The oligosaccharide component of α_1 -adrenergic receptors from BC₃H₁ and DDT₁ muscle cells

Studies with glycosidases and photoaffinity labelling of intact cells

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In this study, we clarify the structural aspects of the oligosaccharides associated with the α_1 -adrenergic receptor in two muscle cell lines. Photoaffinity labelling of intact BC₃H₁ or DDT₁ muscle cells with 2-{4-(4-azido-3-[¹²⁵I]iodobenzoyl)piperazin-1-yl}-4-amino-6,7-dimethoxyquinazoline ([¹²⁵I]azidoprazosin) followed by SDS/polyacrylamide-gel electrophoresis (PAGE) and autoradiography revealed specifically labelled proteins of molecular mass = 87000 and 81000, respectively. Treatment of photoaffinity-labelled receptors in DDT₁ cells with 33 u. of endoglycosidase F/ml for 24 h resulted in the loss of the 81 kDa receptor and the appearance of a 52.5 kDa protein. When lower concentrations of glycosidase or shorter incubation times were used, the 81 kDa receptor was converted to a 66 kDa protein. Treatment of the photoaffinity-labelled BC₃H₁ receptor with endoglycosidase F resulted in the appearance of a 50.5 kDa protein. Neither α -mannosidase nor endoglycosidase H had an effect on the photoaffinity labelling patterns of the receptor from the two cell types. α_1 -Adrenergic receptors, solubilized from membranes prepared from BC₃H₁ and DDT₁ cells, bound to wheat germ agglutinin–Sephadex and were displaced by *N*-acetylglucosamine. Taken together, these results indicate that α_1 -adrenergic receptors in BC₃H₁ and DDT₁ cells contain complex, but not high, mannose oligosaccharide chains; differences in the composition or number of chains partially accounts for the different molecular mass of the receptor in the two cell lines. The results further indicate that the oligosaccharide chains contribute substantially to the apparent molecular mass of α_1 -adrenergic receptors, as detected by SDS/PAGE, and that the protein backbone of these receptors is likely to be ~ 50 kDa.

INTRODUCTION

Catecholamines interact with α_1 -adrenergic receptors to promote a large number of important physiological responses, including metabolic regulation and vascular smooth muscle contractility. These receptors act by mobilization of intracellular calcium [1,2]. Unlike the progress which has been made in clarifying the molecular details by which β -adrenergic and other receptors couple to adenylate cyclase [3,4], understanding of the relation between structural and functional aspects of α_1 -adrenergic receptors is much more rudimentary. The development of radioactively labelled photoaffinity antagonists has allowed for identification of the receptor by SDS/polyacrylamide-gel electrophoresis (PAGE) and autoradiography. Using the irreversible probe 4-amino-6,7-dimethoxy-2-{4-[5-(4-azido-3-[¹²⁵I]iodophenyl)pentanyl]}-1-piperazinoquinazoline ([¹²⁵I]-APDQ), Leeb-Lundberg *et al.* [5] found that a single protein of *M_r* 78000–85000 was specifically labelled in a variety of different tissues. Seidman *et al.* [6] using [¹²⁵I]-azidoprazosin reported three specifically labelled proteins (77 kDa, 68 kDa, 59 kDa) in rat liver membranes; others have used this probe to identify the α_1 -adrenergic receptor

as a 77 kDa protein in rat ventricular membranes [7]; and as a 81 kDa protein in DDT₁ smooth muscle membranes [8]. Thus, while it is clear that photoaffinity labelling is a valuable technique for studying structural aspects of the α_1 -adrenergic receptor, available data have indicated considerable differences in the molecular sizes of these receptors.

