Inhibition of Glucose Trimming with Castanospermine Reduces Calnexin Association and Promotes Proteasome Degradation of the α-Subunit of the Nicotinic Acetylcholine Receptor*

(Received for publication, September 15, 1997, and in revised form, April 14, 1998)

Steven H. Keller‡, Jon Lindstrom§, and Palmer Taylor‡

From the ‡Department of Pharmacology, University of California, San Diego, La Jolla, California 92036-0636 and the §Departments of Neurosciences and Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6074

To identify factors involved in the expression of ligand-gated ion channels, we expressed nicotinic acetylcholine receptors in HEK cells to characterize roles for oligosaccharide trimming, calnexin association, and targeting to the proteasome. The homologous subunits of the acetylcholine receptor traverse the membrane four times, contain at least one oligosaccharide, and are retained in the endoplasmic reticulum until completely assembled into the circular arrangement of subunits of δ-α-γ-α-β to enclose the ion channel. We previously demonstrated that calnexin is associated with unassembled subunits of the receptor, but appears to dissociate when subunits are assembled in various combinations. We used the glucosidase inhibitor castanospermine to block oligosaccharide processing, and thereby inhibit calnexin’s interaction with the oligosaccharides in the receptor subunits. Castanospermine treatment reduces the association of calnexin with the α-subunit of the receptor, and diminishes the intracellular accumulation of unassembled receptor subunit protein. However, treatment with castanospermine does not appear to alter subunit folding or assembly. In contrast, co-treatment with proteasome inhibitors and castanospermine enhances the accumulation of polyubiquitin-conjugated α-subunits, and generally reverses the castanospermine induced loss of α-subunit protein. Co-transfection of cDNAs encoding the α- and δ-subunits, which leads to the expression of assembled α- and δ-subunits, also inhibits the loss of α-subunits expressed in the presence of castanospermine. Taken together, these observations indicate that calnexin association reduces the degradation of unassembled receptor subunits in the ubiquitin-proteasome pathway.

The nicotinic acetylcholine receptor is a prototype molecule of a family of ligand-gated ion channels, which include GABA<sub>A</sub> receptors, glycine, and 5HT<sub>3</sub> receptors (1, 2). Following binding by agonists to these receptors, a conformational change increases cation permeability through the central pore of the receptor, eliciting depolarization of the cell membrane (1, 2). Peptide backbones of each of the four subunits of the acetylcholine receptor traverse the membrane four times, and possess at least one Asn-X-Ser/Thr glycosylation signal (2, 3). The subunits are thought to undergo a maturation pathway which includes oligosaccharide attachment (4), formation of disulfide bonds (5–7), proline isomerization (8), and intersubunit contacts at specific interfaces (9–11). Members of this family of receptors are composed of a multisubunit complex of glycoproteins which are retained and assembled in the endoplasmic reticulum prior to transport to the cell surface (9, 12). Acetylcholine receptor subunits at the neuromuscular junction assemble into a circular orientation of subunits of δ-α-γ-α-β, to enclose the central ion channel (2, 13; but see Ref. 14).

The endoplasmic reticulum localized protein, calnexin, is associated with unassembled subunits of the acetylcholine receptor (15–17), but calnexin appears to be absent with combinations of assembled subunits (16). Connolly et al. (18) demonstrated that calnexin is associated with subunits of the GABA<sub>α</sub> receptor, indicating that calnexin association might be involved in the biogenesis of other multisubunit ion channels. Numerous investigations have established that calnexin associates primarily with monoglucosylated oligosaccharides, which are intermediates in the processing of nascent oligosaccharides or products of reglucosylation by the enzyme UDP-glucose:glycoprotein glucosyltransferase, and that treatment with the glucosidase inhibitor castanospermine can disrupt the interaction (reviewed in Refs. 19 and 20).

Using transient expression of acetylcholine receptor subunits in HEK cells, we find a role for calnexin association in reducing degradation of unassembled subunits by the proteasome, since treatment with castanospermine reduces the subunit-calnexin association and increases the polyubiquitination of the α-subunit. Additionally, co-treatment with proteasome inhibitors blocks the degradation. Although unassembled α-subunits are degraded at a rapid rate (5), treatment with glucosidase inhibitors substantially promotes degradation (4). Our data also indicate that castanospermine treatment does not cause the α-subunit to misfold, as detected by a conformationally sensitive antibody. Therefore, increased degradation by the proteasome appears to be independent of the nascent peptide undergoing misfolding in this system. In contrast, a recent study indicated that treatment of chick myotubes with castanospermine disrupts α-subunit folding and assembly (17). Our data also indicate that calreticulin and ERp57, the two other proteins known to have glycoprotein-associating properties similar to calnexin (19, 21), do not appear to be bound to the α-subunit.

Assembly with the δ-subunit reduces the degradation of the α-subunit when α- and δ-subunits are co-expressed in the presence of castanospermine, indicating that glucose trimming, calnexin association, assembly and entrance into the proteasome are linked in the expression of the receptor. Connections among these phenomena are also likely to be critical to the fidelity of expression of other multisubunit glycoproteins.

* This work was supported by United States Public Health Service Fellowship NS09715 (to S. H. K.), National Institutes of Health Grant NS11323, MDA, and Smokeless Tobacco Research Council, Inc. grants (to J. L.), and United States Public Health Service Grant GM18360 (to P. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MATERIALS AND METHODS
Castanospermine Treatment and Transfections—Castanospermine (Calbiochem, San Diego, CA) was solubilized in Dulbecco’s modified Eagle’s medium at a concentration of 100 μg/ml, and immediately added to 10-cm plates of cells. Cells were treated with castanospermine 2 h prior to transfection, transfected with receptor subunit cDNAs, allowed to grow for 16 h, or replenished with fresh castanospermine in Dulbecco’s modified Eagle’s medium and raised for another 24 h.

Transfections employed the calcium phosphate precipitation method, as described in Keller et al. (16). Generally, 15 μg of plasmid DNA encoding each receptor subunit were added to plates of cells, unless noted otherwise. In transfections where α- to δ-subunit ratios were varied, α-subunit cDNA was transfected at 15 μg of plasmid DNA/plate, and the mass of plasmid DNA encoding the δ-subunit was varied (Figs. 5 and 6).

Detergent Solubilization, Immunoprecipitation, Electrophoresis, and Western Blots—In experiments involving immunoprecipitations with antibodies to calnexin, calreticulin, ERP57, and the receptor subunits, the solubilization buffer consisted of 0.5% CHAPS, 150 mM NaCl, 1 mM CaCl₂, 20 mM HEPES, pH 8.0, and the protease inhibitors: benzamidine, aprotinin, leupeptin, and pepstatin A. The characteristics of the antibodies used for these immunoprecipitations, mAb 35 or mAb 61 to precipitate the α-subunit, mAb 111 to precipitate the β-subunit and mAb 137 to precipitate the δ-subunit, were described previously (22).

