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Secretory glycoproteins of the rat subcommissural organ are N-linked complex-type glycoproteins. Demonstration by combined use of lectins and specific glycosidases, and by the administration of Tunicamycin*

H. Herrera and E.M. Rodríguez**
Instituto de Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile

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Summary. Two experimental protocols were used to investigate the secretory glycoproteins of the subcommissural organ (SCO).
Protocol I: Lectins, specific exoglycosidases and immunocytochemistry were sequentially applied to the same section or to adjacent semithin sections of the rat SCO fixed in Bouin's fluid and embedded in methacrylate. Lectins used: concanavalin A (con A), wheat germ agglutinin, *Limulus polyphemus* agglutinin, *Ricinus communis* agglutinin and *Arachis hypogeae* agglutinin. Glycosidases used: neuroaminidase, β-galactosidase, α-mannosidase, α-glucosidase and β-*N*-acetyl-glucosaminidase. For immunocytochemistry an antiserum against bovine Reissner's fiber (AFRU) was used. Lectins and glycosidases were used in sequences that allowed the cleaved sugar residue to be identified as well as that appearing exposed as a terminal residue. This approach led to the following conclusions: (1) the terminal sugar chain of the secreted glycoproteins has the sequence sialic acid-galactose-glucosamine-; (2) the con A-binding material present in the rough endoplasmic reticulum corresponds to mannose; (3) the apical secretory granules and Reissner's fibers displayed a strong con A affinity after removing sialic acid, thus indicating the presence of internal mannosyl residues in the secreted material; (4) after removing most of the sugar moieties the secretory material continued to be strongly immunoreactive with AFRU. *Protocol II:* Rats were injected into the lateral ventricle with Tunicamycin and killed 12, 24, 50 and 60 h after the injection. The SCO of rats from the last two groups showed a complete absence of con A binding sites. The results from the two experiments confirm that the secretory glycoproteins of the rat SCO are *N*-linked complex-type glycoproteins with the conformation previously suggested (Rodríguez et al. 1986).

Introduction

The subcommissural organ (SCO) is a circumventricular organ displaying a high secretory activity. The first evidence for the presence of a selectively stainable ("Gomori-positive") secretory materal in the ependymal cells of the SCO was provided by Stutinsky (1950). This finding in the frog was confirmed and extended in other species. Bargmann and Schiebler (1952) and Wislocki and Leduc (1952) demonstrated that in mammalian species the secretory material of the SCO was stainable with both, the Gomori and the periodic acid-Schiff (PAS) methods. A more comprehensive histochemical study led Oksche (1962), Naumann (1968) and Diederen (1970) to conclude that the secretory material of the SCO is a polysaccharide-protein complex. Meiniel and Meiniel (1985) used a series of lectins to investigate the SCO of several vertebrate species and concluded that the secretory material has a high content of mannose. The combined use of lectins an immunocytochemistry employing an anti-Reissner's fiber serum led Rodríguez et al. (1986) to suggest that the SCO secretes *N*-linked complex-type glycoproteins. This possibility was further supported by findings obtained by Meiniel et al. (1988). These authors also discussed the possibility that sialytated *O*-linked oligosaccharides branch on the protein backbone in addition to *N*-linked oligosaccharides.

Lectins, although binding selectively to certain sugar residues, usually display affinity for more than one of these residues. It appears that the only way to establish to which of these sugar residues the lectin is actually binding is to use specific glycosidases prior to the incubation of the glycoprotein with the lectin. This approach has been used for in vitro studies of isolated glycoproteins. With the exception of the combined use of lectins and neuroaminidase, an exoglycosidase cleaving terminal residues of sialic acid, the removal of sugar residues by specific glycosidases prior to lectin binding has not been used as histochemical tool for the precise identification of sugar moeities. Although the evidence presented by Rodríguez et al. (1986) and Meiniel et al. (1988) strongly suggested that the secretory proteins of the SCO are *N*-linked, complex-type glycoproteins, the evidence was not conclusive enough. The present investigation was designed to obtain new evidence that would further clarify the nature of the carbohdydrate component of the SCO-secretory glycoproteins. For this purpose we used two methodological approaches. One was the study of the rat SCO by the combined use of specific glycosidases, lectin histochemistry and immunocytochemistry using an antiserum specific for the SCO secretion. The other was the in vivo administration into the cerebrospinal fluid of rats of Tunicamycin, an antibiotic known to inhibit the synthesis

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** To whom offprint requests should be sent

Table 1. Enzymatic treatment of semithin sections of the rat SCO using specific glycosidases

Enzyme	Source	Buffer	Unit per section [U]	Maximum incubation time [h]
Neuroaminidase	*Clostridium perfringens*	0.1 *M* sodium acetate pH 5.6, containing 0.004 *M* calcium chloride	0.2	18
β-Galactosidase	Jack bean	0.05 *M* citric acid, pH 3.5	0.08	48
β-Galactosidase	*Aspergillus niger*	0.05 *M* citric acid, pH 4.0	0.08	60
α-Mannosidase	Jack bean	0.05 *M* citric acid, pH 4.5	0.08	18
α-Glucosidase	Yeast	0.05 *M* citric acid, pH 4.5	0.08	48
β-Glucosidase	Almonds	0.05 *M* sodium acetate, pH 5.0	0.5	48
β-*N*-Ac. glucosaminidase	*Aspergillus niger*	0.05 *M* citric acid, pH 4.0	0.1	48

of the dolichol diphosphate-high mannose core of the *N*-linked glycoproteins (Mahoney and Duksin 1979). The SCO of these treated rats was then investigated histochemically by use of concanavalin A, to evaluate core-glycosylation, and immunocytochemically to evaluate synthesis of the proteinaceous component of the SCO-secretion. Preliminary results of this investigation have been published elsewhere (Rodríguez et al. 1987).

