Effect of Tunicamycin, an Inhibitor of Protein Glycosylation, on the Biological Properties of Acetylcholine Receptor in Cultured Muscle Cells

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We have studied the effect of tunicamycin (TM), an antibiotic which inhibits the glycosylation of nascent proteins, on the properties of the acetylcholine receptor (AChR) at the surface of embryonic chick skeletal muscle cells. The use of two separate assays, specific binding of $^{125}$I-$\alpha$-bungarotoxin and carbamylcholine-activated $^{22}$Na$^+$ uptake, has allowed us to monitor the effects of impaired glycosylation on the metabolic and functional properties of AChR. A significant decrease in the amounts of surface AChR elaborated in the presence of TM is detected by both measurements. This decrease has been found to reflect an enhanced proteolytic degradation of the underglycosylated AChR. The underglycosylated AChR, expressed on the cell surface in the presence of TM, retains the capability of mediating agonist-activated ionic permeability changes, but displays quantitatively altered interactions with receptor ligands. We conclude that the carbohydrate moiety of AChR may play a role in determining the folding of newly synthesized polypeptides to form a conformation compatible with the metabolic properties and ligand interactions characteristic of glycosylated AChR.

The nicotinic AChR is a well characterized transmembrane glycoprotein that mediates the reception of neural impulses by muscle cells at vertebrate neuromuscular junctions (for review, see Refs. 1 and 2). The biosynthesis and incorporation of AChR into surface membranes of embryonic muscle cells differentiating in culture have been extensively studied (reviewed in Refs. 3 and 4). The binding of the activator carbamylcholine to AChR on the surface of cultured muscle cells triggers a large increase in Na$^+$ permeability. This AChR activity can be effectively assayed by the measurement of carbamylcholine-induced linear uptake of $^{22}$Na$^+$ (5, 6).

The contribution of oligosaccharide chains to the biological properties of glycoproteins is currently under extensive investigation. TM, an antibiotic that specifically inhibits protein glycosylation by blocking the synthesis and transfer of core oligosaccharides to asparaginyl residues (7-9), has provided a useful experimental approach to the study of the functional significance of the carbohydrate components of glycoproteins. Studies utilizing TM have indicated a role for carbohydrate moieties in regulating the processing and turnover of specific glycoproteins (10-21).

The carbohydrate moieties of AChR represent approximately 5% of its total weight (2, 22, 23). The impairment of protein glycosylation by TM has recently been shown to diminish the accumulation of AChR in cultured muscle cells (24-26).

In the present study, we have examined the consequences of impaired protein glycosylation on the functional properties of AChR. We provide evidence that the inhibition of protein glycosylation by TM treatment results in the expression of functionally altered AChR on the surface of cultured muscle cells.

EXPERIMENTAL PROCEDURES

Materials—TM was obtained from The Natural Products Branch, Division of Cancer Treatment, National Cancer Institute. $^{125}$I-$\alpha$-Bgt and $^{22}$NaCl were purchased from New England Nuclear. Carbamylcholine chloride, d-tubocurarine chloride, decamethonium bromide, cycloheximide, concanavalin A, chloroquine, $\alpha$-methyl-D-mannoside, phenylmethylsulfonyl fluoride, and ouabain were purchased from Sigma. Triton X-100 was purchased from Calbiochem. Concanavalin A-Sepharose 4B was purchased from Pharmacia. Leupeptin was kindly supplied by the United States-Japan Cancer Program. Tissue culture media were purchased from Gibco.

Cell Culture—Primary cultures of skeletal muscle cells were prepared from breast of 12-day-old chick embryos as described (27, 28). Cells were plated on collagen-coated culture dishes at an initial density of $1.8 \times 10^6$ cells/60-mm culture dish. The cultures were grown in DME supplemented with 25 mM Hepes (pH 7.4), 10% horse serum, and 2% embryo extract, at 37°C in an atmosphere of 92% air, 8% CO$_2$. At 2 days after plating, cultures were treated with 10 µM cytosine arabinoside for a 48-h period to minimize fibroblast proliferation (29).

Assay Procedures—Forty eight hours after plating, cultures were treated with TM (0.05 µg/ml) in growth medium for a 24-h period. Unlabeled $\alpha$-Bgt ($2 \times 10^{-5}$ M) was present during the initial 3 h of TM treatment to eliminate the contribution of AChR elaborated before TM addition. Cultures were then washed 5 times with DME to remove unbound $\alpha$-Bgt, and incubated in growth medium containing TM (0.05 µg/ml) for the remainder of the 24-h incubation period.