Anticalnexin, antipolyubiquitin, and anti-calreticulin were purchased from Stressgen (British Columbia, Canada). Solubilization of cells and immunoprecipitations using anti-polyubiquitin were in 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.4% deoxycholate, 1% Nonidet P-40, 0.1% SDS, phenylmethylsulfonyl fluoride, N-ethylmaleimide, and the other protease inhibitors listed above. The ratio of solubilization buffer volume relative to the number of cell plates were the same for each sample within an experiment. Following solubilization, samples were centrifuged at 10,000 × g for 5 min. Dilution ratios for antibody in all immunoprecipitations were approximately 1:100.

Equivalent volumes of immunoprecipitated samples, consisting of approximately 5% of the total immunoprecipitated material, were loaded in each gel lane. All samples were resolved on 10% Novex gels (San Diego, CA) and transferred to nitrocellulose for Western blot detection. The antibodies used to detect the receptor subunits were mAb 210 for α, mAb 111 for β, and mAb 137 for δ. The antibody to ERP57 was a gift from Dr. Jordan Holtzman (University of Minnesota). Calreticulin was detected on Western blots with the same antibody used in immunoprecipitations. All primary antibodies were diluted 1:1000 to probe Western blots, which were developed by chemiluminescent techniques (Pierce). Immunoprecipitation and Western blot experiments were replicated usually three or more times.

Proteasome Inhibitors—Proteasome inhibitors were solubilized in Me₂SO and added 3 h after transfection. Equivalent concentrations of Me₂SO were added to untreated cells. The final concentration of Me₂SO in a plate of cells was at most 0.3%. Cells were grown for an additional 16 h and then solubilized. The proteasome inhibitor benzoxylcarbonyl-Leu-Leu-phenylalanine (Z-LLF-CHO) was obtained from Calbiochem (San Diego, CA) and used at a final concentration of 30 μM. MG-132 (carboxybenzoyl-leucyl-leucyl-leucyl-leucinal) and lactacystin were purchased from Calbiochem (San Diego, CA) and used at 50 and 10 μM, respectively. Calpain inhibitor I (N-Ac-Leu-Leu-norleucinal; Calbiochem, San Diego, CA) was used at final concentration of 100 μM.

RESULTS
Castanospermine Diminishes Accumulation of α-, β-, and δ-Subunits in the Cell, without Generally Influencing the Accumulation of Other Cellular Proteins—Cells were treated with the glucosidase inhibitor castanospermine, to identify roles for glucose trimming in the biogenesis of acetylcholine receptors expressed in HEK cells. Treatment with castanospermine, prior to and during the transfection period, inhibits the glucosidase I and glucosidase II enzymes, leaving oligosaccharides capped with three glucose residues. Castanospermine treatment, therefore, maintains larger sized oligosaccharides which are attached to glycoproteins confined to the endoplasmic reticulum. Since unassembled receptor subunits and α-δ dimers are retained intracellularly (23, 24), treatment with castanospermine should maintain larger oligosaccharides attached to the receptor subunits. A side reaction, which may result from a residual fraction of glucosidase enzymes remaining active, is that, nascent oligosaccharide chains whose glycosyl residues have been trimmed, in turn, become reglucosylated by UDP-glucose:glycoprotein-dependent glucosyltransferase. Reglucosylated receptor subunits may accumulate transiently because most glucosidase enzymes are inhibited (20).

Cells were untreated or treated with castanospermine before transfection with cDNAs encoding the α-, β-, or δ-subunits. Following detergent solubilization, receptor protein was immunoprecipitated, resolved on gels, transferred to nitrocellulose and detected with appropriate antibodies. The Western blot in Fig. 1A demonstrates that castanospermine decreases the migration in gels of the α- (compare lanes 1 to 2), β- (lanes 3 to 4), and δ-subunits (lanes 5 and 6), indicating untrimmed oligosaccharides predominate. The δ-subunit often appears as a doublet when resolved toward the bottom of a gel, which is presumably due to partial phosphorylation. Similarly, the α-subunit often appears as a doublet, with a faint unglycosylated lower molecular weight band and a more abundant higher molecular weight protein, which is glycosylated (25). γ-Subunits were not included in these experiments due to the unavailability of the appropriate antibodies.

The banding densities in Western blots of α-, β-, and δ-subunits are significantly diminished when expressed in castanospermine (Fig. 1A), indicating that the intracellular accumulations of these unassembled subunits are reduced. Although treatment with castanospermine at 100 μg/ml diminishes the accumulation of receptor protein, and presumably other glycoproteins, it does not influence the accumulation of most cellular proteins detected with a general stain or antibody (Fig. 1B). To establish this, untransfected cells were treated or untreated with castanospermine, and grown in a similar manner to transfected cells. Cells were harvested, washed 3 × in phosphate-buffered saline, and solubilized in the 0.5% CHAPS buffer as described earlier. Samples were resolved in gels, which were then stained with Coomassie Blue (Fig. 1B, lanes 1 and 2). Other samples were resolved in gels, transferred to nitrocellulose, and exposed to an excess of anti-rat IgG-conjugated peroxidase to detect cellular proteins nonspecifically (Fig. 1B, lanes 3 and 4). Proteins of similar banding densities align in the gels (Fig. 1B), indicating that the loss of acetylcholine receptor protein observed in Fig. 1A, is not due to a general reduction in protein synthesis caused by castanospermine treatment.

Polypeubiquitinated α-Subunits Accumulate when Cells Are Treated with Proteasome Inhibitors—To identify mechanisms which regulate receptor subunit degradation, experiments were designed to ascertain whether treatment with castanospermine increases conjugation of polyubiquitin to α-subunits. Cells were treated or untreated with castanospermine, trans-
Fig. 1. Castanospermine treatment reduces the intracellular accumulation of α-, β-, and δ-subunits but not a random sample of cellular proteins. A, cells were treated or untreated with castanospermine and transfected (TFT) separately with plasmid DNAs encoding α-subunits (lanes 1 and 2), β-subunits (lanes 3 and 4), or δ-subunits (lanes 5 and 6). Detergent extracts were immunoprecipitated with antibodies specific for α (mAb 61), β (mAb 111), or δ (mAb 137), and receptor subunits were detected using mAb 210 for α-subunits, mAb 111 for β-subunits, and mAb 137 for δ-subunits. Western blots were developed with chemiluminescent techniques. Equivalent volumes of proteins were loaded in each lane, consisting of approximately 5% of the immunoprecipitation mixture. Note in this and all subsequent blots that castanospermine treatment decreases slightly the mobilities of the receptor sub unit bands, consistent with inhibition of glucose trimming.

