvern, PA). UDP-[^{14}C]galactose (300 Ci/mmol), UDP-[^{3}H]galactose (300 Ci/mmol), and [α^{32}P]dCTP and dTTP (3,000 Ci/mmol) were purchased from Amersham (Arlington Heights, IL). Removal of sialic acid from mucin was accomplished by mild acid hydrolysis as described previously [19]. The 4β-galactosyltransferase antibody used in these studies was shown elsewhere [13] to react with determinants of 42 and 48 kilodaltons (kDa) in Western blots of bovine milk, the molecular weights established for soluble 4β-galactosyltransferase [19]. In Western blots of parotid membranes it reacted solely with a determinant of about 54 kDa [13]. Reagents for agarose gel electrophoresis and buffers were obtained through commercial sources and were of analytical quality. Wistar strain rats were purchased from Charles River Breeding Farm (Wilmington, MA).

Preparation of Isolated Parotid Gland Acinar Cells and Membranes

Male Wistar rats weighing 175–225 g received twice-daily intraperitoneal injections of 0.5 ml of 10 mg/ml d,l-isoproterenol for up to 10 days. Parotid glands were

identified by gross morphology following anesthesia with pentabarbital and death by exsanguination. Intact cells were freed from connective tissue and dissociated using collagenase and hyaluronidase as described by Oliver et al [22].

Alternatively, total membrane fractions were prepared at 4°C by homogenization of intact parotid tissue from either isoproterenol-treated or untreated animals in 10 mM Tris/HCl buffer, pH 8.0, with a Dounce apparatus. Low-speed centrifugation at 1,000 g was then performed to remove connective tissue as well as unlysed cells. The resulting slurry was then centrifuged at 100,000 g for 1 hr to pellet total membrane. Protein assays were performed by a modification of the Lowry method with bovine serum albumin as the standard [23]. Plasma membranes were isolated by the procedure of Arvan and Castle [20], a protocol designed specifically to isolate plasma membranes from rat parotid gland. The total membrane pellet was resuspended in 0.5 mM $MgCl_2$, 1 mM $NaHCO_3$ (pH 7.4) containing 0.7 mM EDTA. The membrane slurry was centrifuged at 825 g for 15 min, and the pellet resuspended in one-half volume of the above buffer. The suspension was overlayed onto 5 ml of the same buffer containing 0.3 M sucrose and recentrifuged at 12,500 g for 15 min. The pellet was adjusted to 1.38 M sucrose (125 ml), overlayed with 0.3 M sucrose, and centrifuged for 2 hr in a Beckman swinging bucket rotor SW27 at 50,000 g. Plasma membranes were removed from the interface, diluted to 0.35 M sucrose in 0.5 mM $MgCl_2$, 1 mM $NaHCO_3$ (pH 7.4) containing 1.7 mM EDTA, and centrifuged in an SW41 rotor for 2 hr at 150,000 g. Fractions enriched for Golgi membranes were obtained from the supernatant of the 12,500 g centrifugation [20]. This supernatant was diluted to 12 ml in 0.3 M sucrose and centrifuged at 100,000 g for 1 hr. The resulting pellet is enriched for Golgi membranes [20]. The final membrane pellets were resuspended in 10 mM Tris buffer (pH 8.0) for subsequent assays for protein and 3β- and 4β-galactosyltransferase activities.

Galactosyltransferase Assays

The activity of two galactosyltransferases were measured as previously described by Humphreys-Beher et al [19]. In brief, membrane fractions were obtained as described above and resuspended in 10 mM Tric/HCl, pH 8.0, to give a final protein concentration of 200 μg/ml. The assay mixture (total volume 50 μl) contained 0.1 M 2-(N-morpholino) ethane sulfonic acid (MES) (pH 6.3), 25 mM $MnCl_2$, 0.5% Triton X-100, 1 mM UDP-[1-^{14}C] galactose (2 mCi/mmol), 0.5 mg ovalbumin (for 4β) or asialo-bovine submaxillary mucin (for 3β) as acceptor, and 0–0.5 mg of the membrane preparation. Assays for sialyltransferases and N-acetylglucosaminyltransferases were performed using the same buffer conditions. One millimolar CMP-[9-^{3}H] sialic acid (New England Nuclear, 2 mCi/mmol) and 10 mg asialofetuin were included as sugar donor and acceptor molecule, respectively, for the assay of sialyltransferase. UDP-[1-^{14}C] N-acetylglucosamine (New England Nuclear, 2 mCi/mmol) and 10 mg asialo-agalacto-a-N-acetylglucosaminyl-fetuin were used to assay N-acetylglucosaminyltransferase. Sialic acid was removed from glycoprotein acceptors by acid hydrolysis [24]. The removal of galactose and N-acetylglucosamine was accomplished by incubation with β-N-acetylgalactosidase (Sigma, 20–40 U/mg) and β-glucosaminidase (Sigma, 35 U/mg) in 50 mM sodium acetate, pH 5.0 [25,26]. Free galactose and N-acetylglucosamine were removed by dialysis against 10% acetic acid. After lypholization, the glycosidase activity was destroyed by heat denaturation. Any residual enzyme activity should be minimal in subsequent transferase assays owing to

