Androgen status and expression of glycoconjugates and lectins in the epithelial cells of the mouse ventral prostate. A glycohistochemical approach

F. Akif[1], H.-J. Gabius[2] and A. Danguy[1]
[1]Unite de Morphologie Fonctionnelle, Laboratoire de Biologie Animale et Histologie Comparee,
Universite Libre de Bruxelles, Bruxelles, Belgique and [2]Institut für Physiologische Chemie,
Ludwig-Maximilians Universität, Tierärztliche Fakultät, München, Germany

Summary. We investigated histochemically the expression of glycans and endogenous sugar-binding proteins (endolectins) in the ventral prostate of normal and androgen-deprived mice. The avidin-biotin-peroxidase complex technique was used with a panel of biotinylated lectins and neoglycoproteins, respectively, on paraffin sections. Characteristic binding patterns depending upon the type of lectin were observed in the glandular epithelium and basement membranes. This report also initiates the histochemical study of endogenous glycan-binding proteins in this male-accessory sex organ. Remarkably, the endocrine status was found to affect the distribution of lectin-reactive determinants of glycoconjugates as well as endolectins. The histophysiological significance of these modifications in the glandular cells are discussed in the light of previous biochemical findings obtained mainly in humans and the rat.

Key words: Ventral prostate, Neoglycoproteins, Lectins, Glycohistochemistry, Androgen

Introduction

Biological importance of glycoconjugates derives from the fact that, in theory, an enormous variety of glycans can be generated from a relatively limited number of monosaccharides. Thus, they offer a high capacity for carrying biologically relevant information (Kobata, 1992; Blithe, 1993; Hunt and Hoe, 1993; Lis and Sharon, 1993; Sharon and Lis, 1993).

During the last decade, there has been a vast expansion in our knowledge of the distribution of glycoproteins in nature. Detection and localization of

glycosubstances has been facilitated thanks to the availability of an increasing range of lectins with a wide spectrum of specificities which are able to detect specific terminal carbohydrates and internal sugars, as well as anomeric and positional linkages of sugar oligomers in complex glycoforms (Spicer and Schulte, 1992; Akif et al., 1993; Coppee et al., 1993; Gabius and Gabius, 1993; Danguy and Gabius, 1993; Pajak and Danguy, 1993; Spicer, 1993; Danguy et al., 1994). When carbohydrates are viewed as potential lingads in a recognitive interplay with endogenous receptor sites like tissue lectins (endolectins), their capacity to bind assumed receptors also needs to be determined. This question requires another class of tool. These tools, termed neoglycoproteins, comprise chemically-coupled glycan moieties that are attached to an inert carrirer protein (Stowell and Lee, 1980; Gabius and Bardosi, 1991; Danguy and Gabius, 1993; Gabius et al., 1993; Lee and Lee, 1993).

Prostate cancer is one of the major causes of mortality in males in the Western world, thus warranting further efforts in the quest to enhance our knowledge of this entity (Wilson, 1980, 1987). A prominent aspect, the responsiveness of the prostate gland to hormones, is well established (Aumüller, 1983; Isaacs, 1983; Montpetit et al., 1986; de Launoit et al., 1988). The rodent ventral prostate has been widely used as a model system to investigate the androgen-dependent processes involved in prostatic pathology (Rennie et al., 1978; Page and Parker, 1982; Tenniswood et al., 1984; Montpetit et al., 1986).

In the male accessory sex glands, complex glycans are important for the functional activities of these organs. There are few reports describing the distribution of lectin-reactive determinants of glycoconjugates of the rodent prostate (Tsukise and Yamada, 1981; Chan and Wong, 1991, 1992). Notably, there have apparently been no studies concerning the effects of androgens on the expression of distinct sugar sequences in cellular glycoconjugates and endogenous sugar-binding proteins

(endolectins), respectively, of the mouse prostate. Therefore, the aim of this paper was to gain insight into these glycobiological aspects of the alveolar secretory epithelium of the ventral prostate of normal as well as of steroid hormone-deprived mice. To this end we used a panel of biotinylated lectins and neoglycoproteins on Bouin-fixed paraffin-embedded tissue sections (Danguy et al., 1991; Akif et al., 1993; Coppee et al., 1993; Danguy and Gabius, 1993; Pajak and Danguy, 1993).