Equivalent volumes of detergent extract were loaded in each lane. (lanes 1 and 3) or treated with 100 μg/ml CST (lanes 2 and 4), grown for 18 h, cells were washed and detergent extracts were resolved in gels. Gels were either developed with Coomassie Blue stain (lanes 1 and 2) or protein was transferred to nitrocellulose and exposed to a high concentration of antinause IgG-peroxidase to detect a random sample of cellular proteins (lanes 3 and 4). Equivalent volumes of detergent extract were loaded in each lane.

Fig. 2. A, castanospermine treatment enhances polyubiquitination of the α-subunit. Cells were incubated with or without castanospermine, and transfected with plasmid DNA encoding the α-subunit. All cells were treated with the proteasome inhibitors: calpain inhibitor I (CI) and Z-LLF-CHO. Extracts were immunoprecipitated with an antibody to polyubiquitin and the Western blot was developed with mAb 210, which detects the α-subunit. B, similar experiment to that described in A, but Z-LLF-CHO was omitted.

In other experiments with proteasome inhibitors, treatment with 50 μM MG-132 resulted in the detection of a distinct, but fainter high molecular weight ladder pattern compared with that observed with Z-LLF-CHO treatment; the density of the high molecular weight ladder pattern was also enhanced when cells were treated with castanospermine (data not shown).

Treatment with the Proteasome Inhibitors MG-132 or Lactacystin Inhibits the Degradation of α-Subunits—To further characterize the mechanisms of degradation, cells were treated with the proteasome inhibitors lactacystin or MG-132, to examine whether degradation of α-subunits is reduced. Lactacystin is an irreversible inhibitor which binds specifically to the proteasome, as demonstrated by affinity labeling and peptide sequencing (31). MG-132 is a congener of Z-LLF-CHO, also a peptide-aldehyde that blocks the proteolytic activities of the proteasome (32). Cells were treated or untreated with castanospermine, transfected with the same plasmid DNA transfection mixture in all plates, and then treated or untreated with a proteasome inhibitor. Following an incubation period, cells were solubilized, and α-subunits were immunoprecipitated and detected on Western blots. As displayed in Fig. 3, lanes 3 and 4, and in previous experiments, treatment with castanospermine results in a loss of α-subunit protein; however, inclusion of lactacystin diminishes the loss of α-subunit protein (Fig. 3, lanes 1 and 2). Similar results were obtained with MG-132 (Fig. 4C, lanes 3 and 4). These data indicate that α-subunits, which may have been polyubiquitinated and subjected to isopeptidase activity (33), are degraded in the proteasome in cells treated with castanospermine.

Treatment with lactacystin does not appear to enhance the accumulation of α-subunits expressed in the absence of castanospermine (Fig. 3, compare lanes 1 and 3), at a concentra-
Intracellular Accumulation of Acetylcholine

The degradation rate for α-subunits may be substantially lower when expressed in the absence of castanospermine, preventing detection of an enhanced accumulation in cells not treated with castanospermine. In support of a substantially lower degradation rate for α-subunits expressed in the absence of castanospermine, the conjugation of polyubiquitin chains is substantially reduced when α-subunits are expressed without castanospermine (Fig. 2A, compare lane 1 to 2).

To examine whether α-subunits are more prone to aggregate in cells treated with castanospermine, and whether this contributes to the loss of α-subunits, we analyzed insoluble fractions following detergent solubilization of cells. Cells were solubilized in the 0.5% CHAPS buffer described above, subjected to low speed centrifugation (5 min, 2,000 g), and the resulting supernatant was subjected to high speed centrifugation (30 min, 16,000 g). Western blots, of insoluble materials in the final fraction, displayed equivalently dense α-subunit bands in samples treated or untreated with castanospermine (data not shown). Therefore, we were unable to establish aggregation as a factor contributing to the loss of α-subunit protein expressed in the presence of castanospermine.

**Treatment with Castanospermine Disrupts an Interaction between Calnexin and the α-Subunit**—To ascertain whether glucose trimming influences interactions between calnexin and α-subunits, cells were treated with castanospermine, and then transfected with various amounts of plasmid DNA encoding the α-subunit (Fig. 4). In some experiments, cells were also treated with proteasome inhibitors to reduce degradation and to facilitate the distinction between altered recognition by calnexin from increased degradation. Sequential immunoprecipitations were conducted with an antibody to calnexin (c), followed by immunoprecipitation of the unbound material with an anti-α-subunit antibody (mAb 61), to assess the extent of calnexin-α-subunit recognition. In Fig. 4, sequentially precipitated α-subunits are displayed, with lane 1 in sections A-C displaying α-subunits bound to calnexin, and lane 3 exhibiting the α-subunits not cleared with calnexin; likewise, lane 2 in sections A-C displays α-subunits bound to calnexin and lane 4 exhibits α-subunits not cleared with calnexin. Densities of α-subunit bands were quantified, and density ratios for α-subunits expressed in the presence relative to the absence of castanospermine were calculated. Each histogram bar was calculated separately for α-subunits precipitated with anti-calnexin or anti-α-subunit antibodies; for example, in Fig. 4, A-C, the histogram bars designated TFT: α and IP to: c, were calculated for α-subunits co-immunoprecipitated with calnexin by anti-calnexin antibody, by dividing the band densities for α-subunits expressed in the presence (shown in lane 2) and absence of castanospermine (shown in lane 1). Similarly, the histogram bar designated as TFT: α and IP to: α, was calculated for α-subunits immunoprecipitated with anti-α-subunit antibody, by di-

**FIG. 3.** Lactacycstin, a selective inhibitor of the proteasome, inhibits the degradation of α-subunits expressed in the presence of castanospermine. Cells were treated (+) or untreated (−) with CST, transfected with plasmid DNA encoding the α-subunit, treated (+) or untreated (−) with 10 μM lactacystin (LAC); an equivalent concentration of Me2SO was added to untreated cells, since LAC was solubilized in Me2SO. Samples were immunoprecipitated with mAb 61 and the Western blot developed with mAb 210. Equivalent numbers of cells were solubilized and subjected to immunoprecipitation and equivalent volumes were loaded in lanes, which consisted of approximately 5% of the total sample.