Materials and methods

Protocol 1. Ten male rats of the Holtzman (Sprague-Dawley) strain were used. They were normal, untreated rats which had been kept under constant temperature and photoregime (L:D = 12:12), with food and water provided ad libitum. Under ether anesthesia the animals were transcardially perfused first with a washing solution and then with Bouin's fixative. A tissue block containing the SCO and adjacent structures was obtained from each animal, and immersed in the same fixative to complete 48 h of fixation. The blocks were dehydrated in increasing concentrations of ethanol and pure acetone and embedded in butyl-methyl methacrylate according to a procedure previously described (Rodríguez et al. 1984b). The tissue blocks were oriented to obtain frontal sections through the SCO. Serial sections of approximately 2 μm in thickness were individually mounted on separate slides. After removing the embedding medium with xylene, the sections were hydrated and then immersed for 15 min in the corresponding buffer according to the staining method to follow.

Immunocytochemistry. The immunoperoxidase method of Sternberger et al. (1970) was used. An antisera raised in rabbit against bovine Reissner's fiber extracted in a medium containing urea (AFRU; see Rodríguez et al. 1984a) was used as primary antibody. The sections were sequentially incubated in (1) AFRU diluted 1:1000, for 18 h; (2) the secondary antibody (Sigma, St. Louis, Mo, USA) diluted 1:50, for 30 min and, (3) PAP (Bioproducts, Bruxel, Belgium) diluted 1:75, for 30 min. All antibodies were diluted in *Tris* buffer, pH 7.8, containing 0.7% lambda carrageenan (Sigma, USA). The peroxidase reaction product was visualized through the diamino-benzidine reaction.

Lectins. The following lectins labeled with fluoresceine isothiocyanate (FITC) or peroxidase (Sigma, St. Louis, Mo, USA; Polyscience, Warrington, Pa, USA) were used: concanavalin A (con A, affinity: mannose, glucose), wheat germ agglutinin (WGA, affinity: sialic acid, glucosamine). *Limulus polyphemus* agglutinin (LPA, affinity: sialic acid), *Ricinus communis* agglutinin (RCA II, affinity: galactose), *Arachis hypogeae* agglutinin (PNA, affinity: galactose). Lectins were dissolved in *Tris*-buffer, pH 7.8 to obtain a final concentration of 50–80 μg/ml for the FITC-labeled lectins, and 2–5 μg/ml for those labeled with peroxidase. The sections were exposed to the lectin for 45 min, at 22° C. After incubation the sections were washed with *Tris*-buffer. Those sections which had been incubated in a FITC-labeled lectin were mounted in a medium containing *Tris*-buffer and glycerol. They were investigated with a Zeiss photomicroscope equipped with an epifluorescence condenser. When peroxidase conjugates were used, the enzyme was visualized through the diaminobenzidine reaction.

The con A and WGA binding sites were checked by pre-incubating the lectin with the corresponding binding sugar, namely, 0.05, 0.1, 0.2 and 0.4 M of D (+) mannose (Merck, Darmstadt, FRG) and D (+) glucose (May and Baker, Dagenham, England) for con A, and 0.2 *M* *N*-acetyl-D-glucosamine, for WGA. The lectins thus treated were used to incubate the sections.

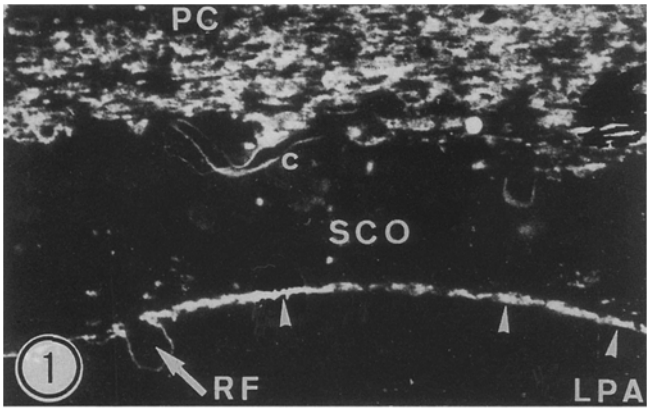
Acid hydrolysis. Hydrated sections were immersed in 0.1 *M* sulphuric acid, for 1 h at 80° C, according to Gibbons (1963).

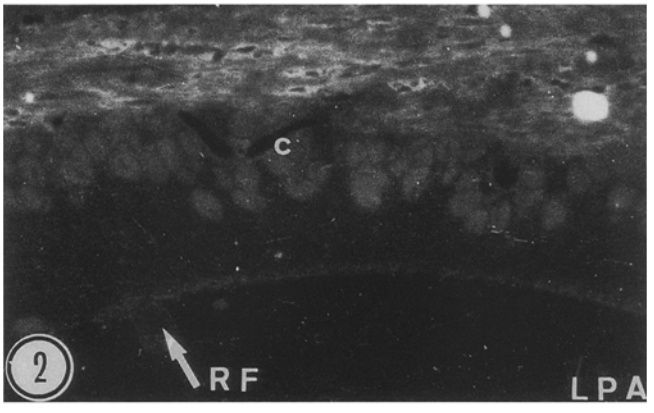
Glycosidases. After hydration the sections were immersed for 15 min in the same buffer used to dissolve the enzyme in which they were subsequently incubated. The sections were covered with the solution containing the enzyme and kept in a moist chamber at 37° C. The enzyme used (Sigma, USA), the source, dissolving buffer, final working concentration and time of incubation are shown in Table 1. Several trials were carried out before establishing the parameters indicated in Table 1.