In this report, we combine photoaffinity labelling and glycosidase treatment to characterize the oligosaccharide component of α_1 -adrenergic receptors. Special emphasis is placed on testing whether variability in the oligosaccharide component of the receptor derived from different cells can account for the differences in observed molecular mass. For these studies, we chose two muscle cell lines that grow in continuous culture: BC₃H₁ and DDT₁. BC₃H₁ cells were initially isolated from a neoplasm extracted from the offspring of pregnant mice that had received injections of nitrosoethylurea [9]. DDT₁ cells were isolated from a leiomyosarcoma of a Syrian hamster vas deferens [10]. Each cell contains a high concentration of α_1 -adrenergic receptors; the pharmacological properties of these receptors [11,12], as well as the functional consequences of agonist binding to these cells, have been well documented [13–18].

Abbreviations used: [¹²⁵I]azidoprazosin, 2-{4-(4-azido-3-[¹²⁵I]iodobenzoyl)piperazin-1-yl}-4-amino-6,7-dimethoxyquinazoline; [¹²⁵I]APDQ, 4-amino-6,7-dimethoxy-2-{4-[5-(4-azido-3-[¹²⁵I]iodophenyl)pentanyl]}-1-piperazinoquinazoline; SDS/PAGE, SDS/polyacrylamide-gel electrophoresis; WGA, wheat germ agglutinin; GLcNAc, *N*-acetylglucosamine; DMEM, Dulbecco's modified Eagle's medium; TMS buffer, see text for composition.

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EXPERIMENTAL

Materials

[¹²⁵I]Azidoprazosin, [³H]prazosin (82 Ci/mmol), and endoglycosidases F and H were purchased from New England Nuclear, acrylamide and other chemicals used for SDS/PAGE were from Bio-Rad, X-ray film (XAR-5) and intensifying screens were from Kodak, wheat germ agglutinin-Sepharose 4B was obtained from Pharmacia. All other biochemicals were from Sigma.

Cell culture

BC₃H₁ smooth muscle cells were routinely grown in Falcon T-flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum and maintained at 37 °C in a humidified atmosphere of CO₂/air (1:9). The cells were subcultured at 3–4 day intervals using cold ATV (5 g of trypsin and 0.2 g of EDTA disodium salt per litre) solution (Irvine Scientific, Irvine, CA, U.S.A.) to detach them from the plastic substrate. For experimental purposes, cells were seeded into 35 mm or 100 mm diameter culture dishes so that they reached confluence in 3–4 days, at which time they were used for the experiments. DDT₁ stock cultures were propagated in 200 ml spinner cultures in DMEM supplemented with 10% (v/v) fetal bovine serum. Cultures were inoculated at 5 × 10⁴ cells/ml and subcultured twice per week. For experimental purposes, exponentially growing cells were used within 2 or 3 days after subculture.

Photoaffinity labelling and SDS/PAGE autoradiography

BC₃H₁ cells grown on plastic dishes or DDT₁ cells grown in suspension and placed on dishes before the experiment, were incubated with 50–150 pM [¹²⁵I]-azidoprazosin for 1 h at 37 °C in the dark. Photoaffinity labelling was done by either resting a u.v. mineral lamp directly above the plates containing the cells or by placing the plates into a u.v. light chamber (88 W, C.B.S. Scientific, Del Mar, CA, U.S.A.). In either case, photolysis was for 15 min. The photoaffinity labelling patterns were identical for the two u.v. light sources though the efficiency of incorporation was ~25% greater for the u.v. light chamber. After photolysis, BC₃H₁ cells were washed while still attached to the plates with 20 mM-Tris/HCl, pH 7.4, 5 mM-MgCl₂, 0.3 M-sucrose (TMS) to remove unbound ligand. The cells were then scraped from the dishes and spun (16000 g for 5 min) in a microcentrifuge; an identical protocol was used to wash cells in other experiments. Photoaffinity-labelled DDT₁ cells were scraped from the dishes, spun (16000 g for 5 min) in a microcentrifuge and washed twice with TMS buffer. Washed and pelleted BC₃H₁ and DDT₁ cells were either prepared directly for SDS/PAGE or treated so as to study the effects of glycosidases (see below). In the former case, the pelleted cells were suspended in 70 µl of gel sample buffer containing 50 mM-Tris/HCl, pH 6.9, 2% (w/v) SDS, 10% (v/v) glycerol, and 0.02% (w/v) Bromophenol Blue. The samples were sonicated for 30 s to disperse the pellet and were then boiled for 3 min. They were then loaded on to 7% or 10% (w/v) acrylamide gels, prepared and run according to Laemmli [19]. The gels were run until the tracking dye left the bottom of the gel. They were then stained, destained, and dried with a gel dryer. Autoradiography was carried out by exposing Kodak XAR-5 film at -70 °C, typically for