**FIG. 4.** Characteristics of calnexin recognition of α-subunits. A, cells were treated or untreated with CST and transfected (TFT) with 15 μg of plasmid DNA encoding the α-subunit or also co-transfected with 15 μg of plasmid DNA encoding the δ-subunit. Cells were not treated with a proteasome inhibitor. Sequential immunoprecipitation (IP to: c) was first to calnexin (c), followed by immunoprecipitation of the unbound material with anti-α (mAb 61). Lanes 1 and 3, 2 and 4, 5 and 7, and, 6 and 8 are pairs of sequentially immunoprecipitated samples. The Western blot was developed with mAb 210 to detect α-subunits. The density ratios were calculated by scanning and integrating the density of α-subunit bands on the Western blot and dividing the CST-treated sample by the CST-untreated sample for α-subunits precipitated by one of the antibodies. B, similar to A, except cells were transfected with 3.0 μg of plasmid DNA encoding the α-subunit to reduce expression levels; cells were also treated with lactacystin. C, similar to A, except cells were treated with 50 μM MG-132. Blot was exposed to film 10 times longer than in A and B.
viding the band densities for α-subunits expressed in the presence and absence of castanospermine (displayed in lane 3). A comparison in the density ratios for α-subunits co-immunoprecipitated with calnexin relative to those immunoprecipitated with anti-α-antibody provides an estimate of the extent of calnexin-α-subunit recognition.

When cells are transfected with cDNA encoding the α-subunit at 15 μg of plasmid DNA/plate and are not treated with proteasome inhibitors, high levels of expression are observed, as evidenced by dense α-subunit bands for relatively short exposures of the blot to film (Fig. 4A, lanes 1–4). The density ratios for α-subunit bands (+CST–CST) are similar when co-immunoprecipitated with calnexin or immunoprecipitated with anti-α subunit antibody (histogram, Fig. 4A), indicating treatment with castanospermine does not appear to repress the formation of the calnexin-α-subunit complex in this experiment. However, treatment with castanospermine causes a substantial loss of α-subunit protein (Fig. 4A, compare lanes 1 and 3 with 2 and 4), which may have included α-subunits that were not bound to calnexin because oligosaccharides were untrimmed.

When cells are co-transfected with cDNAs encoding the α- and δ-subunits at a 1:1 ratio of plasmid DNA masses, the association of calnexin with α-subunits is minimal (Fig. 4A, lanes 5 and 6), presumably because calnexin associates only with the small fraction of unassembled α-subunits present in these cells (16). Interestingly, when cells are treated with castanospermine and transfected with cDNAs encoding α- and δ-subunits at a 1:1 ratio of plasmid DNA masses, the loss of α-subunits in the cell is also minimal (Fig. 4A, compare lanes 7 and 8 to lanes 3 and 4). Since co-transfection of α- and δ-subunits at a 1:1 ratio of plasmid DNAs leads to the biogenesis of assembled α-δ dimers (11), these data indicate that the accumulation of assembled α-subunits is not impacted as significantly by aberrant glucose trimming. Data displayed in Fig. 4A also appear to indicate that calnexin association is more highly dependent on the extent of α-subunit assembly than treatment with castanospermine. This is evident because the α-subunit band isolated from cells co-transfected with plasmid DNAs encoding α- and δ-subunits, and co-immunoprecipitated with calnexin (Fig. 4A, lane 6), is attenuated relative to the α-subunit band isolated from cells expressing only α-subunits (Fig. 4A, lane 2). The assembly of α- and δ-subunits as dimers may occlude the association sites for calnexin.

In contrast to the above results, when receptor expression is lowered by transfection of 3 μg of plasmid DNA/plate encoding the α-subunit, and α-subunit degradation is reduced by treating cells with 10 μM lactacystin, the impaired glucose trimming by castanospermine appears to partially inhibit the formation of the α-subunit-calnexin complex (Fig. 4B). Treatment with castanospermine diminishes the fraction of α-subunits co-immunoprecipitated with calnexin (Fig. 4B, compare lanes 1 and 2), relative to α-subunits immunoprecipitated with anti-α-subunit antibody (Fig. 4B, compare lanes 3 and 4; histogram), illustrating an interaction between calnexin and the oligosaccharide on the α-subunit under these experimental conditions. Treatment with castanospermine similarly reduced the association of calnexin with α-subunits in cells transfected with 1.5 μg of plasmid DNA encoding the α-subunit, and treated with 10 μM lactacystin (data not shown).

Interactions between calnexin and the α-subunit oligosaccharide is further evidenced when α-subunits are expressed in the presence of 50 μM MG-132 (Fig. 4C). In this experiment, treatment with castanospermine appears to reduce substantially the ability of calnexin to associate with the α-subunit (Fig. 4C, lane 2), although α-subunits were readily detected in these cells (Fig. 4C, lane 4). The accumulation of α-subunits in cells was low in this experiment, since a 10-fold increase in the exposure time of this blot to film was necessary to detect a α-subunit band of comparable density. With this longer exposure, background protein bands are manifest in this experiment.

Recognition by calnexin of an appropriately trimmed oligosaccharide in the α-subunit becomes apparent in experiments using proteasome inhibitors, because the degradation of α-subunits not bound to calnexin and recovered by immunoprecipitation with anti-α-subunit antibody is reduced. Lowering receptor subunit expression may further facilitate the ability to detect an interaction between calnexin and an appropriately trimmed oligosaccharide in the α-subunit, by diminishing the probability for lower affinity interactions between polypeptide segments of calnexin and the receptor subunit. In summary, when expression levels are low and degradation is inhibited with proteasome inhibitors, an interaction between the α-subunit oligosaccharide and calnexin becomes apparent. However, additional interactions between calnexin and the α-subunit polypeptide backbone are also evident, especially when expression levels are high.

Assembly with δ-Subunits Reduces the Loss of α-Subunits Expressed in the Presence of Castanospermine—To examine whether α-subunit assembly with the δ-subunit ameliorates the loss of α-subunits expressed in the presence of castanospermine, the mass of cDNA encoding the α-subunit was kept constant at 15 μg of plasmid DNA/plate and the plasmid DNA encoding the δ-subunit was transfected at two different ratios to α-subunit. Accumulation of α-subunits was measured using 125I-α-Bgt binding to permeabilized cells (Fig. 5). 125I-α-Bgt binding was also quantified in a similar manner with untreated cells. Standardized values for 125I-α-Bgt binding are displayed in Fig. 5, where α-toxin binding to untransfected cells is subtracted from the values in transfected cells.