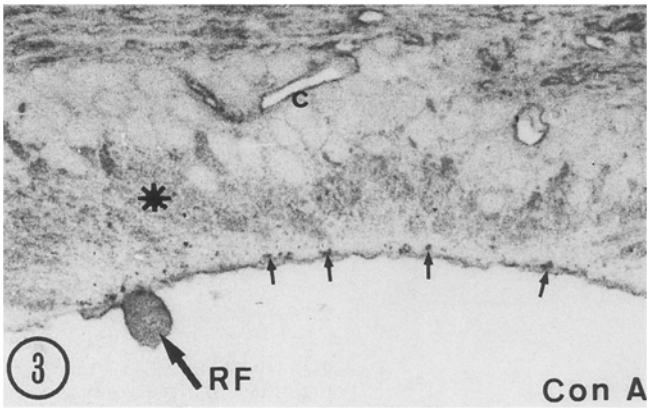
Combined use of glycosidases, lectins and immunocytochemistry. As shown in Table 2, the same semithin section was sequentially processed for: (1) lectin binding; (2) enzymatic treatment or acid hydrolysis; (3) lectin binding; (4) in some cases, immunocytochemistry. When this sequence was applied, FITC-labeled lectins were used. After each lectin incubation the sections were analysed under a fluorescence microscope and microphotographed. Then they were washed in distilled water for 2 h (several changes). An alternative approach was to treat a section with (1) an specific glycosidase and then (2) a peroxidase-labeled lectin. In this case an adjacent and untreated semithin section was stained with the same lectin, and used as a control.

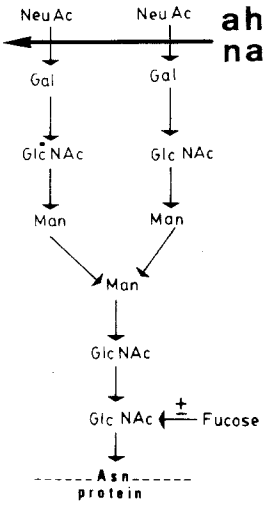
Protocol 2. Twenty-five male rats of the Holtzman strain were used. Two groups were prepared. *Group 1.* Under anesthesia with Nembutal (6 mg/100 body weight) 20 rats were slowly injected (1 μl/min) into a lateral brain ventricle with 50 μg of Tunicamycin (Calbiochem Herring) dissolved in 50 μl of sodium chloride adjusted to pH 7.4 with ammonium bicarbonate. The rats were killed 12 h ($n=5$), 24 h ($n=4$), 50 h ($n=3$) and 60 h ($n=3$) after the injection. The remaining 5 rats died during the second or third postinjectional day. *Group 2.* Five rats were injected into the lateral ventricle with 50 μl of vehicle (saline-ammonium bicarbonate) and killed 60 h after the injection.

Under ether anesthesia the rats were transcardially perfused with a washing solution and then with Bouin's fluid. The brains were embedded in Paraplast and the SCO region was serially cut in the frontal plane. Adjacent sections were used for immunostaining using AFRU, and con A binding.









Figs. 1, 2. The same semithin section through the subcommissural organ (SCO) has been processed for LPA (affinity-sialic acid) binding, before (Fig. 1) and after (Fig. 21) acid hydrolysis. *RF,* Reissner's fiber; *arrowheads* apical granules and pre-FR material; *C,* capillary, *PC,* posterior commissure. × 320

Fig. 3. Semithin adjacent section to that of Figs. 1, 2. Con A binding after acid hydrolysis. Con A binding sites are present in the supranuclear region (RER) of the ependymal cells (*asterisk*) and in addition in apical secretory granules (*arrows*) and Reissner's fiber (RF). *C,* capillary. × 320

Top right. Proposed conformation of the complex-type olygosaccharide of the secretory glycoproteins located in post-Golgi structures, i.e., apical secretory granules, pre-RF and RF. *Arrow* points to site of cleavage of terminal sialic acid residues (Neu Ac) by neuroaminidase or acid hydrolysis. *Gal,* galactose, *GlnNAc,* glucosamine, *Man,* mannose, *Asn,* asparagine residue in the nascent protein

Results

Acid hydrolysis and neuroaminidase.

Both treatments gave the same results with the methods used.

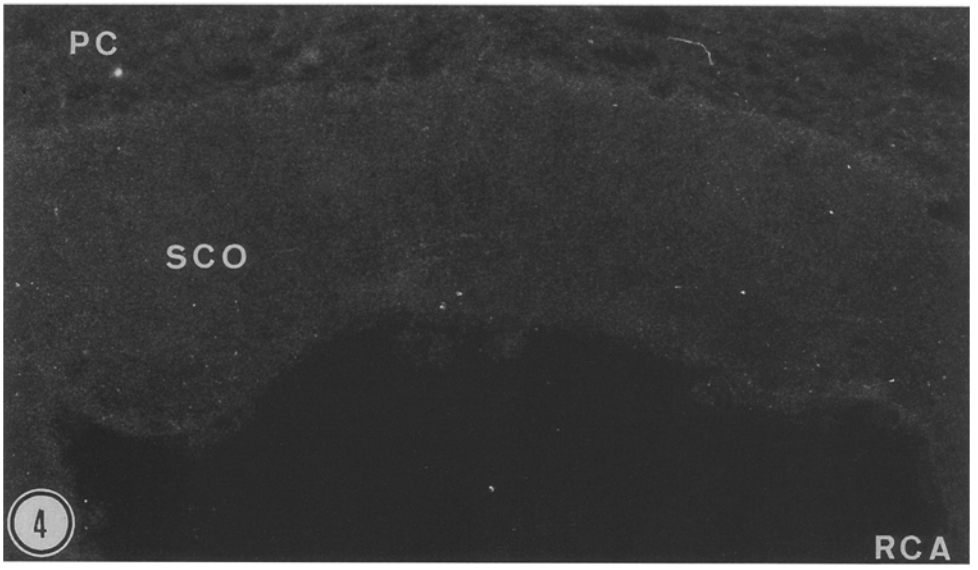
In untreated sections of the SCO LPA (binding to sialic acid) displayed a strong affinity for the apical secretory granules, pre-RF material (cf. Rodríguez et al. 1986) and the periphery of RF (Fig. 1). The perivascular basal lamina and elements of the posterior commissure also bound LPA (Fig. 1). Pretreatment of the section with neuroaminidase or acid hydrolysis completely abolished the binding of LPA in the SCO and RF (Fig. 2). Only some elements of the posterior commissure retained some affinity for LPA.