7 days. Molecular mass standards were also loaded on to the gels. The molecular mass of photoaffinity-labelled proteins was calculated from the centre of the bands on the autoradiographs. Protein was determined as described [20].

Glycosidase treatment

For studies using endoglycosidases F and H, four 100 mm plates of BC₃H₁ cells were grown to confluence, the cells were scraped from the dishes, centrifuged as described, resuspended in 1.5 ml of DMEM and placed in a 24 mm dish before the experiment. When DDT₁ cells were used, 10 ml of cells (10⁶ cells/ml) were centrifuged as described, resuspended in 1.5 ml of DMEM, and placed in a 24 mm dish. Incubation of the cells with the photoaffinity probe and photoaffinity labelling were carried out as described above. For studies using endoglycosidase F, the labelled cells were spun in a microcentrifuge, washed twice with 1.4 ml of 100 mM-sodium phosphate, pH 6.1, 50 mM-EDTA, and finally resuspended in 1.0 ml of buffer containing 100 mM-sodium phosphate, pH 6.1, 50 mM-EDTA, 200 mM-sodium chloride, 1.0% (w/v) digitonin, 1.0% (v/v) Nonidet P-40, 0.1% (w/v) SDS, 10 µg of leupeptin/ml, 5 µg of pepstatin A/ml and 0.01% (w/v) phenylmethanesulphonyl fluoride (endoglycosidase F buffer). For studies using endoglycosidase H, the samples were solubilized in an identical buffer except that 100 mM-sodium citrate, pH 5.5, was used instead of sodium phosphate (endoglycosidase H buffer). The samples were sonicated for 15 s and incubated at room temperature for 1 h. They were then spun (16000 g for 15 min) in a microcentrifuge and the supernatants were recovered. Mercaptoethanol [0.2% (v/v)] was added and the samples were boiled for 3 min. Aliquots (70 µl) were placed into the appropriate number of tubes and endoglycosidase F or H was added as described in the text. The samples were incubated at 37 °C for the appropriate periods of time; the reactions were stopped by adding 1.0 ml of ice cold 10% (w/v) trichloroacetic acid. The samples were spun (16000 g for 15 min) in a microcentrifuge and the resulting pellets were washed twice with acetone to remove any remaining trichloroacetic acid and prepared for SDS/PAGE as described above. α-Mannosidase (Sigma M-7257) treatment of photoaffinity-labelled receptor was carried out as described in the legend to Fig. 5.

Wheat germ agglutinin-Sepharose chromatography of α₁-adrenergic receptors

Membranes were prepared from BC₃H₁ and DDT₁ cells and α₁-adrenergic receptors were solubilized with digitonin essentially as described by Leeb-Lundberg *et al.* [16]. Wheat germ agglutinin (WGA)-Sepharose columns (1.65 mg of WGA/ml of Sepharose) of 0.5 or 1.0 ml gel volume were equilibrated in 150 mM-sodium chloride, 5 mM-EDTA, 50 mM-Tris/HCl, 0.05% (w/v) digitonin, pH 7.4. Soluble α₁-receptor samples (1.3–5.0 ml, 0.3–2.0 pmol of receptor) were applied to the column at 4 °C. The eluted sample buffer was collected in 0.5 ml fractions and pooled ('flow-through fraction'). In a similar fashion, the column was washed with at least 5 bed-volumes of the above buffer containing either 150 mM- or 1 M-sodium chloride ('wash fractions'). Adsorbed receptor was then eluted with 5 bed-volumes of 300 mM-N-acetylglucosamine (GlcNAc; 'eluted fraction') in the