When the ratio of plasmid DNAs encoding the α- and δ-subunits is 1:2 in the transfection mixture, a 30% loss of α-subunit accumulation is observed in the castanospermine-treated cells (Fig. 5). However, when DNAs encoding the α- and δ-subunits are co-transfected at a 8:1 ratio, a 62% loss in the accumulation of α-subunits is evident in cells treated with castanospermine (Fig. 5). These data indicate that assembly of α-subunits with δ-subunits results in the stabilization of subunits coexpressed

**FIG. 5.** Increasing the mass of plasmid DNA encoding the δ-subunit in the transfection increases the accumulation of α-subunits when subunits are coexpressed in the presence of castanospermine. Accumulation of folded α-subunits in the absence (−) or presence (+) of CST is measured by 125I-α-Bgt binding to permeabilized cells. α:δ ratios refer to the masses of plasmid DNAs in the transfection mixture, where the amount of plasmid DNA encoding the α-subunit is kept constant at 15 μg of DNA/plate and plasmid DNA encoding the δ-subunit is varied. Radioactivity (cpm) is standardized to the binding of 125I-α-Bgt to untransfected cells, and each histogram bar displays an average from two or three samples. The maximum counts/ min are calculated as the average among samples untreated with castanospermine, determined separately for the two transfection ratios.
in the presence of castanospermine, and that the accumulation of assembled α-subunits is less dependent on the state of glucose trimming than is the accumulation of unassembled α-subunits.

**Accumulation of α-δ Dimers Expressed in the Presence of Castanospermine Is Dependent on the Transfected Ratios of α- and δ-Subunits in CDnas**—To ascertain whether treatment with castanospermine and the stoichiometric ratios of cDNAs encoding α- and δ-subunits in the transfection influence the accumulation of α-δ dimers, cells were treated with castanospermine and co-transfected with plasmid DNAs encoding the α- and δ-subunits at ratios of 8:1 and 3:1. The mass of plasmid DNA encoding the α-subunit was kept constant at 15 μg of DNA/plate and plasmid DNA encoding for the δ-subunit is varied. A, lanes 1 and 2 display samples transfected at a 8:1 ratio and lanes 3 and 4 display samples transfected at a 3:1 ratio of plasmid DNAs encoding the α- and δ-subunits. The numbers of cells, antibody dilutions for immunoprecipitations, and volumes loaded in each lane were the same among samples, and consisted of approximately 5% of the total sample. The Western blot was developed first with mAb 210 to detect the α-subunit and then reprobed with mAb 137 to detect the δ-subunit, and the exposures on films were overlaid to show α- and δ-subunits together. The δ-subunits displayed in the Western blot are assembled with α-subunits, since the immunoprecipitating antibody recognized the α-subunit (mAb 61). B, Western blot revealing assembled δ-subunits from a similar experiment as displayed in A, developed with mAb 137.

**Fig. 6.** The influence of castanospermine on the accumulation of α-δ subunit dimers depends on the transfected ratios of plasmid DNAs encoding the α- and δ-subunits. The transfected α:δ-subunit ratios refer to the masses of plasmid DNAs present in the transfection mixture, where the amount of plasmid DNA encoding the α-subunit is kept constant at 15 μg of DNA/plate and plasmid DNA encoding for the δ-subunit is varied. A, lanes 1 and 2 display samples transfected at a 8:1 ratio and lanes 3 and 4 display samples transfected at a 3:1 ratio of plasmid DNAs encoding the α- and δ-subunits. The numbers of cells, antibody dilutions for immunoprecipitations, and volumes loaded in each lane were the same among samples, and consisted of approximately 5% of the total sample. The Western blot was developed first with mAb 210 to detect the α-subunit and then reprobed with mAb 137 to detect the δ-subunit, and the exposures on films were overlaid to show α- and δ-subunits together. The δ-subunits displayed in the Western blot are assembled with α-subunits, since the immunoprecipitating antibody recognized the α-subunit (mAb 61). B, Western blot revealing assembled δ-subunits from a similar experiment as displayed in A, developed with mAb 137.

in the presence of castanospermine, and that the accumulation of assembled α-subunits is less dependent on the state of glucose trimming than is the accumulation of unassembled α-subunits.

**Accumulation of α-δ Dimers Expressed in the Presence of Castanospermine Is Dependent on the Transfected Ratios of α- and δ-Subunit cDNAs**—To ascertain whether treatment with castanospermine and the stoichiometric ratios of cDNAs encoding α- and δ-subunits in the transfection influence the accumulation of α-δ dimers, cells were treated with castanospermine and co-transfected with plasmid DNAs encoding the α- and δ-subunits at ratios of 8:1 and 3:1. The mass of plasmid DNA encoding the α-subunit was kept constant at 15 μg of plasmid DNA/plate and the mass of plasmid DNA encoding the δ-subunit was varied. The α-subunit was immunoprecipitated with anti-α-antibody (mAb 61), and the Western blot was developed with antibodies to the α- and δ-subunits (mAb 210 and 137, respectively, Fig. 6A). The appearance of co-immunoprecipitated α- and δ-subunit bands appear to display correspondingly similar changes in density when coexpressed in the absence or presence of castanospermine, indicating that subunit assembly is not disrupted (Fig. 6A). The density of the δ-subunit band on the blot provides an indication of the accumulation of α-δ dimers. When cells are co-transfected with a large stoichiometric imbalance of subunits, using plasmid DNAs encoding α- and δ-subunits at a 8:1 ratio, there are significantly diminished α- and δ-subunit bands in the sample expressed in the presence of castanospermine, indicating α-δ dimers are less prevalent (Fig. 6A, compare lanes 1 to 2). However, treatment with castanospermine has a smaller impact on altering the density of α- and δ-subunit bands when plasmid DNAs are co-transfected at a 3:1 ratio, a closer stoichiometric balance of subunits (Fig. 6A, compare lanes 3 to 4). Similar results with respect to changes in the accumulation of assembled δ-subunits are displayed in Fig. 6B, in an experiment where plasmid DNAs encoding α and δ are co-transfected at ratios of 8:1 and 1:1. These data indicate that when α-subunits have a higher probability of contacting δ-subunits, glucose trimming has a smaller impact on the accumulation of α-δ dimers. These data also indicate that the ratios of nascent unassembled subunits and glucose trimming have a combined influence on the accumulation of assembled receptor subunits, which should ultimately dictate the expression levels of fully assembled receptors at the cell surface.