Materials and methods

Animals and tissue preparation

The present investigation was carried out using sexually mature male NMRI mice (8 weeks old) with an average weight of 30-40 g. They were castrated under sodium pentobarbital anaesthesia, and used for experiments eight weeks later. Control mice (sham operated) were used at the same age. Mice were maintained on a regular photoperiod of 12 hour light-12 hour dark at 23 °C in clear plastic cages with food and water available ad libitum. Animals were anaesthesized with ether. The accessory sex glands were exposed and bathed with the fixative during dissection. The prostates were removed, fixed in Bouin's solution, embedded in paraffin and sectioned at a thickness of 5 µm. After dewaxing, lectin and neoglycoprotein histochemical stainings were performed using the avidin-biotin method, following optimized procedures (Danguy and Gabius, 1993). Some sections were stained with Masson's trichrome for visualization of the general histology.

Lectin histochemistry

Nine types of biotinylated lectins, purchased from Vector (Burlingame, CA USA) were used. Their full names, abbreviations, natural sources, saccharide specificities and binding inhibitors are listed in Table 1. The sections were dewaxed with toluene, rehydrated in graded alcohol solutions and incubated with 0.3%

methanolic hydrogen peroxide for 10 min at room temperature to inhibit endogenous peroxidase activity. Sections were then washed in PBS (phosphate buffered saline, 0.15M NaCl containing 0.01M phosphate buffer, pH 7.3±0.01) and incubated with biotinylated lectins (5 µg/ml) for 10 min at room temperature. Then the sections were rinsed in PBS, and incubated for 30 min with an avidin-biotin-peroxidase complex (ABC kit, Vector). After being washed with PBS, the sections were developed in 0.1% 3,3'-diaminobenzidine 4 HCl- 0.1% $CoCl_2$-$6H_2O$ (DAB: Sigma, $CoCl_2$: Merck)-H_2O_2 medium under microscopic control at room temperature to visualize the activity of peroxidase. The sections were rinsed with tap water, dehydrated, cleared and mounted with DPX. Controls for lectin binding included: a) omission of the respective lectin; b) omission of the ABC kit reagents; and c) incubation of the sections with lectin solutions to which 0.2-0.3M of the specific sugar (Janssen Chemica, Beerse, Belgium) (Table 1) had previously been added. In order to block binding of kit reagents to endogenous biotin, which would lead to a considerable background, sections were incubated with blocking kit substances (Vector lab) just prior to the addition of lectin conjugates. The localization of lectin-specific carbohydrates in the tissues by the probes was examined by a light microscope (Leitz, Wetzlar, FRG). The intensity of binding was evaluated from absent (0) to very strong (3) (see Table 2).

Protocol for reverse lectin glycohistochemistry

For detection of the pattern of endogenous sugar receptors with different carbohydrate specificities, the following biotinylated neoglycoproteins (chemically glycosylated derivatives of bovine serum albumin; BSA) were used. For α-galactoside-specific receptors: Melibiose-BSA-biotin; N-acetyl-D-glucosamine-(BSA-biotin) and N-acetyl-D-galactosamine-(BSA-biotin) for receptors with respective specificity for these two naturally occurring N-acetylated sugars; for ß-xyloside-specific receptors: ß-D-xylose-(BSA-biotin); for mannoside- and fucoside-specific receptors: D-mannose-

Table 1. Lectins used for histochemical characterization of glycans.

LECTIN/LATIN NAME (COMMON NAME)	ACRONYM	SUGAR RESIDUES OR SEQUENCES/ OLIGOSACCHARIDES BINDING PREFERENCE	SUGARS FOUND TO INHIBIT HISTOCHEMICAL BINDING
Galanthus nivalis (snow drop bulb)	GNA	Man α(1-3) Man > Man α(1-6) Man > Man α(1-2) Man	Man
Ulex europaeus (gorse seed)	UEA-I	L-Fuc	L-Fuc
Sambucus nigra (elderberry bark)	SNA	Neu5Ac α(2-6) Gal = Neu5Ac α(2-6) GalNAc	Neu5Ac
Maackia amurensis	MAA	Neu5Ac α(2-3) Gal = Neu5Ac α(2-3) GalNAc	Neu5Ac
Arachis hypogaea (peanut)	PNA	Terminal ßGal (1-3) GalNAc	Lactose
Ricinus communis (castor bean)	RCA-I	Gal ß(1-4) GlcNAc > ßGal > αGal	Gal
Glycine maximus (soybean)	SBA	Terminal (α,ß)GalNAc > (α,ß)Gal	GalNAc
Triticum vulgare (succinylated wheat germ)	s-WGA	GlcNAc- (ß1-4-D-GlcNAc)$_{1-2}$	GlcNAc
Phaseolus vulgaris (red kidney bean)	PHA-L	Tri- and tetraantennary non-bisected N-linked sequences containing Gal ß(1-4) GlcNAc- N-acetyllactosamine linked units at non-reducing end	Complex

GlcNAc: N-acetylglucosamine; GalNac: N-acetylgalactosamine; Gal: galactose; Man: mannose; Glc: glucose; L-Fuc: fucose; Neu5Ac: neuraminic acid.