above buffer. The pooled fractions were either stored frozen at -70° or assayed immediately for [^3H]prazosin binding. Relative enrichment of the eluted receptor and recoveries of α_1 -adrenergic receptor and total protein in each of the pooled fractions were calculated relative to the applied sample. Binding activity was assayed by incubating 0.20 ml aliquots of receptor with [^3H]prazosin (1 nM final concentration) in a total assay volume of 0.25 ml for 30 min at room temperature. Non-specific binding was defined in replicate samples containing $10\ \mu\text{M}$ -phentolamine/HCl. The samples were then chilled on ice for 10 min, and bound [^3H]prazosin was collected from the excluded volume of 2.5 ml Sephadex G-75 columns equilibrated in assay buffer. [^3H]Prazosin was counted in a liquid scintillation counter at 35 % efficiency.

RESULTS

Photoaffinity labelling of α_1 -adrenergic receptor on intact BC_3H_1 and DDT_1 cells

Previous studies using the photoaffinity-labelled antagonist [^{125}I]azidoprazosin have indicated that the molecular mass of the α_1 -adrenergic receptor is dependent upon the tissue source [5–8]. Each of these studies used membranes as a starting material; thus, proteolysis of the receptor during cell disruption, membrane isolation

or incubation of the membranes with the probe may have contributed to the variability in molecular mass. To avoid potential problems owing to proteolysis and to assess more accurately the molecular mass of the receptor, we performed photoaffinity labelling of the receptor in intact cells. Fig. 1(a) shows that an 87 kDa protein is specifically labelled in intact BC_3H_1 cells. Incorporation of probe was blocked by $10\ \mu\text{M}$ -phentolamine (an α_1 -adrenergic antagonist). Photoaffinity labelling of the α_1 -adrenergic receptor from another muscle cell line, DDT_1 cells, revealed specific labelling of an 81 kDa protein (Fig. 1b). The difference in molecular mass of the receptor from the two cell lines is more apparent when the labelling patterns are compared on the same gel (compare lanes 1 and 2, Fig. 4).

The extent of covalent labelling of the α_1 -adrenergic receptor by the probe was dependent upon both the concentration of probe and the time of photolysis. While increasing the concentration of photoaffinity probe increased the covalent labelling of the α_1 -adrenergic receptor, increased labelling of non-specific proteins was seen as well (compare Fig. 1, panel A, with Fig. 2, lane 1, for the effect of 150 pM- versus 50 pM-[^{125}I]azidoprazosin on labelling of BC_3H_1 cells). Increasing the time of photolysis increased the degree of labelling (Fig. 2). As shown in this Figure, increased incorporation

Fig. 1. Photoaffinity labelling of the BC_3H_1 and DDT_1 α_1 -adrenergic receptor

(a) BC_3H_1 cells were grown to confluence on 35 mm dishes as described in the text. [^{125}I]Azidoprazosin was added to a final concentration of 150 pM to both samples; $10\ \mu\text{M}$ -phentolamine was added to one of them. After a 60 min incubation in a 37°C incubator in the dark, a u.v. mineral lamp was placed over the dishes and the samples were photolysed for 15 min. The medium was aspirated from the cells which were washed twice with ice-cold 0.3 M-sucrose, 5 mM- MgCl_2 , 20 mM-Tris/HCl, pH 7.4. The samples were scraped from the dishes, spun ($16000\ g$ for 5 min) in a microcentrifuge, and resuspended in gel sample buffer. SDS/PAGE autoradiography was performed as described in the text. A 10% (w/v) acrylamide gel was used. (b) Photoaffinity labelling of DDT_1 cells was carried out as described above for BC_3H_1 cells, but with the following modifications. Cells were grown in suspension as described in the text. An aliquot ($180\ \mu\text{l}$) of cells was placed in each of two wells of a 96-well plate. [^{125}I]Azidoprazosin (50 pM) was added to both samples and phentolamine ($10\ \mu\text{M}$) to one. The cells were incubated in the dark and photolysed as described for BC_3H_1 cells. The cells were pipetted into microcentrifuge tubes, pelleted, washed twice with sucrose-containing buffer and finally suspended in gel sample buffer.