the higher pH (6.5) of the reaction mixture versus that of the glycosidase reaction. After incubation at 37°C for 1 hr the reaction was terminated by the addition of 1 ml ice cold 10% trichloroacetic acid (TCA), and the precipitate was recovered on glass fiber filters.

Cell Surface 4β-Galactosyltransferase Assay

Intact cells from stimulated and control animals, isolated by the protocol described above, were washed into Ca^{++}-, Mg^{++}-free Hanks' solution containing 10 mM $MnCl_2$. They were resuspended in the same buffer and approximately 10^6 cells were added to incubation tubes that also contained 200 μM UDP-[^{3}H]Gal (0.2 Ci/mmol unless specified) in a final volume of 50 μl. The tubes also contained ovalbumin (10 mg/ml final concentration) or GlcNAc (30 mM final concentration) or neither so as to assess activity towards exogenous or endogenous acceptors, respectively. Cell protein was determined using the Bio-Rad Protein Assay. Owing to the larger size of the isoproterenol-treated cells, normalization on the basis of protein resulted in about 15% fewer cells being present in assays with treated cells. Enzyme assays were performed at 37°C for 1 hr with intermittent mixing of the cells. Lactate dehydrogenase assays on postincubation supernatants and sonicated cells were performed [27] to provide an estimate of enzyme leakage. The reactions were terminated by the addition of 1 ml cold 10% TCA and the incorporation of [^{3}H]galactose determined by precipitation onto glass fiber filters followed by scintillation counting. In assays utilizing GlcNAc as an acceptor the incorporation of [^{3}H]galactose into disaccharide was determined using high-voltage borate paper electrophoresis [28]. Zero time controls were subtracted from all reported values.

Cell Surface Immunostaining

Dissociated cells for immunofluorescent studies of intact nonpermeabilized cells were washed into a balanced salt solution containing 5% fetal calf serum and 0.02% sodium azide (included to hinder energy-requiring rearrangements of cell surface constituents). The IgG fractions from the anti-4β-galactosyltransferase antiserum or a pre-immune serum were added at a concentration corresponding to a 1:20 dilution of the initial serum. The incubation was carried out on ice for 30 min with periodic resuspension, and the cells subsequently were washed three times in the calf serum-containing buffer. Commercially available fluorescein isothiocyanate (FITC)-conjugated antirabbit IgG antiserum (1:20) was then added to the cells, and following incubation and washing as described above, the cells were either cytocentrifuged and then fixed with 2% formalin for microscopic examination or fixed directly for cell sorting. Acinar cells from the different conditions were examined with a Leitz microscope equipped with epifluorescent illumination and appropriate filters for fluorescein fluorescence to determine surface labeling.

A Becton-Dickenson FACStar Fluorescence-Activated Cell Sorter with a 2-W argon ion laser tuned to 488 nm was used to quantitate cell surface labeling of 4β-galactosyltransferase on control and stimulated cells [29]. Routinely 10,000 cells per condition were quantitated for immunofluorescence.

In experiments in which cells were to be permeabilized, the dissociated cells were cytocentrifuged, and then fixed, permeabilized with Triton X-100, stained, and washed as described by Berger and Hesford [30].