The specific binding of $^{125}$I-$\alpha$-Bgt was used to measure AChR on the surface of intact muscle cells, as described previously (34). After 2 washes with DME, cultures were incubated with $^{125}$I-$\alpha$-Bgt (10$^{-5}$ M) in 1 ml of DME containing 1 mg/ml of bovine serum albumin for 1 h at 37°C. Unbound toxin was removed by 5 washes with DME, and cells were suspended in 1 N NaOH containing 1% Triton X-100. Radioactivity was determined by $\gamma$ spectroscopy. Nonspecific binding, established in replicate cultures in the presence of the competitive ligand decamethonium (10 µM) was subtracted and did not account for more than 10% of total labeling.

$^{22}$Na$^+$ uptake was measured essentially as described by Catterall (30, 31). Cultures were preincubated in 1 ml of Na$^+$-free medium consisting of 135.5 mM KCl, 50 mM Hepes (adjusted to pH 7.4 with

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Trich base), 5.5 mM glucose, 0.8 mM MgSO4, and 0.1 mM ouabain for 30 min at 37°C. At the end of this period, medium was removed and cells were rinsed twice with 2.5 mL of medium consisting of 5.4 mM KCl, 130 mM choline chloride, 50 mM Hepes (pH 7.4), 5.5 mM glucose, and 0.8 mM MgSO4, at room temperature. Uptake of 22Na was then assayed at room temperature in 1 mL of medium consisting of 168 mM choline chloride, 50 mM Hepes (pH 7.4), 5.5 mM glucose, 0.8 mM MgSO4, 0.1 mM ouabain, and 22NaCl (5 μCi/mL). Under these conditions, 22Na uptake is linear with time for 20 s (see Fig. 4A). Linear 22Na uptake was assayed for 15 s and terminated by washing 5 times within 25 s with 2 mL of ice-cold medium consisting of 168 mM choline chloride, 50 mM Hepes (pH 7.4), 5.5 mM glucose, 0.8 mM MgSO4, 1.8 mM CaCl2, and 1 mM d-tuloburarine chloride. The cells were suspended in 1 N NaOH containing 1% Triton X-100 and radioactivity was determined by γ-spectroscopy. 22Na uptake measured in replicate cultures as described above, but in the absence of carbamylcholine, was subtracted from total uptake. Results are expressed as nmol of 22Na taken up/min/culture plate.

Concanavalin A-Sepharose Column— Cultures were treated with TM (0.05 μg/ml) or with TM and leupeptin (100 μM) for 24 h and labeled with 125I-a-Bgt as described above. Unbound toxin was removed by rinsing with DME. After an additional rinse with phosphate-buffered saline, cells were scraped from culture plates with a solution of 50 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1 mM PMSF, and 100 μM leupeptin at 4°C and Triton X-100 was added to a final concentration of 1%. After incubation at 4°C for 1 h, insoluble material was removed by centrifugation for 30 min at 100,000 × g. The supernatant (Triton extract) was adsorbed batchwise for 3 h at 4°C to 0.2 mL of Con A-Sepharose 4B that had been prewashed twice with 2.5-mL volumes of 1% Triton X-100 in 50 mM NaCl, 50 mM Tris-HCl, pH 7.4. The beads were then washed successively in batch with 8-mL volumes of 50 mM NaCl, 50 mM Tris-HCl, pH 7.4, containing 1 mM PMSF, and 1% Triton X-100, until no radioactivity was detected in the washes. Elution was carried out in batch with 0.4 M α-methyl-D-mannoside in 50 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1% Triton X-100 at 4°C for 40 h.

RESULTS

We have monitored the expression of AChR on the surface of muscle cells by two distinct measurements i.e. the specific binding of 125I-a-Bgt and the linear uptake of 22Na+ induced by the AChR activator carbamylcholine. Fig. 1 shows the time course of expression of AChR during muscle differentiation as monitored by these two assays. Highly similar kinetics of developmental appearance are observed. These results confirm that the linear uptake of 22Na+ induced by carbamylcholine reflects the amount of cell surface AChR.