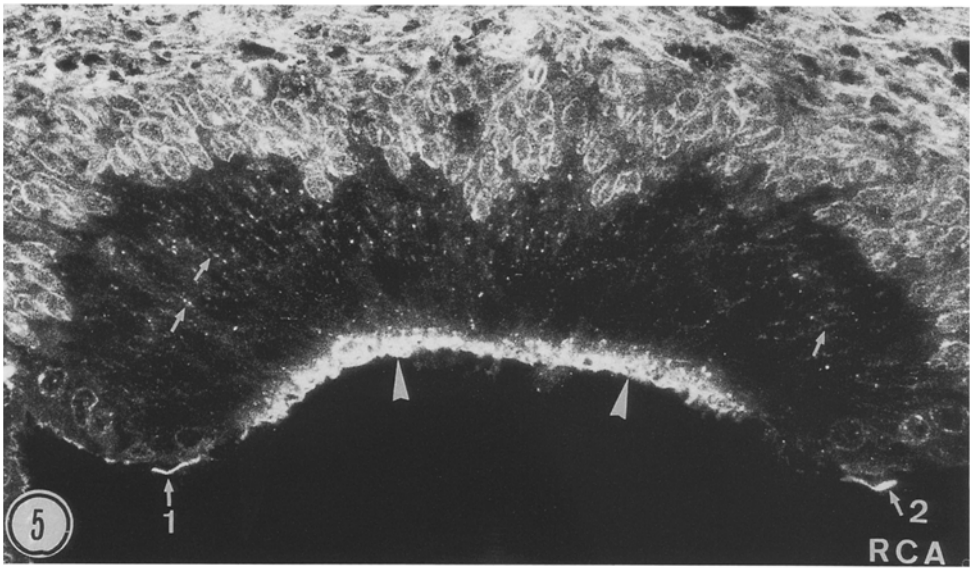
Con A, which in untreated sections only binds to material located within cisternae of the rough endoplasmic reticulum (cf. Rodríguez et al. 1986), after neuroaminidase or acid hydrolysis, also bound to apical secretory granules, pre-RF and RF (Fig. 3).

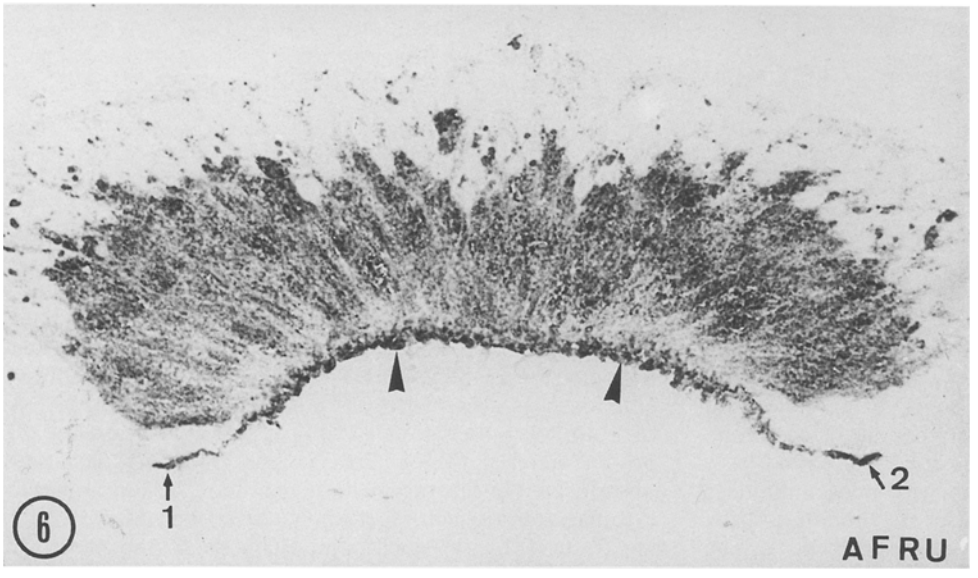
After acid hydrolysis the affinity of WGA for the secretory granules, pre-RF material and RF was even stronger than in untreated control sections.

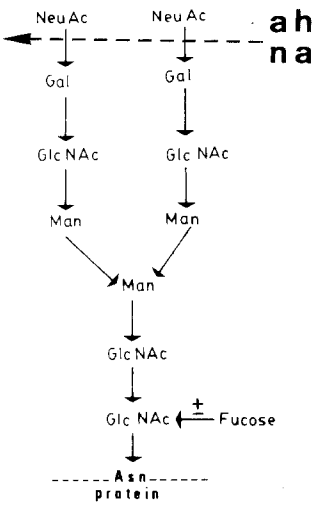
RCA and PNA (both binding to galactose) did not bind to untreated control sections of the SCO (Fig. 4). When the same or an adjacent section was treated with neuroaminidase or acid hydrolysis a strong labeling with RCA or PNA was observed in (1) apical secretory granules, (2) pre-RF material, (3) RF (Figs. 5, 6) and (4) elongated structures located in the intermediate region of the supranuclear cytoplasm and which most likely correspond to the Golgi apparatus (Fig. 5). Nuclei and elements of the posterior commissure also became RCA (Fig. 5) and PNA positive.

The affinity of the secretory granules, pre-RF material and RF to RCA developed after acid hydrolysis was drastically diminshed when the sections were subsequently sub-