RESULTS

Assays of Membrane Fraction for Galactosyltransferase Activities

As reported previously [19], total membrane-associated 4β-galactosyltransferase specific activity in parotid glands was found to increase following isoproterenol stimulation, while 3β-galactosyltransferase activity remained unchanged (Table I). In addition, it was determined that membranes enriched for Golgi apparatus [20] exhibited no changes in either enzyme's specific activity following treatment. However, plasma membrane fractions prepared from isoproterenol-treated animals showed a 40-fold stimulation in 4β-galactosyltransferase specific activity, while other Golgi transferases such as sialytransferase, N-acetylglucosaminyl transferase, and 3β-galactosyltransferase remained low. These transferases were, however, enriched in the Golgi fraction relative to the specific activity obtained in total membrane preparations. The resulting specific activity for the 4β-galactosyltransferase in the stimulated plasma membranes was nearly two-thirds that of the Golgi-enriched fractions.

Intact Cell Surface 4β-Galactosyltransferase Assays

In order to investigate further the enhancement in 4β-galactosyltransferase activity seen with isoproterenol, intact parotid gland acinar cells were isolated from treated and control rats and assayed for surface enzyme activity. As shown in Table II, the incorporation of [^{3}H]-Gal into endogenous acceptors by intact cells increased nearly four-fold after treatment with isoproterenol. When the exogenous acceptor ovalbumin was present the increase was even more pronounced. A tenfold stimulation of treated versus nontreated cell surface transferase was also seen when 30 mM GlcNAc was utilized as an exogenous acceptor (data not shown). This level of incorporation could not be attributed to cell breakage and the release of 4β-galactosyltransferase to the medium since assays for solubilized transferase following a mock incubation were negative and since leakage of lactate dehydrogenase to the medium was only 4% of the activity released by sonic disruption of the cells. It is also difficult to attribute the incorporation to internal utilization of the isotope since the inclusion of excesses of unlabeled Gal or αGal-1-phosphate had no effect on incorporation, while excess unlabeled UDP-Gal significantly diluted macromolecular label. These results, when coupled with the general impermeability of membranes for intact sugar nucleotides [31], strongly suggest the presence of externally oriented surface 4β-galactosyltransferase.

As early as 3 hr after a single isoproterenol challenge 4β-galactosyltransferase activity is present at the cell surface in elevated quantities (Table III). A further increase in surface expression of enzyme activity was seen 19 hr after treatment with isoproterenol and, with continued drug treatment, increased further at 10 days. Pretreatment of animals with a dosage of cycloheximide sufficient to inhibit most protein synthesis [32] prevented the increase in surface 4β-galactosyltransferase activity. This would suggest that at least the bulk of cell surface 4β-galactosyltransferase is the result of de novo protein biosynthesis.

Immunofluorescent Analysis of Intact Cells

Isolated parotid acinar cells were also assessed for surface 4β-galactosyltransferase by indirect immunofluorescence using a monospecific rabbit antibody to 4β-

TABLE I. Glycosyltransferase Activities in Parotid Membrane Fractions

Membrane fraction	4β-Galactosyltransferase[a]		3β-Galactosyltransferase[b]		Sialyltransferase[c]		N-acetylglucosaminyltransferase[d]	
	Control	IPR-treated	Control	IPR-treated	Control	IPR-treated	Control	IPR-treated
Total membranes	0.36 ± 0.07	1.69 ± 0.17	1.93 ± 0.14	2.06 ± 0.2	0.86 ± 0.12	0.53 ± 0.21	0.16 ± 0.05	0.12 ± 0.07
Golgi-enriched	5.41 ± 0.23	5.04 ± 0.36	4.23 ± 0.17	4.33 ± 0.15	10.24 ± 0.31	8.16 ± 0.21	2.22 ± 0.11	2.28 ± 0.10
Plasma membranes[e]	0.07 ± 0.05	3.27 ± 0.05	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.23 ± 0.02	0.04 ± 0.02	0.03 ± 0.01

[a]Values expressed as nmol/min/mg protein utilizing 10 mg/ml ovalbumin as an exogenous acceptor in these assays.
[b]Values expressed as nmol/min/mg protein utilizing 10 mg/ml asialo-bovine submaxillary mucin as exogenous acceptor.
[c]Values expressed as nmol/min/mg protein utilizing 10 mg/ml asialo-fetuin as exogenous acceptor.
[d]Values expressed as nmol/min/mg protein utilizing 10 mg/ml asiasol-agalacto-1-N-acetylglucosaminyl-fetuin as exogenous acceptor.
[e]Enrichment of plasma membrane fractions was determined by the assay of the parotid gland acinar cell plasma membrane marker enzyme γ-glutamyltranspeptidase [20]. Plasma membranes prepared as described resulted in a ten-fold enrichment in the specific activity for this enzyme over that present in the total membrane preparation (3,700 U/mg protein and 350 U/ml, respectively).