With the aim of achieving maximal expression of underglycosylated AChR, cultures were treated with TM (0.05 μg/ml) at 48 h after plating for a 24-h interval. As can be seen (Fig. 1), during this period a major increase in AChR elaboration occurs. Under these experimental conditions, TM treatment inhibits incorporation of [3H]mannose into trichloroacetic acid precipitable material by over 80%, while causing less than 20% inhibition of [14C]leucine incorporation into protein (data not shown). To investigate the consequences of impaired protein glycosylation, the effects of TM and protease inhibitors on carbamylcholine-activated 22Na+ uptake have been studied. Table I shows that the linear uptake of 22Na+ mediated by AChR elaborated in the presence of TM is inhibited by approximately 80% as compared to untreated cultures. This inhibitory effect of TM is attenuated in the presence of the protease inhibitors leupeptin and chloroquine. In addition, Table I shows that the effects of TM and protease inhibitors on 22Na+ uptake and 125I-a-Bgt binding, measured in replicate cultures, are quantitatively similar. These results suggest that the TM-induced decrease in linear uptake of 22Na+ primarily reflects the reduced amounts of surface AChR. Furthermore, the partial reversal of the effect of TM by protease inhibitors is consistent with the possibility that the TM-induced loss of AChR activity is associated with enhanced proteolytic degradation.

![Fig. 1. Time course of appearance of AChR during the differentiation of cultured muscle cells. AChR on the surface of intact cells was monitored by the specific binding of 125I-a-Bgt (○) and by the linear uptake of 22Na+ activated by carbamylcholine (△). For binding measurements, cultures were labeled with 125I-a-Bgt (106 cpm/mL) for 60 min at 37°C. Nonspecific binding, determined in the presence of the competitive ligand decamethonium (10 μM), was subtracted from total labeling and did not account for more than 10% of total labeling. 22Na+ linear uptake was measured in replicate cultures by incubation for 15 s at room temperature in the presence of carbamylcholine (10 mM). Uptake of 22Na+, measured under the same conditions but in the absence of carbamylcholine, was subtracted. Points represent the averages of 2 experiments each consisting of 3 determinations.](http://www.jbc.org/appendices/)
significantly enhanced, with a half-time of approximately 4 h (Fig. 2 and inset). These values are in close agreement with the degradation rates of $^{125}$I-a-Bgt-AChR complexes in TM-treated and untreated cultures reported previously (24).

ConA, a lectin with high affinity for glucose and mannose residues on glycoproteins, has been shown to interact with surface AChR in muscle cells (33). To monitor the effect of TM on the glycosylation of surface AChR, intact TM-treated and untreated cells were labeled with $^{125}$I-a-Bgt. The $^{125}$I-a-Bgt-AChR complexes were then detergent-solubilized and analyzed by ConA-Sepharose affinity chromatography. A comparison of the capacity for specific adsorption to ConA-Sepharose of $^{125}$I-a-Bgt-AChR complexes from TM-treated and untreated cultures is shown in Table I. As can be seen, the fraction of surface AChR from TM-treated cells that is adsorbed to ConA-Sepharose is significantly lower than that from untreated cells. In addition, the fraction of the adsorbed $^{125}$I-a-Bgt-AChR complexes that is specifically eluted by $\alpha$-methyl-D-mannoside is considerably smaller in extracts from TM-treated cells. Similar results are obtained with extracts from cultures exposed to TM in the presence of the protease inhibitor leupeptin (Table II), a treatment that results in a partial reversal of the TM-induced depletion of surface AChR (Table I). There is no detectable adsorption to ConA-Sepharose of the nonspecific component of $^{125}$I-a-Bgt bound to muscle cells in the presence of decamethonium (see "Experimental Procedures"). These results indicate that the AChR expressed on the surface of muscle cells during exposure to TM both in the absence and presence of leupeptin is under-glycosylated. Since TM blocks the synthesis and transfer of core oligosaccharides to asparaginyl residues (7-9), this under-glycosylated state of AChR apparently corresponds to the presence of fewer asparagine-linked core oligosaccharides.

To further investigate if the carbamylcholine-induced Na$^+$ uptake in TM-treated cells is mediated by AChR deficient in glycosylation, we have compared the effects of ConA on AChR activity in TM-treated and untreated cultures. As shown in Fig. 3 (inset), ConA inhibits carbamylcholine-activated $^{22}$Na$^+$ uptake in a dose-dependent manner, to a maximum of approximately 70% at 0.5 $\mu$M ConA. This inhibitory effect of ConA is almost abolished in cultures previously exposed to TM (Fig. 3).