(BSA-biotin) and L-fucose-(BSA-biotin). respectively; for receptors specific for sugars that contain a phosphate group: D-mannose-6-phosphate-(BSA-biotin). The sulphated glycosaminoglycan, heparin, was also employed after mild cyanogen bromide activation, aminoalkylation and biotinylation. The batch of BSA, used for neoglycoprotein synthesis, had additionally been treated with periodate to destroy any traceable contamination with carbohydrates. Chemical preparation, quality controls and properties of labelled carbohydrate-protein conjugates are described elsewhere (Stowell and Lee, 1980; Gabius and Bardosi, 1991). Deparaffinization and inhibition of endogenous peroxidase were performed as above. After rehydratation in graded alcohol the sections were preincubated with 0.1% BSA + 0.1M phosphate buffer (PBS, pH 7.4) solution for 15 min to minimize the unspecific binding of BSA-biotin derivatives by protein-protein interaction used in the following steps. Incubation was performed using the biotinylated carbohydrate-BSA conjugates dissolved in 0.1% BSA + 0.1M PBS (10 μg lyophilized derivative in 1 ml of 0.1% BSA + 0.1M PBS) at room temperature for 15 min. For the control studies sections were preincubated at room temperature with homologous un-labelled carbohydrate-BSA conjugates at a concentration of 20 μg/ml to mask sugar-specific receptors. Incubation was then performed for 90 min with a mixture (in relation of 1:100) of labelled carbohydrate-BSA conjugate (10 μg) and the corresponding unlabelled neoglycoprotein as competitive inhibitor. Absence of staining with the carbohydrate-free labelled carrier protein was also ascertained at the same concentration.

To assess the contribution of glycosyltransferases to binding of probes, incubation of the biotinylated markers was performed in the presence of nucleotides cytidine-5'-diphosphate and uridine-5'-diphosphate which have been shown to suppress binding to glycosyltransferases (Straus and Keller, 1986). The visualization and the intensity of neoglycoprotein binding was carried out, as described in detail for lectin histochemistry.

Results

Overview of tissue binding sites of each biotinylated lectin

The binding patterns observed with each lectin are summarized in Table 2.

Mannosyl-specific lectin

Galanthus nivalis (GNA):
Normal males: Staining with this lectin revealed weak binding sites restricted to apical border and moderate reactivity of perinuclear areas of epithelial cells. This area was characterized by the presence of positive granular (Fig. 1A) material. The basement membrane of the epithelium was also moderately labelled with this lectin.
Castrated males: With GNA luminal border, cytoplasm and the basement membrane of epithelial cells exhibited a strong staining (Fig. 1B).

Fucosyl-specific lectin

Ulex europaeus (UEA-I):
Normal males: The apical surface of the epithelial cells showed a moderate reactivity with the lectin. The cytoplasm and basement membrane were weakly stained.
Castrated males: The same pattern was disclosed in the castrated animals

Complex carbohydrate-specific lectin

Phaseolus vulgaris leukoagglutinin (PHA-L):
Normal males: A moderate reaction was only seen in the apical surface bordering the lumen of epithelial cells, whereas no evidence for any reactivity was obtained in cytoplasm. The basement membrane was lightly stained.
Castrated males: Androgen deprivation completely abolished the staining of the apical area of epithelial cells.

Table 2. Semiquantitatively-determined intensity of lectin binding to the mouse ventral prostate.

TYPE OF LECTIN	NORMAL MALES (epithelium)				CASTRATED MALES (epithelium)			
	A	C	BM	%	A	C	BM	%
GNA	1	2*	2/3	100	3	3-3*	2/3	100
UEA-I	2	1	1	100	2	1	1	100
SNA	0	0	2	0	2	0	2	30
MAA	0	0	1	0	0	0	1	0
PNA	1/2	0	1	10	3	0	1	100
RCA-I	2	1-1*	2	100	3	2-2*	2	100
SBA	2	2	1	100	2	2	1	100
s-WGA	1	0	1	100	1	0	1	100
PHA-L	2	0	1	100	0	0	1	0

A: apical region (including microvilli); C: cytoplasm; BM: basement membrane; %: percentage of labelled cell; *: perinuclear labelling; n/n: variable reactivity in the same structure. Numbers indicate intensity on an estimated scale from 0 (unreactive) to 3 (strongly reactive).