TABLE II. Cell Surface 4β-Galactosyltransferase Assays of Intact Parotid Cells

	[^{3}H]Gal transferred to macromolecular acceptors	
	Noninjected controls (cpm)	5-Day isoproterenol treatment (cpm)
Endogenous acceptors only	1,200	4,600
+ 10 mg/ml ovalbumin	2,500	15,800
Ovalbumin + excess unlabeled UDP-Gal (2 mM)	200	1,200
Ovalbumin + unlabeled αGal-1-P (2 mM)	2,400	17,000
Ovalbumin + unlabeled Gal (2 mM)	2,600	17,200
Leaked activity toward ovalbumin in supernatant[a]	200	700
Sonicated cell activity toward ovalbumin	32,000	71,000

[a]Cells removed by centrifugation following a mock assay. Supernatant then assayed for leaked transferase.

TABLE III. Induction of Parotid Gland Acinar Cell Surface 4β-Galactosyltransferase Enzyme Activity*

	[^{3}H]Gal transferred to macromolecular acceptors	
Treatment	Endogenous (cpm)	Exogenous (cpm)
Experiment 1[a]		
Control	6,800	8,000
3 hr isoproterenol	16,100	78,400
3 hr isoproterenol + 5 mg/kg cycloheximide[b]	5,800	11,700
19 hr isoproterenol	23,800	181,800
Experiment 2[c]		
Control	1,300	1,900
19 hr isoproterenol	2,500	8,100
10 days isoproterenol	4,100	15,900

*All results are the average of two experimental animals, each assayed in duplicate.
[a]5 μM UDP-Gal (2×10^6 cpm) input label.
[b]Cycloheximide was injected 2 hr prior to isoproterenol. This concentration has previously been shown to inhibit 90% of protein synthesis in rats [29].
[c]200 μM UDP-Gal (2×10^6 cpm) input label.

galactosyltransferase [13]. Because of the recent report by Childs et al [33] that carbohydrate epitopes on bovine milk 4β-galactosyltransferase can cross-react with unrelated glycoproteins in certain other tissues, plasma membranes from parotid glands were probed with this antiserum. In Western blots reactivity was seen solely with a determinant of about 54 kDa [13], the molecular weight for membrane-associated 4β-galactosyltransferase previously reported by Strous and Berger [34] in human epithelial carcinoma (HeLa) cells. Using this antiserum and an FITC-labeled secondary antibody we obtained results on nonpermeabilized cells that correlated well with the enzyme assays in a variety of experiments and served to further confirm the surface expression of this enzyme. While there was no detectable surface staining seen with pre-immune serum on either untreated or isoproterenol-stimulated cells

(Fig. 1A), very light staining was observed with the anti-4β-galactosyltransferase using untreated control cells (Fig. 1B). Significantly brighter staining was seen with isoproterenol-stimulated cells (Fig. 1C). Optical sections in the plane of the cell surface revealed a patchiness in staining on the isoproterenol-treated cells, despite a constant 4°C temperature and the presence of azide in the medium. A light fixation in formalin prior to the application of the first antibody did not alter this pattern (data not shown).

A fluorescence-activated cell sorter was used to quantitate the results of the immunofluorescence studies [29]. Following initiation of the isoproterenol injections, mean fluorescence owing to 4β-galactosyltransferase staining of the cell surface increased by 3 hr and remained elevated during a 10-day drug regimen (Fig. 2). Two populations of cells seemed to be apparent in the nontreated cells (Fig. 3), and by 3 hr following isoproterenol administration, the majority of the cells were shifted to a higher level of fluorescence. At later times nearly all the isolated cells were highly fluorescent (data not shown). The treatment of animals with the protein synthesis inhibitor cyclohexamide blocked significant increases in the staining of acinar cells stimulated with isoproterenol, thus confirming the results of the enzyme assays.