Fig. 2. Photoaffinity labelling of BC₃H₁ α_1 -adrenergic receptor

BC₃H₁ cells were grown to confluence on four 35 mm tissue culture plates. The cells were incubated for 1 h in the dark with 50 pM-[¹²⁵I]azidoprazosin, at which time they were placed into a C.B.S. u.v. light chamber. Each of the samples was photolysed for the period of time indicated in the Figure. The samples were prepared for SDS/PAGE as described for Fig. 1. A 10% (w/v) acrylamide gel was used.

of probe (or increased time for autoradiography) led to a broadening of the band of the labelled protein.

Endoglycosidase F treatment of α_1 -adrenergic receptors of BC₃H₁ and DDT₁ cells

We undertook a series of experiments to determine whether variations in the oligosaccharide component of the receptor could account for the observed differences in molecular mass seen between α_1 -adrenergic receptors on intact BC₃H₁ and DDT₁ muscle cells. The strategy taken was to test whether similar molecular masses would be observed after treatment of photoaffinity-labelled receptors with endoglycosidase F. Fig. 3 shows the effect of endoglycosidase F treatment on the photoaffinity labelling pattern of the DDT₁ α_1 -adrenergic receptor. Increasing either the time of incubation with the glycosidase or the concentration used, resulted in the disappearance of the 81 kDa protein and the appearance of first a 66 kDa and then a 52.5 kDa protein. In three separate experiments, we calculated the molecular mass of the photoaffinity-labelled receptor treated with endoglycosidase F as 53 kDa, 52.5 kDa and 51.8 kDa. In each case, the molecular mass was calculated from the centre of the photoaffinity-labelled band.

Comparison of control BC₃H₁ and DDT₁ cell receptors on the same gel [Fig. 4, lane 1 (BC₃H₁ cells) and lane 2 (DDT₁ cells)] emphasizes the difference in molecular masses of the receptors from the two cell types.

Fig. 3. Endoglycosidase F treatment of photoaffinity-labelled DDT₁ α_1 -adrenergic receptors

DDT₁ cells (8 ml) were pelleted, resuspended in 1.0 ml of medium, and photolabelled with [¹²⁵I]azidoprazosin (150 pM) as described in the text. The cells were washed, solubilized (in 0.5 ml) and denatured as described in the text. Aliquots (0.75 ml) were treated with endoglycosidase F at 37 °C as follows (u./ml): lane 1, none, for 24 h; lane 2, 5, for 12 h; lane 3, 10, for 12 h; lane 4, 10, for 24 h; lane 5, 33, for 24 h. After treatment, the samples were prepared for SDS/PAGE as described in the text. A 7% (w/v) acrylamide gel was used.

Interestingly, the photoaffinity-labelled receptors of these cells did not precisely co-migrate after endoglycosidase F treatment (lanes 3, 4). Even though the α_1 -adrenergic receptor from untreated BC₃H₁ cells has an apparent molecular mass on SDS/PAGE which is ~ 6 kDa greater than that from DDT₁ cells, the BC₃H₁ α_1 -adrenergic receptor treated with endoglycosidase F migrates to an apparent molecular mass that is slightly less than that of the DDT₁ α_1 -adrenergic receptor. In three experiments using endoglycosidase F, the molecular mass of the deglycosylated BC₃H₁ receptor was calculated as 51 kDa, 50.5 kDa, and 50.5 kDa.

These results indicate that variations in the oligosaccharide component partially account for the difference in apparent molecular mass on SDS/PAGE of the α_1 -adrenergic receptors from the two cell types. Further, the residual 1–2 kDa difference in molecular mass of the receptors from the two cell types seen after endoglycosidase F treatment suggests that other structural properties may also differ between the two receptors; this is discussed in more detail later.