N-acetylgalactosaminyl-or galactosyl-specific lectins

Arachis hypogaea (PNA):
Normal males: Staining with PNA uncovered a weak to moderate presence of binding sites restricted to luminal border of some epithelial cells. The basement membrane disclosed a faint labelling.
Castrated males: Following castration the luminal border of all the epithelial cells was heavily labelled.

Ricinus communis (RCA-I):
Normal males: A moderate reaction was evidenced both in the apical surface bordering the lumen of epithelial cells and in the basement membrane. The cytoplasm was weakly labelled.
Castrated males: Following castration an increase in stainability was seen at the epithelial cell cytoplasm and apical surface.

Glycine maximus (SBA):
Normal males: This lectin moderately reacted with the apical surface and cytoplasm of the epithelial cells. A weak reaction was seen at the basement membrane level.
Castrated males: Castration did not noticeably change lectin binding.

N-actylglucosaminyl specific lectin

Succinylated Triticum vulgare agglutinin (s-WGA):
Normal males: This lectin slightly reacted with the apical surface and basement membrane of the epithelial cells, whereas the cytoplasm was devoid of affinity for this lectin.
Castrated males: The same pattern was disclosed in the castrated animals.

N-acetylneuraminic acid-specific lectin

Sambucus nigra (SNA):
Normal males: This lectin did not bind at all at any site of the epithelial cells except the basement membrane.
Castrated males: Following castration a moderate reaction was noticed in the apical area of epithelial cells.

Maackia amurensis (MAA):
Normal males: No positive reaction was obtained in epithelial cells. This lectin reacted slightly with the basement membrane of the epithelium.
Castrated males: Androgen deprivation did not change lectin binding pattern.

Reverse lectin histochemistry

The neoglycoprotein-binding capacity of the alveolar epithelial cells in normal and castrated mice is summarized in Table 3. A careful examination of this table shows that following androgen deprivation epithelial receptors for all the neoglycoproteins were enhanced, but to a variable degree (Fig. 2A,B). Furthermore, only 20 to 80% of the epithelial cells

Table 3. Semiquantitatively-determined intensity of neoglycoprotein and heparin binding in the mouse ventral prostate.

TYPE OF NEOGLYCO-PROTEIN AND HEPARIN	NORMAL MALES (epithelium)				CASTRATED MALES (epithelium)			
	A	C	BM	%	A	C	BM	%
Heparin	0	0-(2)	0	0	3	3-(2)	1	50
Xylose-BSA	2	1	2	100	3	2/3	2	80
Fucose-BSA	1	1	1	100	2	1/2	1	30
GalNAc-BSA	0/1	0/1	1	100	1	1/2	1	80
GlcNAc-BSA	1	1	1	100	1/2	2	1	80
Melibiose-BSA	0/1	0/1	0/1	100	1/2	2	1	20
Mannose-6-P-BSA	1	1	1	100	2	2	2	30
Mannose-BSA	2	1	1	100	2	2/3	1	80

BSA: bovine serum albumine; GalNAc: N-acetylgalactosamine; GlcNAc: N-acetylglucosamine; (): nuclear labelling. For further details, see legend to Table 2.

Fig. 1. Binding sites of GNA in the ventral prostate of normal (A) and castrated (B) mice. **A.** Positive granular material is observable at the basal and perinuclear cytoplasm of the alveolar cells (arrows). A weak binding is restricted to the epithelial apical border (arrowheads). No counterstaining. x 512. **B.** The whole cytoplasm of the alveolar cells is intensely stain. No counterstaining. x 512

Fig. 2. Glycohistochemical staining of mannose binding proteins in the ventral prostate of normal (A) and castrated mice (B). **A.** The apical border of the alveolar cells is moderately labelled (arrows). No counterstaining. x 1,024. **B.** The whole cytoplasm of most cells is moderately to strongly reactive. Some cells remain unstained (arrowheads). No counterstaining. x 512