**Calreticulin and Erp57 Association with α-Subunits Is Not Detectable under Conditions Where Calnexin Association Is Evident**—Similar to the binding properties of calnexin, calreticulin and Erp57 are thought to preferentially associate with newly synthesized glycoproteins which possess oligosaccharides capped with a single glucose residue (19, 21). Immunoprecipitation and Western blotting experiments were performed to ascertain whether calreticulin and Erp57 are associated with α-subunits. Calreticulin is expressed in HEK cells, as detected in a Western blot of a detergent extract (Fig. 7A, lane 1) and upon immunoprecipitation with an antibody to calreticulin (Fig. 7A, lane 4). However, co-immunoprecipitation of α-subunits with calreticulin was not appreciably detected (Fig. 7B, lane 2), although calreticulin was immunoprecipitated in this sample (Fig. 7A, lane 4), α-subunits were present in the extraction mixture (Fig. 7B, lane 4), and these conditions reveal co-immunoprecipitation of α-subunits with calnexin (Fig. 7B, lane 1). Association of α-subunits with calreticulin was also not detected when the detergent extract was immunoprecipitated with anti-α-antibody (mAb 61) and the Western blot developed with an antibody to calreticulin (data not shown). Experiments with the antibodies available to us indicate calnexin is more
intracellular accumulation of acetylcholine

prevalently associated with α-subunits than is calreticulin.

As with calreticulin, ERp57 was not observed to associate with α-subunits, by immunoprecipitating with mAb 61, and developing the Western blot with an antibody to the α-subunit (mAb 210). Cells were transfected with plasmid DNA encoding the α- and δ-subunits at a 1:1 ratio of plasmid masses (15 μg of plasmid DNA/plate for each subunit). Sequential immunoprecipitations initially used mAb 35, which has a higher affinity for folded α-subunits, and then unbound material was immunoprecipitated with mAb 61. Both antibodies, at a concentration of 5 mg/ml, were diluted 1:100 prior to a 1.5-h immunoprecipitation. The intensity of the α-subunit band immunoprecipitated by mAb 35 relative to mAb 61 provides an indication of the fraction of folded α-subunits. B, same Western blot as in A, but reprobed with an antibody to δ (mAb 137), indicating the relative amounts of assembled δ-subunits. Lanes 5–8 in B correspond to lanes 5–8 in A. C, quantitation of the fraction of folded α-subunits in the Western blot displayed in A, calculated as the ratio of densities for α-subunits immunoprecipitated with mAb 35 relative to α-subunits immunoprecipitated with mAb 61; α- and δ- refer to cells transfected with plasmid DNA encoding the α- and δ-subunits, untreated or treated with castanospermine, respectively, and αδ- and αδ+ refer to cells co-transfected with plasmid DNAs encoding the α- and δ-subunits, untreated and treated with castanospermine, respectively.

To examine whether assembly with δ-subunits influences the folding of α-subunits, plasmid DNAs encoding the α- and δ-subunits were co-transfected at a 1:1 ratio of DNA masses, sequential immunoprecipitations were performed with mAbs 35 and 61, and the extent of folding was quantified by density scans of Western blots. The Western blot was developed first with mAb 210 to detect α-subunits (Fig. 8A, lanes 5–8), and then reprobed with mAb 137 to detect assembled δ-subunits (Fig. 8B, lanes 5–8). A larger fraction of the α-subunit pool is immunoprecipitated with mAb 35 when α- and δ-subunits are coexpressed (Fig. 8A, compare lanes 1–4 to lanes 5–8), indicating α-subunits fold, or are maintained in a folded state, when assembled with δ-subunits. Moreover, treatment with castanospermine does not significantly alter the ratio of folded α-subunits when α- and δ-subunits are coexpressed (Fig. 8A, lanes 1–4 to lanes 5–8 and C). Fig. 8D displays δ-subunits assembled with α-subunits, since the same Western blot in Fig. 8A was reprobed with an antibody to δ-subunits (mAb 137). A larger fraction of δ-subunits are associated with α-subunits which are recognized by mAb 35 (Fig. 8E, A and B, compare lane 5 with lane 6 and lane 7 with lane 8), further indicating that assembly promotes α-subunit folding.

MAB 35 displays a corresponding increase in affinity for folded α-subunits during maturation (5). The affinity of α-Bgt for the α-subunit also increases correspondingly with maturation (5). Since mAb 35 recognizes a region distinct from the primary contact sites of α-Bgt (22), it can be used as a global indicator for the folding of the extracellular domain of the α-subunit. At the dilutions and times for antibody incubations used in this study, a clear distinction in Western blot banding densities was observed for unassembled relative to assembled α-subunits, which were immunoprecipitated with mAb 35.

In summary, the data compiled in Fig. 8C indicate that, assembly with the δ-subunit increases the fraction of folded α-subunits which accumulate in cells. Therefore, the δ-subunit may play a chaperone-like role promoting the folding of the α-subunit. An alternative explanation for the observed enhancement in the accumulation of folded α-subunits is that, folded α-subunits are more likely to assemble and α-δ-complexes accumulate because they are more stable. However, our data indicate that the fraction of folded α-subunits is increased relative to the total α-subunit pool expressed in these cells.

We additionally sought to ascertain whether treatment with castanospermine disrupts the assembly of α- with δ-subunits, by co-transfecting plasmid DNAs encoding α- and δ-subunits and estimating the extent of assembly with differential ligand binding (16). The specific binding of 125I-α-Bgt in cells incubated in the presence of carbamoylocholine, which blocks α-subunits assembled with δ-, γ-, or ε-subunits, estimates the accumulation of α-subunits which are unassembled and folded. To estimate the accumulation of assembled and folded α-subunits, the quantity of unassembled and folded α-subunits is subtracted from the total specific 125I-α-Bgt bound in cells (16).
Therefore, the numerical ratio of unassembled α-subunits to assembled α-subunits, as estimated by $^{125}\text{I}-\alpha$-Bgt binding, takes into account both subunit folding and assembly. Based on the mean of four samples for cells treated, and four samples for cells untreated with castanospermine, our data revealed similar ratios of unassembled α-subunits to assembled α-β dimers (without castanospermine, 0.083; with castanospermine, 0.080). These nearly identical numerical values indicate that treatment with castanospermine does not significantly alter both the folding and assembly of α- and δ-subunits. Furthermore, Western blots of co-transfected α- and δ-subunits show by subunit mobility that castanospermine efficiently inhibits glucose trimming (Fig. 6A). However, communoprecipitated α- and δ-subunit bands display correspondingly similar density changes for cells treated and untreated with castanospermine (Fig. 6A), further indicating that glucose trimming does not significantly impact α-δ subunit assembly. Similar results were obtained when plasmid DNAs encoding α- and δ-subunits were transfected at 1:1 ratios (data not shown).