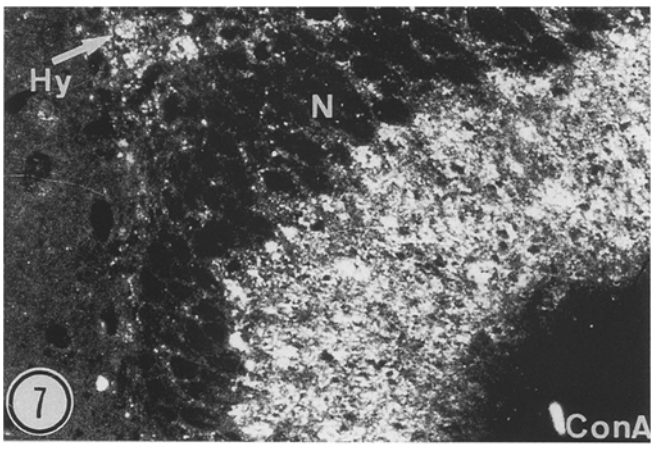


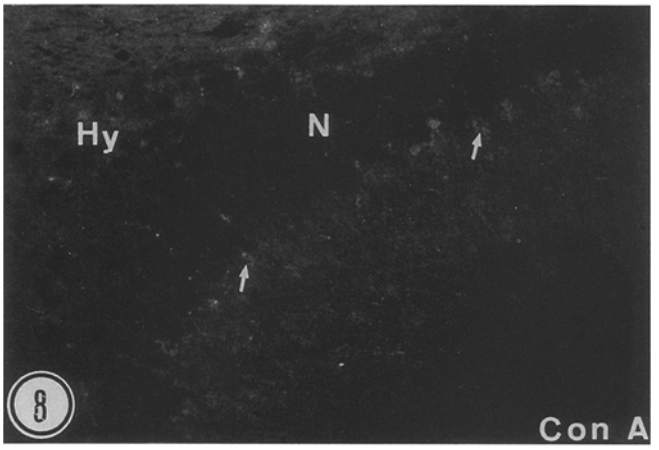


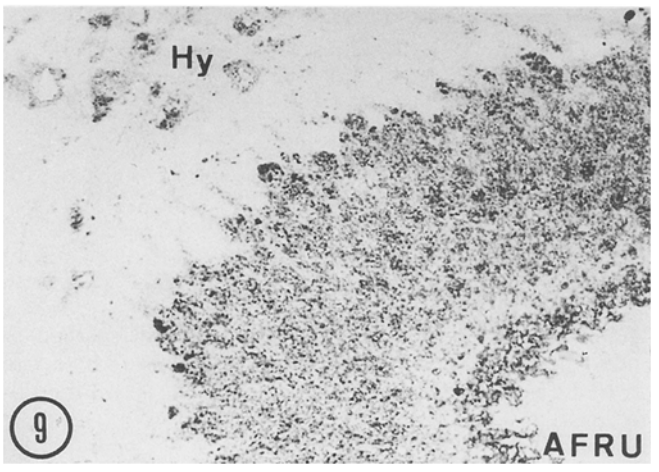


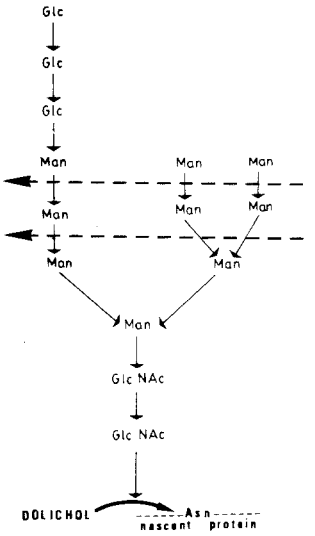
Figs. 4–6. The same semithin section through the subcommissural organ and posterior commissure (*PC*) has been processed for RCA binding, before (**Fig. 4**) and after (**Fig. 5**) acid hydrolysis and then for immunoperoxidase staining using AFRU (**Fig. 6**). After acid hydrolysis the apical secretory granules and pre-RF material (*arrowheads*) and the fibrils originating Reissner's fiber (*arrows 1, 2*) became strongly RCA-positive (**Fig. 5**). The same structures are immunoreactive (compare Figs. 5 and 6). In addition, in the supranuclear region there are elongated structures binding RCA (Golgi apparatus?) (*arrows* in **Fig. 5**) and granular structures (RER) containing immunoreactive material (**Fig. 6**). ×600

Top right. Proposed conformation of the complex-type oligosaccharide of the secretory glycoproteins located in post-Golgi structures. Removal of sialic acid (*arrow*) by acid hydrolysis or neuroaminidase exposed galatose residue, which then displayed affinity for RCA (**Fig. 5**).









Figs. 7, 8. Adjacent semithin sections through the subcommissural organ processed for con A binding, before (**Fig. 7**) and after (**Fig. 8**) treatment with α-mannosidase. Con A binding sites of the material located within the RER disappeared after the enzymatic treatment. Minute amounts of con A-positive material remained in the paranuclear cisternae (*arrows* in **Fig. 8**). *N*, nuclei of ependymal cells. *Hy*, hypendyma. × 600

Fig. 9. Same section as that used for α-mannosidase treatment (**Fig. 8**) immunostained with AFRU. *Hy*, hypendyma. × 600

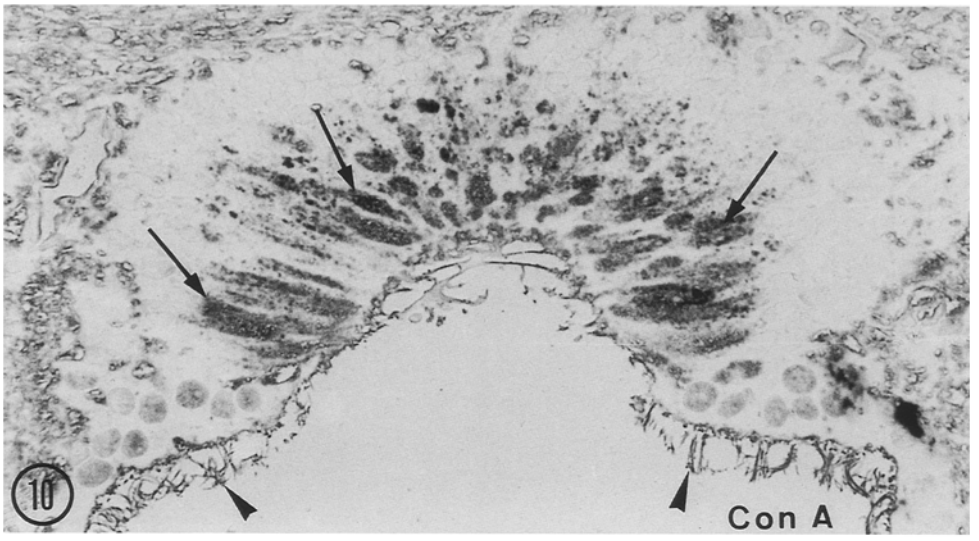
Top right. Proposed configuration of the oligomannosidic core of the secretory glycoproteins stored in the rough endoplasmic reticulum. *Arrows* point to probable sites of action of α-mannosidase. The three glucose residues shown must not actually be present in the glycosylated protein detached from the ribosome (Hickman et al. 1984), thus allowing the complete removal of the α-1,2-, α 1,3- and α 1.6-linked mannose residues by α-mannosidase. The residual Con A binding could correspond to β-linked mannose residues