Fig. 3. Ventral prostate of normal (A) and castrated (B) mice after incubation with heparin-biotin. **A.** Selective staining of many epithelial cell nuclei is observable (arrows). No counterstaining. x 512. **B.** The cytoplasm of half the alveolar cells is heavily labelled. Strong reactivity is also observable in many nuclei (arrows). No counterstaining. x 512
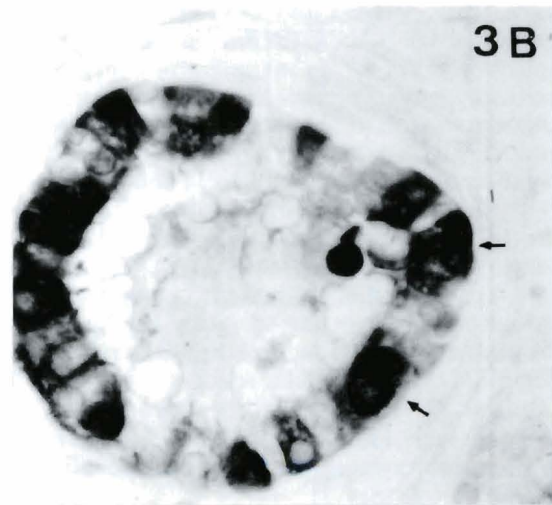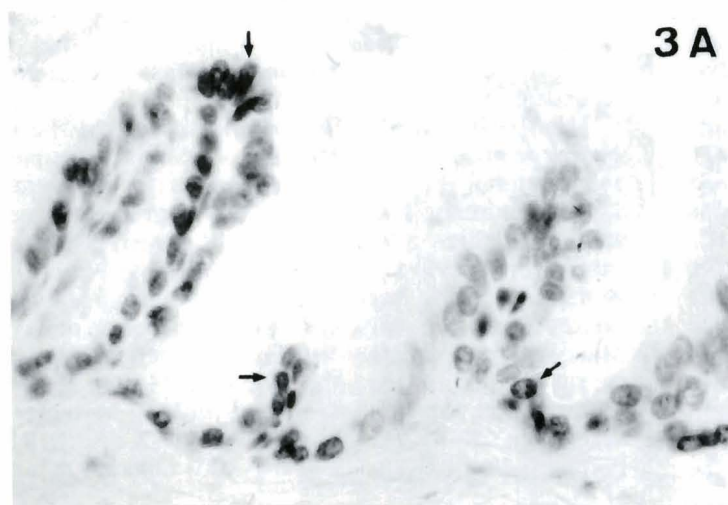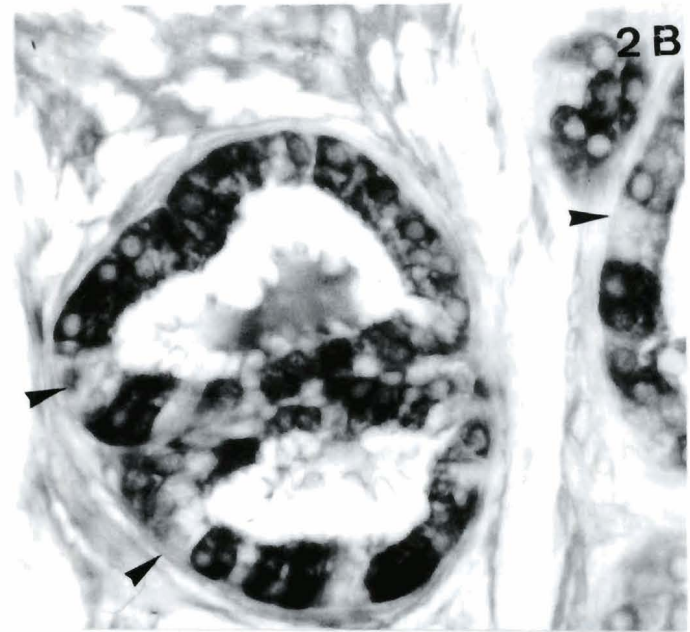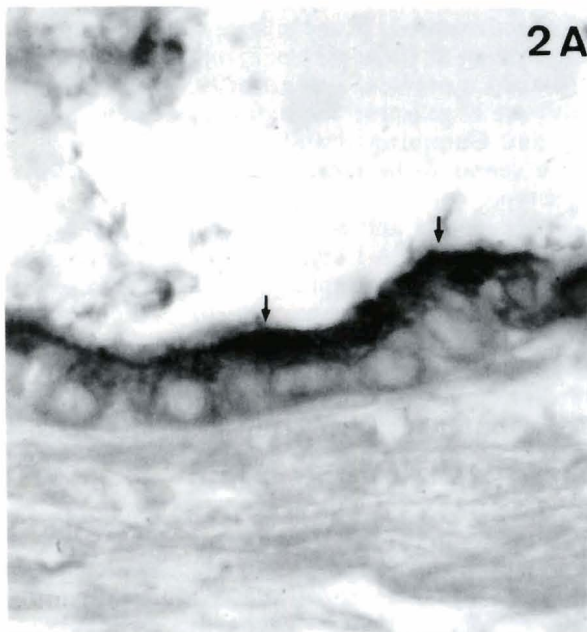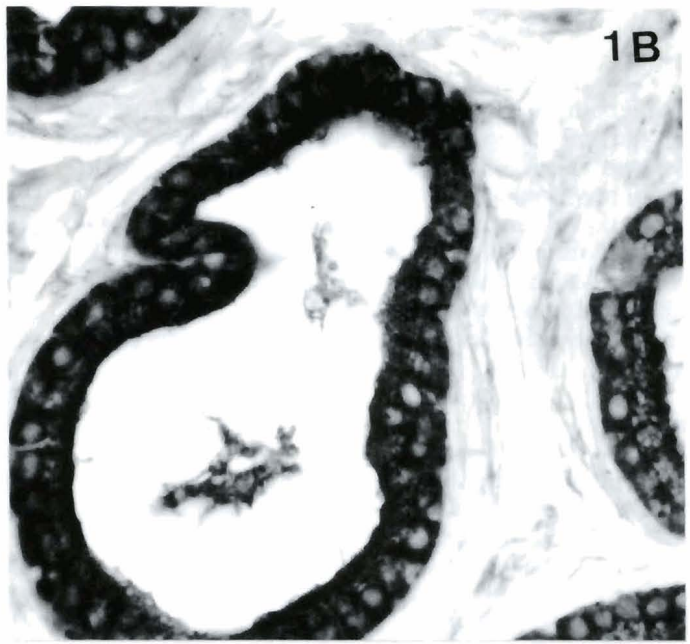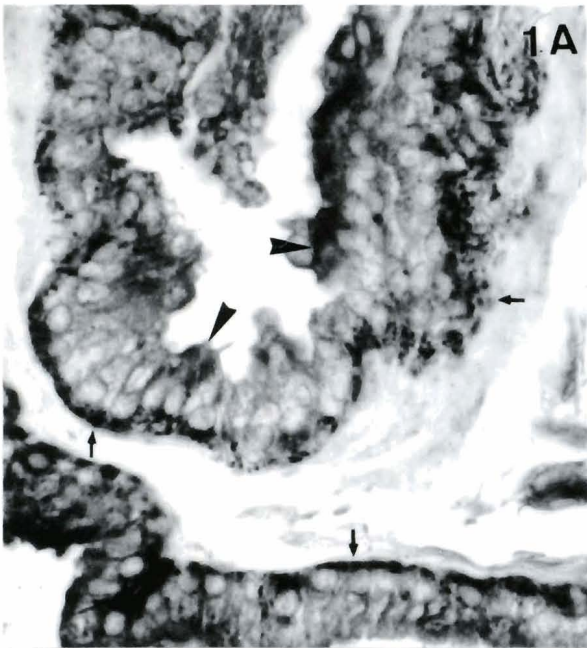
expressed an increased binding (Fig. 2B). In castrated animals only heparin-X-biotin and mannose-6-phosphate-biotin displayed an increased binding at the epithelium basement membrane.