To be sure that the effect of endoglycosidase F on the molecular mass of the receptors was not due to contaminating protease activity in the endoglycosidase F stock solution, we incubated four proteins (phosphorylase b, bovine serum albumin, ovalbumin, and α -chymotrypsinogen) which are not glycosylated with endoglycosidase F under conditions identical to those

Fig. 4. Endoglycosidases F and H treatment of BC₃H₁ and DDT₁ α_1 -adrenergic receptor

BC₃H₁ cells were scraped from four 100 mm plates, pelleted and resuspended in 0.5 ml of medium. DDT₁ cells (6 ml) were pelleted and resuspended in 0.5 ml of medium. The cells were photolysed with 150 pM-[¹²⁵I]azidoprasozin as described in the text. For each cell type, 100 μ l was added to 0.9 ml of 100 mM-sodium citrate, pH 5.5, plus 50 mM-EDTA, pelleted, washed twice in this buffer and resuspended in 0.2 ml of endoglycosidase H solubilization buffer (see text). The rest of the photoaffinity-labelled cells were washed in 100 mM-sodium phosphate, pH 6.1, plus 50 mM-EDTA, and resuspended in 0.4 ml of endoglycosidase F solubilization buffer (see text). The solubilized and denatured receptors were aliquoted and treated for 24 h at 37 °C with glycosidase as follows: lane 1, BC₃H₁ cells (100 μ l in endoglycosidase F solubilization buffer), not treated with glycosidase; lane 2, DDT₁ cells (100 μ l in endoglycosidase F solubilization buffer), not treated with glycosidase; lane 3, BC₃H₁ cells treated with 33 u. of endoglycosidase F/ml; lane 4, DDT₁ cells treated with 33 u. of endoglycosidase F/ml; lane 5, BC₃H₁ cells treated with 33 u. of endoglycosidase H/ml; lane 6, DDT₁ cells treated with 33 u. of endoglycosidase H/ml. The samples were prepared for SDS/PAGE as described in the text. A 7% (w/v) acrylamide gel was used.

used to study the α -adrenergic receptor. The sample was prepared for SDS/PAGE and Coomassie Blue staining revealed no change in molecular mass of the proteins.

WGA chromatography of digitonin-solubilized BC₃H₁ and DDT₁ cell α_1 -adrenergic receptors

α_1 -Adrenergic receptor glycosylation in the two cell lines was further characterized by using lectin affinity chromatography to identify the terminal sugars on the oligosaccharide chains. As shown in Table 1, the α_1 -adrenergic receptor is efficiently and reversibly adsorbed by WGA columns and selectively eluted by GlcNAc, indicating that the receptor oligosaccharides bear GlcNAc or sialic acid residues. Together with the

observed susceptibility of the receptor to endoglycosidase F treatment, these results allow classification of α_1 -adrenergic receptor *N*-linked oligosaccharides as the complex type.

Interestingly, we noted subtle differences in the interaction of receptors from the two cell lines with the WGA columns. α_1 -Adrenergic receptors from BC₃H₁ cells were slightly more completely adsorbed by the column with less receptor in the flow-through or wash fractions and with better quantitative recovery on GlcNAc elution (Table 1). The unexpectedly high receptor recovery relative to the activity of the applied sample from BC₃H₁ cells (Table 1) in part may represent enhanced binding activity of the receptor in elution buffer containing 0.05% (w/v) digitonin compared to

Table 1. WGA-Sepharose chromatography of α_1 -adrenergic receptors

Data are compiled from the number of experiments shown in parentheses (*n*). Experimental procedures are described in the text. Recoveries of total protein and α_1 -adrenergic receptor are calculated relative to the applied sample.