To examine if the exposure of muscle cells to TM produces changes in the functional properties of AChR, we have measured $^{22}$Na$^+$ linear uptake at increasing concentrations of carbamylcholine in TM-treated and untreated cultures. As indicated by the dose-response relationships shown in Fig. 4B, the apparent $V_{max}$ is significantly reduced in TM-treated cells, reflecting the reduced number of functional receptors. Moreover, the apparent affinity of AChR for carbamylcholine is reduced in TM-treated cultures, as shown by the 3-4-fold increase in the apparent $K_a$ (Fig. 4B, inset). To rule out the possibility that the observed difference in apparent $K_a$ reflects the large difference in AChR levels in TM-treated and untreated cultures, these measurements were repeated under

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Effect of TM on the rate of decrease of carbamylcholine-activated Na$^+$ uptake after the addition of cycloheximide. $^{22}$Na$^+$ uptake was measured in control cultures (○) and in cultures pretreated with TM (0.05 $\mu$g/ml) for 24 h (□) at various times after addition of cycloheximide (10 $\mu$g/ml). Results are displayed as linear and semilogarithmic (inset) plots. The initial point in the semilogarithmic plot corresponds to a 4-h interval after cycloheximide addition. Points represent the average of 3 determinations.

![Figure 3](http://www.jbc.org/)

**Fig. 3.** Comparison of the effect of ConA on Na$^+$ uptake in TM-treated and untreated cultures. ConA (50 $\mu$g/ml) was added for a 1-h period to control cultures and to cultures pretreated with TM (0.05 $\mu$g/ml) for 24 h. Na$^+$ uptake was then measured as described under "Experimental Procedures." Results are expressed as percentage of values obtained with replicate cultures not exposed to ConA. Values shown are the averages of 6 determinations. Bars represent the range. Inset, effect of increasing concentrations of ConA on carbamylcholine-activated Na$^+$ uptake. Results are expressed as percentages of Na$^+$ uptake in the absence of ConA and are averages of 3 determinations.

| $^{125}$I-a-Bgt-AChR | Triton extract | Control | TM | TM + Leupeptin | Decamethonium |
|----------------------|----------------|---------|----|----------------|--------------|
|                      | f mol          | $^{22}$  | f mol | $^{22}$         | f mol        |
| Adsorbed             | 498            | 100     | 136 | 100            | 175          |
| Eluted with $\alpha$-methyl-D-mannoside | 318 | 64 | 42 | 31 | 47 | 27 |
|                      | 219            | 44 (69$^a$) | 12 | 9 (29$^a$) | 14 | 8 (30$^a$) |

$^a$ Values in the % column are expressed as percentage of radioactivity counted in the Triton extract. Values shown are the means obtained in a single experiment. Values obtained in 3 separate experiments, though variable, show both adsorption and elution of $^{125}$I-a-Bgt-AChR in TM-treated cells to be significantly lower than that in untreated cells.

$^b$ Amount of radioactivity retained on the column after successive washes (see "Experimental Procedures").

Elution expressed as percentage of adsorption.

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*Table II: Adsorption of $^{125}$I-a-Bgt-AChR to ConA-Sepharose*

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the following conditions that eliminated the possible contribution of the difference in amount of functional AChR. Two days after plating, cultures were treated with α-Bgt (2 × 10⁻⁶ M) for 1 h. Unbound toxin was removed, and cells were allowed to accumulate new AChR for further 4-h period. After this interval, the amounts of new surface AChR, as measured by both [³²P]α-Bgt binding and "Na⁺ uptake, were equivalent to AChR levels in cultures exposed to TM for 24 h. However, the apparent $K_m$ under these conditions (1.6 mm, results not shown) is significantly lower than the $K_m$ in TM-treated cultures (4.5 mm, Fig. 4B), and resembles that of untreated cultures (1.2 mm, Fig. 4B).

The time-dependence of carbamylcholine-activated "Na⁺ uptake is shown in Fig. 4A. The uptake of "Na⁺ is linear during the initial 20 s of exposure to carbamylcholine (10 mm), and subsequently attenuates sharply. This decrease in AChR-mediated Na⁺ uptake in the presence of activator has been attributed to the development of receptor inactivation (5), a process characteristic of AChR (34, 35). To examine the significance of protein glycosylation for the development of AChR inactivation, we compared the time dependence of carbamylcholine-induced "Na⁺ uptake in TM-treated and untreated muscle cells. Fig. 4A shows that, although the maximal uptake is reduced, the duration of the initial period of linear "Na⁺ uptake and its subsequent attenuation are not significantly altered by TM treatment.