Remarkably, receptors for sulphated polysaccharides (heparin) were also present in nuclei of both normal (Fig. 3A) and castrated mice (Fig. 3B) alveolar cells. Furthermore, in castrated animals half the cytoplasm of alveolar cells exhibited a strong binding with this probe (Fig. 3B).

Discussion

Glycoconjugates are among the most prominent substances elaborated and released by the secretory epithelial cells of mammalian accessory sex glands (Mann and Lutwak-Mann, 1981).

However, little attention has been paid to the localization and characterization of glycosubstances in these organs (Tsukise and Yamada, 1984, 1987; Chan and Wong, 1991).

Combining data in Tables 1 and 2 gains access to some interesting initial conclusions on structural aspects of the glycans that are synthesized in the mouse ventral prostate and detected by the panel of lectins.

The two main families of glycoproteins are those having carbohydrate side chains linked N- and O-glycosidically. The only N-glycosidic bond found in glycoproteins is N-acetyl-glucosaminyl-asparagine (GlcNAc [ß1-N] Asn). The O-glycosidic bond presents a variety of linkage structures, the most important being the mucin type, in which the oligosaccharide chain is linked from N-acetyl-D-galactosamine to the hydroxyl groups of L-serine and/or L-threonine (GalNAc [α1-3] Ser or GalNAc [α1-3] Thr). Some lectins may preferentially bind to either O- or N-linked glycoproteins or both.