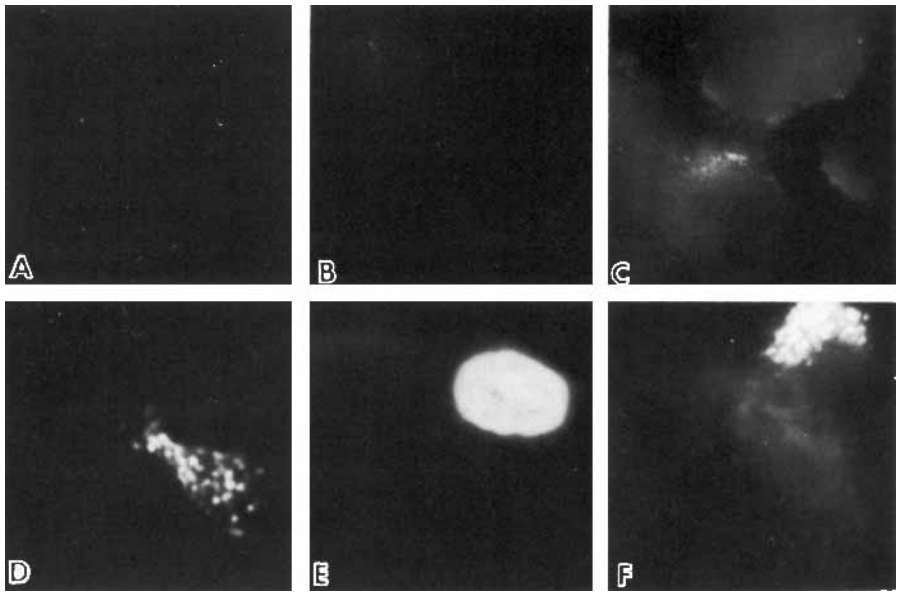

Fig. 1. Immunofluorescent staining of isolated rat parotid gland acinar cells from control animals or following 5-day isoproterenol treatment. **A:** Intact, nonpermeabilized isoproterenol-treated cells exposed to a pre-immune rabbit serum. **B:** Intact, nonpermeabilized control acinar cells exposed to anti-4β-galactosyltransferase antiserum. **C:** Isoproterenol-treated acinar cells exposed to the anti-4β-galactosyltransferase serum. **D:** Control acinar cells fixed and permeabilized with Triton X-100 and then exposed to antiserum to 4β-galactosyltransferase. The antibody revealed perinuclear, vesicular staining presumed to be Golgi apparatus, but no other staining in the cytoplasm nor on the cell surface. **E:** Nuclear staining of the same cell shown in D, using Hoechst reagent. **F:** Permabilized isoproterenol-treated cells exposed to antiserum to 4β-galactosyltransferase. The plane of focus reveals intense vesicular, perinuclear staining, presumed to be Golgi, and more diffuse staining owing to the presence of antibody at the cell surface and mainly out of focus in this optical section.

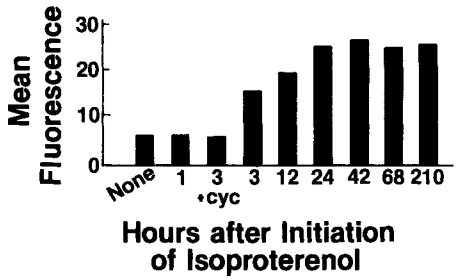

Fig. 2. Levels of cell surface 4β-galactosyltransferase as a function of time after initiation of isoproterenol treatment. Quantitation was determined from data such as that shown in Figure 3. Injections of 5 mg of isoproterenol began at time 0 and were repeated approximately every 12 hr thereafter.

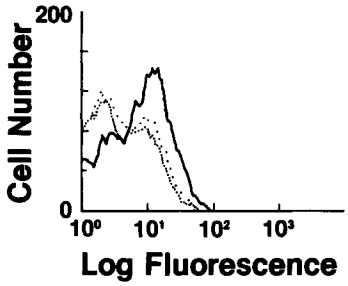

Fig. 3. Numbers of intact cells displaying various levels of fluorescence following staining with anti-4β-galactosyltransferase antibody and an FITC-labeled secondary antirabbit IgG antibody. With no isoproterenol (narrow dotted line) or 1 hr following the injection of 5 mg of isoproterenol (wide spaced dotted line), the majority of the cells displayed fluorescence levels of less than 6 arbitrary units. By 3 hr following injection the majority of the cells have shifted to 10–20 units (solid line).