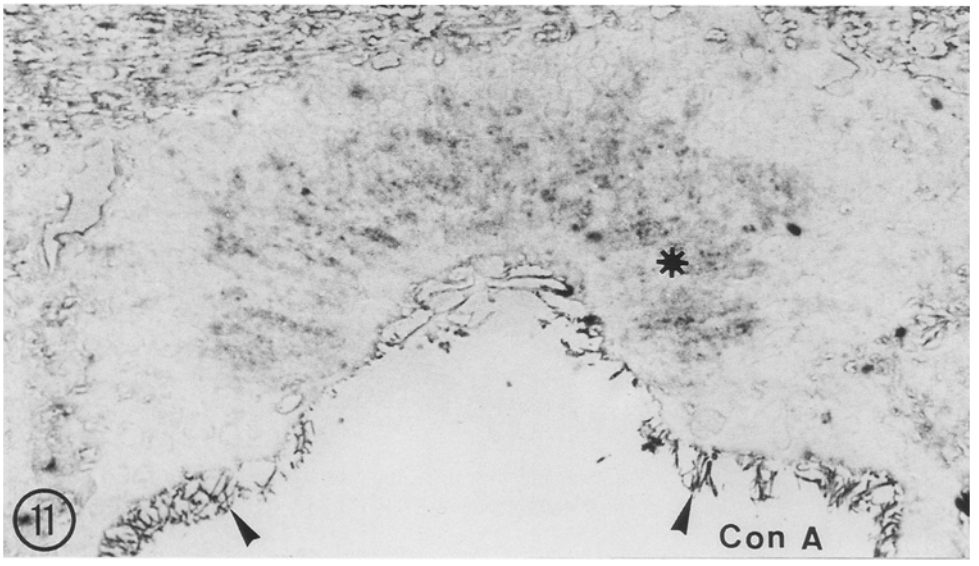
Table 2. Sequential treatments of semithin methacrylate sections of rat SCO

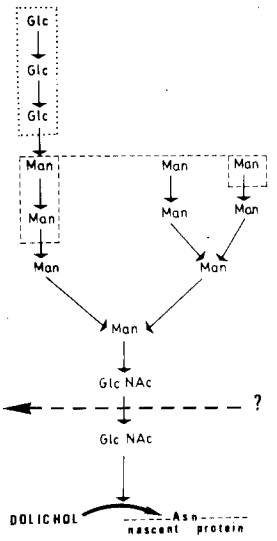
Section	Step 1	Step 2	Step 3	Sugar residues investigated
1	LPA	Neuroaminidase or acid hydrolysis	LPA	Sialic acid
2	LPA	Neuroaminidase or acid hydrolysis	RCA II	Sialic acid, galactose
3	RCA (PNA)	Neuroaminidase or acid hydrolysis	RCA (PNA)*	Galactose
4	–	Neuroaminidase or acid hydrolysis	WGA	Glucosamine
5	–	Neuroaminidase or acid hydrolysis	Con A	Mannose, glucose
6	Acid hydrolysis	β-Galactosidase	RCA II	Galactose
7	Con A	β-Glucosidase	ConA	Glucose
8	Con A	α-Mannosidase	Con A*	Mannose
9	Con A	β-*N*-Acetyl-glucosaminidase	Con A*	Mannose

LPA = *Limulus polyphemus* agglutinin (affinity = sialic acid); RCA II = *Ricinus communis* agglutinin (affinity = galactose); PNA = *Arachis hypogeae* agglutinin (affinity = galactose); WGA = wheat germ agglutinin (affinity = sialic acid, glucosamine); Con A = concanavalin A (affinity = mannose, glucose)

* These sections were further processed for immunocytochemistry using AFRU







Figs. 10, 11. Adjacent semithin sections through the subcommissural organ processed for con A binding without (**Fig. 10**) and with (**Fig. 11**) previous treatment with exo-*β-N*-acetyl-glucosaminidase. Most of con A binding sites located within the RER (*arrows* in **Fig. 10**) disappeared after the enzymatic treatment (*asterisk* in **Fig. 11**). Con A-binding material on the plasma membranes (*arrowheads*) is not affected by this enzymatic treatment. × 600

Top right. Proposed configuration of the oligomannosidic core of the secretory glycoproteins present within the RER. *Arrow:* site of action of the exo-*β-N*-acetyl-glucosaminidase?

jected to acid hydrolysis, treated with jack bean *β*-galactosidase and then incubated with RCA (Table 2).

β-Glucosidase and α-mannosidase (Fig. 7–9)

Binding of con A to the secretory material of the ependymal and hypendymal cells of the SCO was almost completely abolished by pretreating the sections with jack bean-*α*-mannosidase for 18 h. Shorter incubation times in *α*-mannosidase had a partial effect (12 h) or had no effect (3 h) on con A binding to the secretory material. On the other hand, incubation of the sections with yeast *α*-glucosidase or almond *β*-glucosidase for periods longer than 18 h (up to 50 h) did not affect the reactivity of the SCO secretory cells to con A.