In normal mice the lectin binding pattern observed over the microvilli and the apical region of the alveolar epithelial cells demonstrates that both N- and O-linked oligosaccharides of glycoproteins are produced by these cells. Furthermore, the reactivity of this cell part with s-WGA, SBA, UEA-I and GNA is indicative of the presence of GlcNAc, GalNAc, Fuc and Man residues, respectively. As far as mannose is concerned, GNA specifically probes «pure» high-mannose structures and is unreactive with α-glucosyl terminals on glycosyl high mannose glycans and glycogen (Jones et al., 1992). The moderate labelling with RCA-I and PHA-L suggests that the lumenal region of the alveolar cells contains N-acetyl-lactosamine and GlcNAc ß(1-2) Man tri-antennary complex oligosaccharides, respectively. The heterogeneity of this epithelium is emphasized by the fact that only 10% of the secretory cells expressed the terminal disaccharide Gal-ß(1-3)-GalNAc (PNA binding). Many intracellular resident proteins are known to contain O-GlcNAc residues (Hart et al., 1989). Among these molecules are cytoskeletal proteins (Blithe, 1993), which in many cell types are localized in the

apical cytoplasm beneath the cell membrane. The s-WGA binding pattern is in accordance with such localization.

No sialic acid residues were observed over the microvilli and apical area of epithelial cells, as judged by the lack of SNA and MAA binding. Presence of a lectin-reactive sialoglycoprotein had been reported in the rat by Tsukise and Yamada (1981).

In the present study, GNA and RCA-I binding sites were detected in the supranuclear region of secretory cells of the ventral prostate. It has been shown that this area contains the Golgi apparatus. Our results are in agreement with structural studies, reporting Con-A and RCA-I binding sites in the Golgi saccules (Chan and Wong, 1992).

The basement membranes at the epithelial-stromal interface of the prostate alveoli are rich in acidic glycosaminoglycans and acidic glycoproteins with sialic acids are also present in this region in guinea pig (Chan and Wong, 1991). The demonstration of SNA- and MAA-reactive sites is in accordance with these data. The actual binding of these probes is indicative of terminal sialic acid moieties, linked α(2-3) and α(2-6) to penultimate D-galactose residues (Shibuya et. al., 1987; Wang and Cummings, 1988) Above all, the α(2-6) linkage seems to be more frequent than the former linkage type.

SNA receptors and an increase in the intensity of GNA, PNA and RCA-I binding were obviously present on the apical region of epithelial cells after castration, whereas highly branched non-bisected complex sequences disappeared, as judged by the complete lack of PHA-L staining. Moreover, RCA-I labelling was enhanced in androgen-deprived mice both at apical region and in the cytoplasm. The other lectins did not display any apparent hormone status-related modifications in their extent of binding.

It is well known that the activities of the prostate are regulated by androgens (Aumüller, 1983, 1989 for review) and that the activities of numerous enzymes are stimulated by testosterone (Rennie et al., 1978; Tenniswood et al., 1978). Remarkably, in this respect, Parker et al. (1978) have demonstrated in rats by electrophoresis and affinity chromatography that the synthesis of three major glycoproteins is androgen-dependent.

Montpetit et al. (1986) demonstrated for the rat ventral prostate that a number of proteins became more prominent in the translation products of androgen-deprived animals. The same authors suggested that new mRNA species are present in the prostate after castration. Several other investigators, have also identified proteins, or enzyme activities, that appear after castration. In this way, castration-dependent proteins which are approximately 30 KDa have been identified in the rat ventral prostate (Anderson et al., 1983). Prostate regression in rats was associated with an increase of activities in proteolytic enzymes (Lee, 1981). Furthermore, Lee and Sensibar (1987) reported that the

rat ventral prostate, during its castration-induced regression, underwent a drastic modification in the pattern of protein synthesis. In detail, a rapid inactivation in the synthesis of androgen-dependent proteins and a concomitant activation in the synthesis of a new class of proteins, designated as the «castration-induced proteins», were measured. it is at present still unclear, whether or not these castration-induced proteins, perhaps in part degradative enzymes, are glycosylated, thus reactive to lectins.

Many enzymes are produced by the prostate, i.e. prostatic acid phosphatase, prostate specific antigen, ß-microseminoprotein, ß-glucorinidase, esterase, aminopeptidase... and different proteases (plasminogen activator). Among the predominant proteins secreted by the human prostate, a 33 KDa glycoprotein (prostate specific antigen) has been identified as a serine protease (Lilja, 1985). The presence of transglutaminase has also been reported in human and rat semen (Aumüller, 1989).