When cells were fixed and permeabilized with Triton X-100 prior to application of the antibodies, perinuclear vesicular staining was observed in cells that had not been exposed to isoproterenol (Fig. 1D). This staining corresponds to fluorescent staining of the Golgi apparatus seen by Berger and co-workers in other cell types with an independent anti-4β-galactosyltransferase antibody [30,35–38]. Cells from animals treated with isoproterenol also showed putative Golgi staining but in addition showed a patchy, cell surface staining (Fig. 1F).

DISCUSSION

Membrane-associated 4β-galactosyltransferase has classically been localized to the Golgi apparatus [1] but has also been reported to be a cell surface constituent in a variety of cells [2–6]. Galactosyltransferases have previously been found associated with plasma membranes upon fractionation [39–41], and an external orientation has been supported by experiments with intact cells that include controls arguing against enzyme leakage or intracellular utilization [3]. Recently monospecific antibodies against 4β-galactosyltransferase have been utilized to localize the enzyme in both light and electron microscopic studies by Roth, Berger, and co-workers. While Golgi staining with this antibody was detected with all cells examined, a cell surface localization of 4β-galactosyltransferase was found in about half the cell types examined [4,27,35–38, but see also 33].

The mechanism responsible for this apparent change in subcellular localization of this enzyme remains unknown. The ability of cycloheximide to inhibit the appearance of 4β-galactosyltransferase at the cell surface of isoproterenol-stimulated cells, as well as conservation of specific activity in the Golgi, suggests the need for new synthesis of the enzyme. Our results do not, however, rule out the possibility that enzyme present in the Golgi prior to isoproterenol treatment can at least contribute partially to cell surface 4β-galactosyltransferase. One possible explanation for an external localization for 4β-galactosyltransferase is that overproduction of the enzyme is simply too much for the Golgi to accomodate and the excess is defaulted to the cell surface. Strous and Berger [34] presented data suggesting that in HeLa cells 4β-galactosyltransferase moves from the endoplasmic reticulum to Golgi in about 20 min, and then remains in the Golgi for an average of 9 hr. Following this lengthy stay, however, all the enzyme eventually seems to move to the plasma membrane and then is lost from the cell, probably by proteolysis. Immunofluorescent analyses indicate that in this cell type the enzyme's stay at the cell surface is brief, but the authors hypothesize that cleavage and release could occur at reduced rates in cells displaying significant cell-surface 4β-galactosyltransferase. Because of the increase in production of the enzyme in parotid cells stimulated by isoproterenol this balance could shift to favor a shorter stay in the Golgi and accumulation at the cell surface.

Another more complex mechanism for relocalization could involve a post-translational modification of the enzyme. Chronic isoproterenol treatment of rat salivary glands is known to activate cellular protein kinases [42]. Baum and co-workers [43] have recently provided evidence for the phosphorylation of galactosyltransferases of the dolichol pathway in parotid glands as a consequence of chronic isoproterenol treatment. In contrast to the increase in 4β-galactosyltransferase activity reported here, which would seem to be due to an increase in enzyme copy number, these galactosyltransferases appear to increase in activity owing to phosphorylation in response to isoproterenol. It is possible that a similar post-translational phosphorylation of 4β-galactosyltransferase in response to isoproterenol may lead not to a change in activity, but rather to alterations in the subcellular localization of this enzyme. Although such a kinase-mediated change in the subcellular distribution of a protein has not been reported, the transfer of a phosphate group from UDP-GlcNAc to C6 of mannose residues on N-linked oligosaccharides of lysozomal hydrolases directs their compartmentalization to lysosomal vesicles [44].

Because of the increase reported here in cell surface 4β-galactosyltransferase activity accompanying the transition to growth of the parotid glands following isoproterenol stimulation, our laboratories began a series of investigations to determine if this increase were important to the growth process [13]. One approach was to inject intraperitoneally agents that might interact with surface 4β-galactosyltransferase in the parotid. Injection of the 4β-galactosyltransferase modifier protein α-lactalbumin with isoproterenol caused a dose-dependent decrease in the parotid growth normally seen, with twice-daily injections of 5μmol effectively eliminating the increase. This injection resulted in serum concentration of approximately 20 μM. Injections with the control protein lysozyme had no effect on the isoproterenol-mediated increase [13]. In other experiments, co-injection of UDP-Gal (but not UDP-Glc or other sugar nucleotides) and of anti-4β-galactosyltransferase antibody (but not pre-immune serum) along with isoproterenol also led to significant decreases in growth.