**DISCUSSION**

Of the three proteins thought to be involved in the biogenesis of glycoproteins, and to associate primarily with glycoproteins possessing oligosaccharides capped with one glucose residue (19, 21), only calnexin was found to associate with the receptor α-subunits. Calreticulin and ERP57 could be associated with α-subunits in vitro, but the interaction was undetectable in vitro, by using antibodies available to us and techniques which readily detect calnexin in association with α-subunits. Based on data presented in this study, the processing events in receptor biosynthesis which are altered by castanospermine treatment appear to be related to calnexin association.

Recognition of α-subunits by calnexin appears to be rather complex, with calnexin displaying association to both oligosaccharide structures and the polypeptide backbone. Calnexin binding to the α-subunit oligosaccharide is dependent on oligosaccharide trimming, because castanospermine treatment reduces the interaction. The importance for oligosaccharide trimming in this interaction becomes apparent when expression levels are low, and degradation is inhibited with a proteasome inhibitor (Fig. 4A). These observations may indicate that the calnexin-oligosaccharide interaction displays a higher affinity with unassembled subunits, than the calnexin-polypeptide interaction. In fact, several investigations have suggested that calnexin displays a sufficient affinity to bind directly to oligosaccharide structures irrespective of the polypeptide backbone (19, 35).

The additional interaction between calnexin and the α-subunit polypeptide backbone appears to be dependent on subunit assembly, because assembly with the δ-subunit reduces calnexin association, and this interaction is not significantly altered by castanospermine treatment (Fig. 4A, lanes 5–8). Subunit assembly may inhibit the calnexin-polypeptide interaction, because the newly formed contact interface between subunits occludes the lower affinity association with calnexin. Alternatively, α-subunits which fold upon assembly may bury an interface which is required for calnexin attachment.

In agreement with our observations that castanospermine increases receptor degradation, an earlier study with BC3H-1 cells showed that, treatment with the glucosidase inhibitor 1-deoxynojirimycin decreased the stability of unassembled α-subunits expressed from the endogenous gene (4). Investigations on other transmembrane spanning glycoproteins have also revealed a decreased stability of glycoproteins expressed under conditions which impair glucose trimming (36–38). In contrast, studies of secretory protein processing indicate that calnexin association correlates with the instability of these proteins (39, 40).

The ubiquitin-proteasome pathway appears to be involved in the degradation of α-subunits expressed in the presence of castanospermine, because polyubiquitinated α-subunits accumulate in cells treated with proteasome inhibitors. Moreover, the degradation of α-subunits is inhibited when cells are treated with the specific proteasome inhibitor lactacystin (31), localizing the proteasome as the site for the degradation. Several investigations have established that proteins embedded in the ER membrane are susceptible to degradation in the proteasome, with examples including the cystic fibrosis transmembrane conductance regulator (29, 41), major histocompatibility complex subunits (42–44), and T-cell receptor subunits (45). Treatment with proteasome inhibitors can result in the detection of conjugated polyubiquitin chains (29), and an enhanced accumulation of proteins which would otherwise be targeted for degradation (29, 45). Investigations on major histocompatibility complex subunits (42–44) and T-cell receptor α-subunits (45) have also revealed that degradation of misfolded or unassembled proteins follows a pathway which involves retrograde transport through the Sec61 protein translocator channel (43, 44), cytoplasmic deglycosylation, and degradation in the proteasome (43–45). The α-subunit of the acetylcholine receptor has numerous lysine residues positioned in its two cytoplasmic loops (i1–2 and i3–4); these and other lysines positioned at the cytoplasmic and luminal boundaries of the transmembrane domains, and in the extracellular domains, may serve as polyubiquitin attachment sites, which target receptor subunits to the proteasome.

Treatment with castanospermine may perturb the assembly pathway, where calnexin association is a critical step which stabilizes unassembled receptor subunits. Disruption of this interaction, and bi-directional motions of the α-subunit, may lead to increased exposure of ubiquitin attachment sites at the cytoplasmic boundary, and promote targeting of the subunit to the proteasome. Furthermore, the association of calnexin with the α-subunit may occlude the ability of the α-subunit-calnexin complex to move through a relatively narrow Sec61 channel (46). A low affinity interaction between calnexin and unassembled α-subunits, in the absence of artificially impaired glucose trimming with castanospermine, might release proteins to enter the degradative pathway. In support of the notion that retrograde transport is a component of the degradative pathway for acetylcholine receptor subunits, we have preliminary data indicating that treatment with castanospermine and proteasome inhibitors results in an enhanced accumulation of unglycosylated α-subunits.

Ubiquitin-conjugating enzymes have been identified to reside on the cytoplasmic exposure of the ER membrane in yeast (47, 48), and attachment of ubiquitin chains is a requirement for efficient degradation via retrograde transport and proteasome hydrolysis (48). The presence of a homologous enzyme in HEK cells might conjugate ubiquitin molecules to sites on acetylcholine receptor subunits exposed just outside the cytoplasmic face of the ER membrane. In contrast, an attachment of calnexin to secretory proteins may result in their localization near to the ER membrane, and this may contribute to their destabilization. Secretory proteins which are positioned at the ER membrane may become dissociated from calnexin, translocated through the Sec61 channel, and degraded in the proteasome.

Assembly with δ-subunits may further stabilize the α-subunits, by constraining the folded conformation, increase teth-

---

2 S. Keller, unpublished data.
ering in position in the membrane, and physically inhibit the stability of the larger \( \alpha\) subunits with \( \beta\) subunits in COS and HEK cells (17). This hypothesis is consistent with the observation that the accumulation of Glucose trimming and calnexin association may have their greatest impact on the expression of multisubunit glycoprotein complexes when the translation product of any subunit is in a large stoichiometric deficiency to \( \alpha\)-subunits. Using muscle cells, several investigations have revealed that endogenous acetylcholine receptor subunits are expressed in stoichiometric imbalances (24, 50, 51).

Acknowledgments—We thank Dr. J. Holtzman for providing an antibody to ERp57 and Drs. J. DiDonato and F. Mercurio for providing Z-LLF-CHO.