Cell line	Recovery (%)						Enrichment of peak fraction (x-fold)
	Flow-through		Wash		GlcNAc elution		
	Protein	Receptor	Protein	Receptor	Protein	Receptor	
DDT ₁ (<i>n</i> = 6)	89 ± 10	9 ± 7	6 ± 2	4 ± 2	5 ± 1	59 ± 7	14 ± 2
BC ₃ H ₁ (<i>n</i> = 3)	85 ± 6	6 ± 3	3 ± 1	1 ± 0.3	5 ± 0.5	111 ± 6	20 ± 1

the 0.5% (w/v) digitonin used for initial receptor solubilization. However, it should be noted that both cell lines were prepared identically for lectin chromatography. Moreover, the BC₃H₁ receptor required a higher concentration of competing GlcNAc for half-maximum elution from the lectin column (60 mM) than did the receptor from DDT₁ cells (10 mM), when receptors were eluted by a gradient of increasing GlcNAc concentrations (not shown). Thus heterogeneities in receptor glycosylation may contribute to the differences in apparent molecular mass of the two receptors.

Endoglycosidase H and α_1 -mannosidase treatment of α_1 -adrenergic receptors

The difference between the oligosaccharide components of the α_1 -adrenergic receptors from the two cell types could be a difference in the number of chains or variations in the sugars comprising individual chains. To characterize further the carbohydrate moieties, we tested for the presence of high mannose oligosaccharides with endoglycosidase H [21]. Comparing lane 6 with lane 2 of Fig. 4, indicates that endoglycosidase H has no effect on the photoaffinity-labelling pattern of the DDT₁ α_1 -adrenergic receptor. While lane 5 of this experiment was underloaded with BC₃H₁ protein, the results of other experiments (not shown) indicate that endoglycosidase H had no effect on the photoaffinity-labelling pattern of BC₃H₁ α_1 -adrenergic receptors. These results indicate that the α_1 -adrenergic receptors from the two cell lines contain complex, but not high, mannose oligosaccharides. In support of this conclusion, we find that α -mannosidase has no effect on the photoaffinity-labelling patterns of the α_1 -adrenergic receptors from BC₃H₁ cells (Fig. 5) and DDT₁ cells (data not shown). Table 2 summarizes the effects of glycosidase treatment on the molecular mass of photoaffinity-labelled α_1 -adrenergic receptors from the two cell types.

It may be argued that the endoglycosidase H and α -mannosidase are not active under the conditions used. The results of the following experiment (data not shown) would indicate that this is not the case. When BC₃H₁ cells were incubated for 48 h with 200 μ M-1-deoxymannojirimycin, photoaffinity labelling of intact cells indicated loss of the 87 kDa specifically labelled protein and appearance of a 62 kDa protein. 1-Deoxymannojirimycin prevents conversion of high mannose oligosaccharides to complex chains [22] and, as expected, the 62 kDa protein was susceptible to both α -mannosidase and endoglycosidase H treatment [22a].

DISCUSSION

In this study we combined photoaffinity labelling with glycosidase treatment to characterize the asparagine-linked oligosaccharide moieties of α_1 -adrenergic receptors in BC₃H₁ and DDT₁ muscle cells. The receptors from both cells are glycoproteins that appear to contain complex, but not high, mannose carbohydrates. Importantly, the oligosaccharide component of α_1 -adrenergic receptors in these cells appears to contribute substantially to the apparent molecular mass of α_1 -adrenergic receptors observed in intact cells and in most photoaffinity-labelling studies with membranes [5–8]. Thus, endoglycosidase F treatment, which is expected to remove most of the oligosaccharide moiety, produces a substantial decrease in apparent molecular mass of receptors on SDS/PAGE from ~80–87 kDa to 50–52 kDa. This suggests that the size of the protein backbone of α_1 -adrenergic receptors is likely to be considerably smaller than previously thought and much more akin to that recently deduced for β -adrenergic and muscarinic cholinergic receptors [23–26]. Since α_1 -adrenergic receptors also link to guanine nucleotide regulatory (G) proteins (e.g. [13]), it seems likely that this family of G-linked receptors may share common structural determinants.

Our results indicate that the oligosaccharide moieties of α_1 -adrenergic receptors from two muscle cell lines appear to be different and that this difference partially accounts for the difference in molecular mass of the receptors, as determined by SDS/PAGE autoradiography. Variability in the oligosaccharide component of glycoproteins, including membrane receptors from different cell types, has been previously noted [4,27,28]. For example, Heidenreich & Brandenburg [27] observed that endoglycosidase F treatment of the α -subunit of the insulin receptor yielded a 100 kDa protein from both the 115 kDa photoaffinity-labelled rat brain receptor and the 125 kDa rat adipocyte receptor. Stiles *et al.* [4] have reported that even the same cell can show microheterogeneity in the carbohydrate moieties of the β -adrenergic receptor.