The inhibition in parotid weight gain was paralleled by an inhibition in in vivo ^{3}H-thymidine incorporation. In addition, parotid cells were put into culture, and again

these agents selectively led to a decrease in ^{3}H-thymidine incorporation seen following exposure to isoproterenol. Interestingly, these inhibitions in growth were not accompanied by a dimunition of the levels of cell surface 4β-galactosyltransferase [13]. Together with the results reported in this paper, these results may suggest that an increase in 4β-galactosyltransferase is a necessary requisite for cell division in this tissue.

Since there is no evidence for the internalization of UDP-Gal, α-lactalbumin, or anti-4β-galactosyltransferase antibody, all of which inhibited the parotid hyperplasia, we suggest that it is the cell surface portion of this enzymatic activity that is mediating the growth inhibitory effects caused by these agents. One possible model, illustrated in Figure 4, depicts 4β-galactosyltransferase as providing a long-term binding site for GlcNAc-containing acceptor glycoproteins on the cell surface. As long as no UDP-Gal is present, catalysis can not occur and the juxtaposition persists. This juxtaposition may be crucial for maintaining a cell surface glycoprotein, such as a growth factor receptor, in a geometry appropriate for the transduction of signals for growth. The presence of extracellular UDP-Gal would be expected to result in increased catalysis of acceptors by the transferase while the presence of antiserum or α-lactalbumin might, by steric hindrance, also cause dissolution of such a juxtaposi-

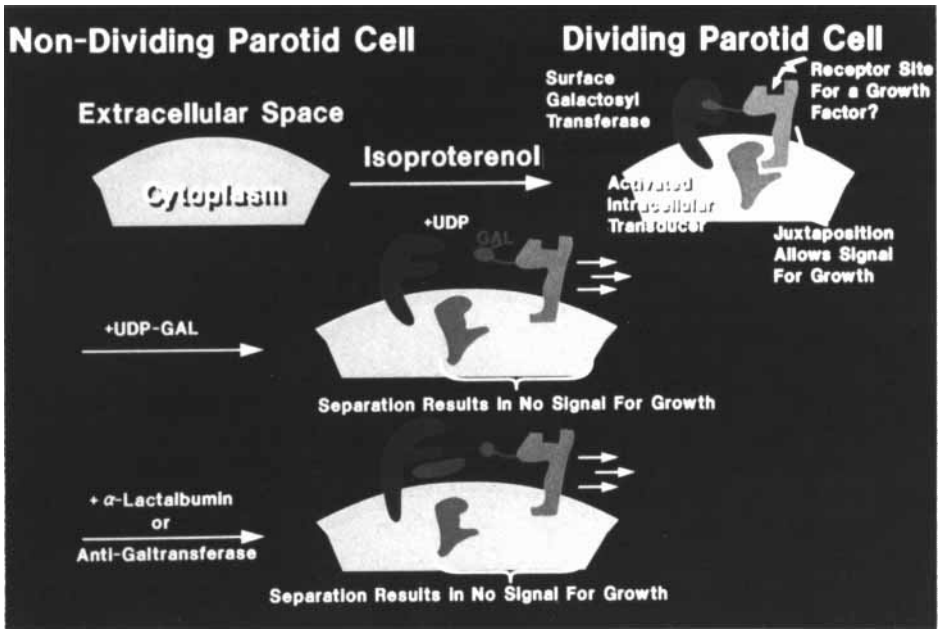

Fig. 4. On the left is shown a nondividing parotid cell with little cell surface 4β-galactosyltransferase. Isoproterenol causes an increase in this enzyme, as shown to the right. We hypothesize that the active site of this transferase is involved in the stable binding to the enzyme of some unknown cell surface glycoprotein via a terminal GlcNAc residue present on this unknown moiety. This juxtaposition is suggested to be crucial for the transduction of a signal for growth in these cells. The exogenous UDP-Gal allows for the addition of Gal to the hypothesized glycoprotein and thus destroys the required juxtaposition. The addition of α-lactalbumin or antibody to 4β-galactosyltransferase also results in separation, in this case owing to steric hindrance. Any of these additions would result in the inhibition of growth seen in vivo and in vitro with these agents [13].

tion. How such a phenomenon could result in inhibition of growth remains to be determined, although experiments to test this model are underway.

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