The effect of various concentrations of d-tubocurarine, a competitive inhibitor of AChR, on carbamylcholine-induced "Na⁺ uptake in TM-treated and untreated muscle cells is shown in Fig. 4C. As can be seen, d-tubocurarine retains its inhibitory effect on carbamylcholine-induced AChR activation under conditions of impaired protein glycosylation. The altered dose-response relationship observed in cultures exposed to TM can reflect the change in apparent affinity towards carbamylcholine, and possibly an additional contribution of a modified interaction of underglycosylated AChR with d-tubocurarine.

**DISCUSSION**

In the present study, the consequences of impaired protein glycosylation on the functional and metabolic properties of AChR in cultured muscle cells have been investigated. We present evidence that AChR expressed on the surface of intact muscle cells treated with TM, an inhibitor of protein glycosylation, displays altered properties.

Under the culture conditions used, myogenesis is marked by a rapid burst of AChR appearance on the surface of newly fused muscle cells. The amount of AChR on the cell surface was measured by the specific binding of [³²P]α-Bgt and AChR activity was monitored by receptor-mediated Na⁺ uptake. The time-course of AChR accumulation is essentially identical when measured by either [³²P]α-Bgt binding or the initial rate of "Na⁺ uptake triggered by the AChR activator carbamylcholine (Fig. 1). This close similarity verifies that the initial rate of carbamylcholine-activated Na⁺ influx reflects the amount of AChR. Furthermore, the Na⁺ flux assay provides a quantitative estimate of functional AChR capable of transducing agonist binding into activation of ionic channels. The simultaneous appearance of toxin-binding and Na⁺ flux activities under conditions of synchronous differentiation (Fig. 1) indicates, in addition, that AChR reaches the cell surface in a functional form.

The AChR elaborated during the 24-h exposure to TM is expressed on the cell surface in reduced amounts but can be readily detected by both [³²P]α-Bgt specific binding and carbamylcholine-activated Na⁺ uptake. In order to restrict our measurements to the AChR synthesized in the presence of TM, the contribution of pre-existing AChR was abolished by exposure of muscle cells to unlabeled α-Bgt during the initial 3 h of TM treatment.

In the context of the present study, it is important to assess...
if the AChR detected on the surface of TM-treated cells is underglycosylated. The impaired glycosylation of surface AChR is suggested by our finding that exposure of muscle cells to TM results in decreased capacity of $[^{125}]$I-$\alpha$-Bgt-AChR complexes to adsorb specifically to ConA-Sepharose (Table II). Furthermore, we observe that ConA significantly inhibits Na$^+$ uptake in control cultures (Fig. 3, and inset) while, in marked contrast, ConA treatment fails to inhibit receptor-mediated Na$^+$ uptake in TM-treated cells (Fig. 3) even at a higher ConA concentration (1 $\mu$m, results not shown). These findings indicate that AChR appearing on the surface of TM-treated muscle cells is underglycosylated. The phenomenon of inhibition of carbachol-activated Na$^+$ uptake by ConA is itself of interest, indicating the proximity of receptor-linked oligosaccharides to either agonist binding sites or channel components.

The most pronounced effect of TM treatment is the extensive reduction in the amounts of AChR present on the cell surface. The net accumulation of AChR represents the balance between receptor synthesis and degradation. An enhanced degradation rate has been demonstrated for several glycoproteins synthesized in the presence of TM (10, 12, 15). The degradation rate of AChR, measured by the release of radioactivity from $[^{125}]$I-$\alpha$-Bgt specifically bound to AChR, was recently shown to increase markedly upon treatment of muscle cells with TM (24). To estimate the extent to which the TM-induced reduction of Na$^+$ flux (Table I) could be accounted for by enhanced degradation, carbachol-activated Na$^+$ uptake was monitored at various intervals after the addition of cycloheximide, an inhibitor of protein synthesis. Under these conditions, a significantly enhanced degradation rate of the surface underglycosylated receptor is observed (Fig. 2). The notion that the reduced Na$^+$ uptake induced by TM treatment reflects depletion of surface AChR due to enhanced receptor degradation is further supported by the partial reversal of the TM effect by the protease inhibitors leupeptin and chloroquine (Table I). These findings are consistent with evidence that the absence of carbohydrate moieties on glycoproteins renders these proteins more susceptible to proteolytic degradation both in vitro and in intact cells (10, 15, 36).