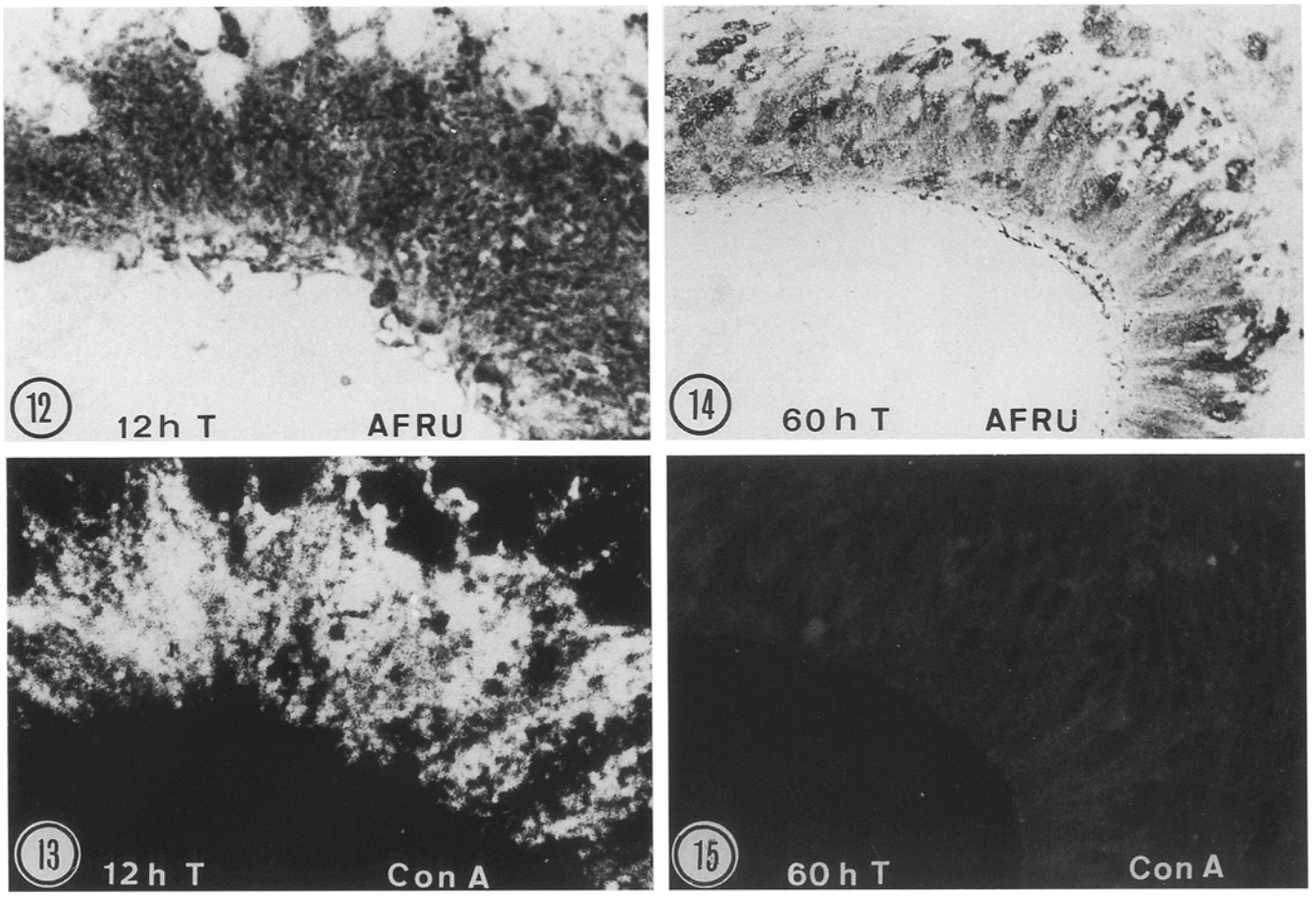
β-N-acetyl-glucosaminidase

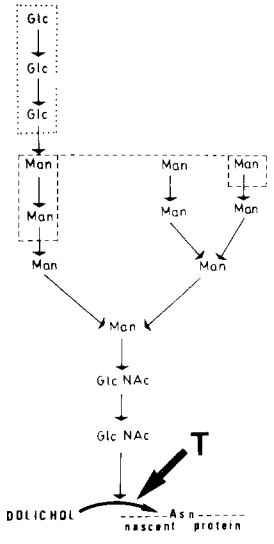
The material located within cisternae of the rough endoplasmic reticulum greatly lost its affinity for con A when the sections had been previously treated with *β-N*-acetylglucosaminidase from *Aspergillus niger* for at least 48 h (Figs. 10, 11). Other structures, such as the apical plasma membrane of ependymal cells and elements of the posterior commissure, retained their affinity for con A after this enzymatic treatment (Figs. 10, 11).

The immunoreactivity of the secretory material to the anti-RF serum was not affected by previous treatment of the section with acid hydrolysis or neuroaminidase (Fig. 6), mannosidase (Fig. 9) or acetyl-glucosaminidase.

Tunicamycin.

Twelve and 24 h after the administration of Tunicamycin no changes were detected in the amount of AFRU-immuno-





Figs. 12, 13. Adjacent sections of the subcommissural organ from a rat killed 12 h after the administration of Tunicamycin and processed for immunocytochemistry using AFRU (**Fig. 12**) and con A binding (**Fig. 13**). ×450

Figs. 14, 15. Rat killed 60 h after administration of Tunicamycin. Adjacent sections immunostained with AFRU (**Fig. 14**) and processed for con A binding (**Fig. 15**). ×450

Bottom left. Site of action of Tunicamycin (*T*) preventing the core-glycosylation of the nascent protein (Hubbard and Ivatt 1981)

reactive material and con A binding sites in the SCO (Figs. 12, 13). The SCO of rats killed 50 and 60 h after drug administration showed a decrease in the amount of AFRU-immunoreactive material (Fig. 14), and complete absence of con A-binding sites (Fig. 15). The SCO of control rats injected with the vehicle (saline-ammonium bicarbonate) was not different from that of untreated rats with respect to amount of immunoreactive material and con A binding sites.