REFERENCES
1. Changeux, J-P. (1995) Biochem. Soc. Trans. 23, 195–205
2. Karlin, A., and Akabas, M. (1995) Neuron 15, 1231–1244
3. Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Tanabe, T., Shimizu, S., Kikoyotani, S., Kayano, T., Hirose, T., Inayama, S., and Numa, S. (1983) Nature 305, 818–823
4. Smith, M. M., Schlesinger, S., Lindstrom, J., and Merlie, J. P. (1986) J. Biol. Chem. 261, 14825–14832
5. Merlie, J. P., and Lindstrom, J. (1983) Cell 34, 747–757
6. Kao, P. N., and Karlin, A., (1986) J. Biol. Chem. 261, 8085–8088
7. Fu, D.-X., and Sine, S. M. (1996) J. Biol. Chem. 271, 31479–31484
8. Helekar, S. A., Char, D., Neff, S., and Patrick, J. (1994) Neuron 12, 179–189
9. Gu, Y., Camacho, P., Gardner, P., and Hall, Z. W. (1991) Neuron 6, 879–887
10. Blount, P., and Merlie, J. P. (1988) J. Biol. Chem. 263, 1072–1080
11. Kreienkamp, H.-J., Maeda, R. K., Sine, S., and Taylor, P. (1995) Neuron 14, 635–644
12. Smith, M. M., Lindstrom, J., and Merlie, J. P. (1987) J. Biol. Chem. 262, 4367–4376
13. Tsigeley, I., Sugiyama, N., Sine, S. M., and Taylor, P. (1997) Biophys. J. 73, 52–66
14. Urowin, N. (1993) J. Mol. Biol. 229, 1101–1124
15. Gelman, M. S., Chang, W., Thomas, D. Y., Bergeron, J. M., and Prives, J. M. (1995) J. Biol. Chem. 270, 15085–15092
16. Keller, S. H., Lindstrom, J., and Taylor, P. (1996) J. Biol. Chem. 271, 22871–22877
17. Chang, W., Gelman, M. S., and Prives, J. M. (1997) J. Biol. Chem. 272, 28925–28932
18. Connolly, C. N., Krishek, B. J., McDonald, B. J., Smart, T. G., and Moss, S. J. (1996) J. Biol. Chem. 271, 89–96
19. Helenius, A., Trombetta, E. S., Herbert, D. N., and Simons, J. P. (1997) Trends Cell Biol. 7, 193–200
20. Van Leeuwen, J. E., and Kearse, K. P. (1997) J. Biol. Chem. 272, 4179–4186
21. Lloyd-Jones, J., and High, S. (1997) J. Biol. Chem. 272, 13849–13855
22. Tsartos, S. J., Rand, D. E., Einhorn, B. L., and Lindstrom, J. M. (1981) J. Biol. Chem. 256, 635–644
23. Saedi, M. S., Conroy, W. G., and Lindstrom, J. M. (1991) J. Cell. Biol. 112, 1007–1015
24. Sine, S. M., and Claudio, T. (1991) J. Biol. Chem. 266, 19369–19377
25. Blount, P., and Merlie, J. P. (1996) J. Biol. Chem. 271, 10316–10322
26. Vinitsky, A. C., Michaud, C., Powers, J. C., and Orlowski, M. (1996) Biochemistry 35, 9421–9428
27. DiDonato, J. A., Mercurio, F., and Karin, M. (1996) Mol. Cell. Biol. 16, 13092–1311
28. DiDonato, J. A., Mercurio, F., Caridad, R., Wu-li, J., Suyang, H., Ghosh, S., and Karin, M. (1996) Mol. Cell. Biol. 16, 1295–1304
29. Ward, C. L., Omura, S., and Kopito, R. R. (1995) Cell 83, 121–127
30. Yeung, S. J., Chen, S. H., and Chan, L. (1996) Biochemistry 35, 13843–13848
31. Fenteany, G., Standaert, R. F., Lane, W. S., Choi, S., Corey, E. J., and Schreiber, S. L. (1995) Science 268, 726–731
32. Lee, D. H., and Goldberg, A. L. (1996) J. Biol. Chem. 271, 27280–27284
33. Ciechanover, A. (1994) Cell 79, 13–21
34. Merlie, J. P., Sebbane, R., Taarts, S., and Lindstrom, J. (1982) J. Biol. Chem. 257, 2694–2703
35. Zapun, A., Petrescu, S. M., Rudd, P. M., Dwek, R. A., Thomas, D. Y., and Bergeron, J. M. (1997) Cell 88, 29–38
36. Herbert, D. N., Foelml, B., and Helenius, A. (1996) EMBO J. 15, 2961–2968
37. Romagnoli, P., and Germain, R. (1995) J. Exp. Med. 182, 2027–2036
38. Kearsie, K. P., Williams, D. B., and Singer, A. (1994) EMBO J. 13, 3678–3686
39. Qu, D., Teckman, J. H., Omura, S., and Perlmuter, D. H. (1996) J. Biol. Chem. 271, 22781–22785
40. McCracken, A. A., and Brodsky, J. L. (1996) J. Biol. Cell 132, 291–298
41. Jensen, T. J., Loo, M. A., Pind, S., Williams, D. B., Goldberg, A. L., and Riordan, J. R. (1995) Cell 83, 129–135
42. Hughes, E. A., Hammond, C. A., and Cresswell, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1896–1901
43. Wiertz, E. J., Tortorella, D., Bego, M., Yu, J., Mothes, W., Jones, T. R., Rappolt, T. A., and Ploegh, H. L. (1996) Nature 384, 432–438
44. Wiertz, E. J., Jones, T. R., Sun, L., Bego, M., Genze, H. J., and Ploegh, H. L. (1996) Cell 84, 769–779
45. Yu, H., Kaung, G., Kohayashi, S., and Kopito, R. R. (1997) J. Biol. Chem. 272, 20600–20604
46. Beckmann, R., Bubeck, D., Grassucci, R., Penczek, P., Vereschov, A., Blobel, G., and Joachim, F. (1997) Science 278, 2123–2128
47. Sommer, T., and Jentsch, S. (1998) Nature 395, 176–179
48. Biederer, T., Volkwein, C., and Sommer, T. (1997) Science 278, 1806–1809
49. Shihrom, S. S., and Hall, Z. W. (1996) J. Biol. Chem. 271, 25506–25514
50. Stover, B. G., Nakamura, C., Blackmer, K., Kinter, C. R., and Burden, S. J. (1991) J. Cell Biol. 110, 487–487
51. Moss, S. J., Darlison, M. G., Beesen, D. M., and Barnard, E. A. (1989) J. Biol. Chem. 264, 20199–20205
Inhibition of Glucose Trimming with Castanospermine Reduces Calnexin Association and Promotes Proteasome Degradation of the α-Subunit of the Nicotinic Acetylcholine Receptor

Steven H. Keller, Jon Lindstrom and Palmer Taylor

J. Biol. Chem. 1998, 273:17064-17072.
doi: 10.1074/jbc.273.27.17064

Access the most updated version of this article at http://www.jbc.org/content/273/27/17064

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 51 references, 31 of which can be accessed free at http://www.jbc.org/content/273/27/17064.full.html#ref-list-1