It is of interest to compare our present findings with a recent study by Merlie et al. (26). These authors utilized pulse-chase and immunoprecipitation procedures in detergent extracts to study the effects of TM on the expression of AChR subunits in a mouse muscle cell line. Their observations that TM treatment results in the elaboration of underglycosylated AChR that displays accelerated degradation kinetics are qualitatively consistent with previous observations (24) and our current findings. However, there are apparent quantitative discrepancies between our findings and the conclusions drawn by Merlie et al. As proposed by these authors, TM treatment results in a significant inhibition of AChR subunit assembly, and the small amounts of the nonglycosylated subunits that undergo assembly are degraded too rapidly to allow their accumulation on the cell surface. In marked contrast, our present findings, based on two independent assays for surface AChR, clearly indicate that underglycosylated receptors reach the surface of cultured muscle cells exposed to TM. How can our current findings be reconciled with the observations of Merlie and co-workers? Firstly, the different types of measurements utilized in each of the studies make meaningful quantitative comparisons difficult. In particular, the values of AChR catabolic half-life obtained by Merlie et al. (26) may have been influenced by several factors including the brief pulse intervals used, the short chase period, and the possible reduced detergent-solubility of the nonglycosylated subunits (18). In addition, the increased susceptibility of nonglycosylated proteins to proteolytic degradation (10, 12, 15, 36) may be amplified by enhanced protease activity in the detergent extract, leading to an overestimate of the degradation rate of underglycosylated AChR as compared to measurements performed in intact cells. Secondly, the AChR elaborated in the different cell type used by Merlie et al. displays different metabolic properties (3), and possibly different kinetics of synthesis, from those of AChR expressed on the surface of cultured embryonic chick skeletal muscle cells. Though it is conceivable that TM-induced inhibition of assembly of AChR subunits, as suggested by Merlie et al. (26), contributes to the reduced accumulation of AChR observed in the present study (Table I), treatment of muscle cells with TM during the period of sharply increased AChR elaboration (2 to 3 days post-plating) allows the accumulation of significant amounts of underglycosylated AChR on the cell surface.

The oligomeric glycoprotein AChR transduces the binding of activators into changes in transmembrane ionic permeability (for review see Refs. 1 and 2). The measurement of surface AChR by carbachol-activated Na$^+$ uptake provides a useful approach for investigating the functional significance of oligosaccharide chains on these receptors. In the present study, we find that AChR activation, desensitization, and susceptibility to inhibition by d-tubocurare are retained under conditions of impaired glycosylation (Fig. 4). However, the apparent affinity of surface AChR for carbacholystine is reduced as a consequence of TM treatment (Fig. 4B), independently of the TM-induced reduction in AChR levels (see "Results"). This finding is of particular interest when contrasted with the absence of detectable differences in the functional properties of several nonglycosylated glycoproteins synthesized in the presence of TM. Examples include the observation that nonglycosylated fibrinectin is as effective as the glycosylated protein in promoting fibroblast attachment and erythrocyte agglutination (38), as well as the finding that nonglycosylated G protein is functional in vesicular stomatitis virus attachment and penetration (18, 37).

Although retaining functional properties, the nonglycosylated G protein synthesized in the presence of TM displays modified physical properties consistent with an altered conformation (18, 38). Evidence for the significance of carbohydrate in maintaining glycoprotein tertiary structure has been advanced by studies utilizing TM (39) as well as enzymatic removal of oligosaccharide chains (40, 41). The notion that conformation is an important determinant of the degradative rates of proteins is well documented (for review see Ref. 42). The findings of increased susceptibility of several nonglycosylated glycoproteins to degradation by intracellular proteases constitute indirect evidence for the influence of carbohydrate moieties on tertiary structure. The association between the conformational features and the function of AChR has been established in studies using covalent modification (43). By analogy, the change in apparent affinity of underglycosylated AChR toward carbacholcholine observed in the present study may reflect the significance of carbohydrate for the conformational features of functional AChR.

In conclusion, we suggest that impairment of protein glycosylation by TM results in the expression of AChR altered in conformation and, as a consequence, changed in its metabolic and functional properties.

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