By comparing the effect of endoglycosidase F on the photoaffinity-labelled receptor in both BC₃H₁ and DDT₁ cells (Fig. 5), we have concluded that some structural differences, in addition to variability in asparagine-linked oligosaccharides, probably exist between the two receptors. It is possible that variability in O-linked oligosaccharides accounts for the 1–2 kDa difference in molecular mass seen after endoglycosidase F treatment of the two cell types. Alternatively, some other

Fig. 5. α -Mannosidase treatment of photoaffinity-labelled BC_3H_1 α_1 -adrenergic receptors

BC_3H_1 cells grown to confluence on one 100 mm cell culture dish were scraped from the dish, pelleted, resuspended in 1.0 ml of cell culture medium, incubated with 150 pM-[^{125}I]azidoprazosin and photolysed as described in the text. The cells were washed, solubilized (in 0.5 ml) in endoglycosidase H buffer and aliquots (200 μ l) were treated with or without 5 u. of α -mannosidase/ml at 30 $^{\circ}C$ for 24 h. The samples were then prepared for SDS/PAGE as described in the text.

form of covalent modification (phosphorylation, lipid acylation, etc.) might account for the difference. As DDT_1 cells were initially isolated from hamster and BC_3H_1 cells from mice, species differences might also account for the differences in apparent molecular mass of the deglycosylated receptors.

The cellular mechanism(s) which account for the differences in oligosaccharide components of BC_3H_1 and DDT_1 α_1 -adrenergic receptors remain(s) unclear. The enzymes involved in processing the carbohydrate moieties on newly synthesized glycoproteins have been characterized in some detail [29,30] and certain mechanisms which regulate the degree of glycoprotein processing have been identified [31,32]. The difference in oligosaccharide components we observed between BC_3H_1 and DDT_1 α_1 -adrenergic receptors could suggest that each cell regulates the glycosylation of its respective receptors

Table 2. Summary of the effects of glycosidase treatment on photoaffinity-labelled α_1 -adrenergic receptors

This Table summarizes the results of a number of experiments. In each case the given molecular mass represents the average of at least three experiments.

Glycosidase treatment	Molecular mass of photoaffinity-labelled receptor (Da)	
	BC_3H_1 cells	DDT_1 cells
None	87000	81000
Endoglycosidase F	50500	52500
Endoglycosidase H	87000	81000
α -Mannosidase	87000	81000

in different ways so as to serve some regulatory or functional purpose that depends upon the degree of glycosylation. This hypothesis is difficult to test, since there is currently no consensus as to the role of carbohydrates in receptor function. In some cases, such as for insulin [33] and epidermal growth factor receptors [34], glycosylation of newly synthesized receptors is required for acquisition of ligand binding; this does not appear to be the case for other receptors, including the β -adrenergic receptor [35]. Proper glycosylation of insulin [36] and acetylcholine [37] receptors is required for their movement to the cell surface, although other receptors do not appear to show this property [38,39].

The potential role of oligosaccharides in movement or trafficking of α_1 -adrenergic receptors is particularly intriguing. These receptors appear to move to and from the cell surface under a number of conditions. For example, newly synthesized receptors probably move from the endoplasmic reticulum via the Golgi apparatus to the cell surface. Incubating cells with catecholamines can down-regulate α_1 -adrenergic receptors [40,41] and this may represent internalization of cell surface receptors [42]. In addition, changes in receptor number at the cell surface are observed under a number of physiological conditions including ischaemia and hypothyroidism [43]. Clearly, some structural aspect of the receptor must be involved in movement to and from the cell surface. It is attractive to speculate that oligosaccharides may contribute to this functionally important aspect of α_1 -adrenergic receptor regulation.

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