Discussion

Neuroaminidase is an exoglycosidase known to specifically cleave terminal sialic acid residues (Spicer et al. 1960). A similar property has been ascribed to acid hydrolysis carried out according to the procedure of Gibbons (1963). On the other hand, the lectin *Limulus polyphemus* agglutinin (LPA) only binds to sialic acid residues (Muresan et al. 1982). Therefore, the affinity of the apical secretory granules and

the released secretory material (pre-RF and RF) of the rat SCO for LPA and the loss of such an affinity after neuroaminidase or acid hydrolysis can only be explained by the assumption that sialic acid is the terminal sugar residue of the secretory glycoproteins of the rat SCO. A similar assumption has been advanced previously based on the affinity of the SCO secretory material for WGA (Rodríguez et al. 1986; Meiniel et al. 1988). However, WGA has affinity for both sialic acid and glucosamine, and consequently its affinity for the SCO-secretory material could be due to glucosamine, with sialic acid residues missing, as has been shown to occur in the glycopeptide of the vasopressin precursor (Rodríguez et al. 1988). As early as in 1958, Olsson, based on histochemical tests, concluded that sialic acid is present in the secretory material of the bovine SCO. Similarly, Sterba and Wolf (1969) demonstrated histochemically the presence of sialic acid in the bovine RF.

The lack of affinity of *Ricinus communis* agglutinin (RCA) and *Arachis hypogeae* agglutinin (PNA) for the secretory material of the SCO, and the strong affinity of these two lectins for the post-Golgi secretory material (apical secretory granules and released secretory material) after acid hydrolysis or neuroaminidase treatment indicate that galactose is the subterminal sugar residue of the SCO secretory glycoproteins. This is further substantiated by the fact that sequential treatment of the section with acid hydrolysis and β-galactosidase drastically diminished the affinity of RCA for the post-Golgi secretory material.

Wheat germ agglutinin (WGA) binds to both *N*-acetylglucosamine and sialic acid (Alroy et al. 1984; Podell and Vacquier 1984). WGA also displays the property to bind to internal sugar residues in addition to terminal sugar moieties (Roth 1978). Therefore, the strong affinity of WGA for the post-Golgi secretory material of the SCO after removal of sialic acid residues by acid hydrolysis can only be ascribed to the presence of internal glucosamine residues. Furthermore, these glucosamine residues must be those added by the Golgi apparatus, and which in the *N*-linked complex-type glycoproteins are conjugated to the mannosyl residues of the core (see configuration next to Fig. 1), since WGA does not bind to the glucosamine residues of the oligomannosidic core (Rodríguez et al. 1986; Meiniel et al. 1988; Peruzzo et al. 1989).

Another unspected finding was the affinity of con A for the post-Golgi secretory material after removal of sialic acid by acid hydrolysis or neuroaminidase. This indicates (1) that sialyl residues interfere with the binding of con A to internal mannosyl residues in tissue sections of fixed material; (2) that in the secretory glycoproteins present in the secretory granules, pre-RF material and RF, mannose is indeed present although as internal residue, thus further supporting the configuration of the carbohydrate component of the SCO secretory glycoproteins proposed by Rodríguez et al. (1986) and Meiniel et al. (1988) (see schematic configuration next to Fig. 1 of present paper).

The drastic reduction of the amount of con A binding sites in the material contained within cisternae of the rough endoplasmic reticulum after treatment of the sections with α-mannosidase indicates that the positive con A reaction is due to mannose and not to glucose (cf. Meiniel and Meiniel 1985; Rodríguez et al. 1986). This conclusion is further supported by the fact that treatment of the sections with glucosidase, even for 48 h, did not affect the reactivity of con A for the content of the rough endoplasmic reticulum of the SCO cells. However, it must be considered that contradictory results with respect to the capacity of this type of glucosidase to remove terminal glucose residues have been reported (Hunt 1979).

The loss of con A affinity of the material present in the RER of the secretory cells after treatment with β-*N*-acetyl-glucosaminidase from *Aspergillus niger* and the lack of effect of this treatment on the con A binding sites of the glycocalix of adjacent cells suggest that this enzyme may trim off glucosamine residues of the oligomannosidic core. This is rather surprising since the cleavage of the core glucosamine residues would be exclusively performed by endoglycosidases, such as the endo-β-*N*-acetyl glucosaminidase (Hunt 1979; Staneloni and Leloir 1982). It should be borne in mind, however, that in the present investigation the glycoproteins have been subjected to drastic treatment, such as fixation with Bouin's fluid (pH 2.0) and paraffin embedding. An alternative explanation would be that the batch of β-*N*-acetyl glucosaminidase used had contaminants such as mannosidase.

The fact that the secretory material of the SCO retains its strong affinity for the anti-RF serum indicates that this antiserum primarily recognizes the proteinaceous component of the secretory glycoproteins.

The results obtained by the use of specific glycosidases conform to the assumption of Rodríguez et al. (1986) that the carbohydrate component of the secretory glycoproteins of the SCO consists of *N*-linked complex-type oligosaccharides. This is strongly supported by the results obtained after the administration of Tunicamycin into the cerebrospinal fluid.

Tunicamycin is known to interfere with the core glycosylation by blocking the synthesis of the dolichol diphosphate-high mannose core through an inhibition of the transfer to the lipid carrier of the phosphate-*N*-acetylglucosamine molecule (Mahoney and Duksin 1979; Hubbard and Ivatt 1981). The disappearance of con A binding sites associated with the secretory material after in vivo administration of Tunicamycin clearly indicates that the SCO secretory glycoproteins are core glycosylated. The complete renewal of the rat CSF takes about 4–6 h (S. Rodríguez et al. 1989). Consequently the longest period in which Tunicamycin would exert its inhibitory effect is 4–6 h. The fact that 12 h after the administration of the antibiotic the secretory material continues to bind con A indicates (1) that most of the secretory glycosylated proteins existing in the SCO before the injection are still present at this post-injection interval and (2) that the newly synthesized secretory proteins devoid of carbohydrates represent only a small proportion. At longer postinjectional intervals the "old" glycoproteins continue to be released and the newly synthesized non-glycosylated proteins accumulate in the SCO. This results in a progressive disappearance of the con A binding sites, which become completed 60 h after the administration of Tunicamycin. This represents a very slow turnover of the secretory material in the rat SCO.

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