Expression of epidermal growth factor and growth factor receptors has also been documented (Aumüller, 1989 for review). As mot of these enzymes and cell receptors are glycoproteins, our glycohistochemical observations may be correlated with these biochemical data. To pursue this line of interpretation, lectin reactivities on extract blots and with purified proteins need to be throughly determined.

Several studies have revealed a complex role for N-linked glycosylation in glycoprotein secretion and cell surface expression (Shakin-Eshelman and Spitalnik, 1993 for review). Evidently, the majority of secreted and cell surface proteins, but not the intracellular ones, were modified by glycosylation. Other investigators have pointed to a complex relationship between N- and O-glycans in glycoprotein transport (Shakin-Eshelman and Spitalnik, 1993). In view of the increase in staining with certain lectins after castration it is tempting to speculate on a role played by N-glycans in influencing transport of affected glycoproteins. As already noted, further clarification of this issue would require the application of these lectins within biochemical assays.

The second class of probe that we have employed in our study to localize complementary binding sites, namely neoglycoproteins, has already proven it value for analysis on fixed tissue specimens (Danguy et al., 1991; Gabius and Bardosi, 1991; Zanetta et al., 1992; Akif et al., 1993; Coppee et al., 1993; Danguy and Gabius, 1993; Gabius et al., 1993). To our knowledge, this report initiates the histochemical study of endogenous glycan-binding proteins in adult male accessory sex organs. Our results clearly demonstrate specific and extensive binding of these synthetic probes to accessible sugar receptors, indicating the presence of carbohydrate-binding proteins associated with alveolar glandular cells of the mouse prostate.

In normal males endogenous receptors with specificity to xylose and mannose proved to be the most abundant at the lumenal compartments of epithelial cells. In the cytoplasm, specific staining with neoglycoproteins was more faintly distributed. Our reverse lectin histochemical method demonstrated a weak affinity of the basement membrane for all the synthetic probes used except for labelled heparin, whereas a moderate extent of receptor expression was visualized using xylose-BSA.

Interestingly, a moderate concentration of receptors with specificity to sulphated polysaccharides (heparin) was observed only in the nucleus of normal mice glandular cells. Such nuclear staining was also recorded in human placental tissues (Gabius and Bardosi, 1991) and fish epidermis (Danguy et al., 1991). Heparin and heparan sulphate play key roles in regulating many physiological processes (Ishiara, 1993). There is an emerging consensus that these substances, serving as important low affinity binding sites for growth factors, besides their protein receptors, may exert effects by modulating the activity of various heparin-binding growth factors. Additionally, the heparin-binding activity may be involved in the translocation of basic fibroblast growth factor into the nucleus. The binding of labelled heparin, reported in nuclei in our study, may be a visual reflection of such an assumption. In this context, it is interesting to note that some internalized heparan sulphate is found in the nucleus (Yamaguchi, 1993).

Remarkably, castration enhanced the binding of all the neoglycoproteins in a variable percentage (20%-80%) of glandular cells. The increased labelling was observed at both the lumenal and intracellular compartments. Although no biochemical data on endolectins is available, it is in principle noteworthy that changes in the level of certain proteins have been documented in the rat. In detail, it has been shown that during its castration-induced regression the rat ventral prostate synthesized 33, 38 and 64 KDa proteins (Lee and Sensibar, 1987). Using a mRNA translation system, Montpetit et al. (1986) and Saltzman et al. (1987) observed the expression of unique proteins in the prostate of androgen-deprived rats. Our data can indicate either a reduced expression of endolectin-specific glycoligands, enhancing lectin accessibility, a quantitative increase in expression of already present endolectins or the expression of additional types of neoglycoprotein-binding receptors. Only further biochemical studies will clarify this problem. At any rate, a precedent for androgen-sensitive modulation of expression of this class of proteins has recently been assessed (Akif et al., 1993).

From the results reported in this paper, it is conceivable that the various glycoconjugates, synthesized by the alveolar epithelial cells of the mouse prostate, are heterogenous in their chemical composition. Furthermore, the hormonal status changed the histochemical expression of lectin-reactive glycans of glycoproteins and, notably, of endolectins, thus modifying both sides of a presumed physiologically salient protein-carbohydrate interplay in this system. So far the precise nature of the glycocompounds and the detected endolectins that are synthesized by the prostate are unknown. Admittedly, it is not even clear whether

they contribute significantly to prostatic physiology. Nonetheless, it is expected that glycohistochemistry will expand our understanding in this area and serve as a guideline for further investigations, attempting to elucidate presently unknown physiological functions played by endolectins in steroid target tissues.

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