Appearance and cellular distribution of lectin-like receptors for α_1 -acid glycoprotein in the developing rat testis

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A histochemical avidin–biotin technique with three different α_1 -acid glycoprotein glycoforms showed pronounced alterations in the cellular localization of two α_1 -acid glycoprotein lectin-like receptors during cell differentiation in the developing rat testis. The binding of α_1 -acid glycoprotein glycoforms to their receptors is inhibited by steroids. Testosterone, oestradiol and progesterone inhibited the binding of α_1 -acid glycoprotein glycoform A to its receptor. Cortisone, aldosterone, oestradiol and progesterone inhibited the binding of α_1 -acid glycoprotein glycoforms B and C to their receptor. A difference in the cellular content of α_1 -acid glycoprotein glycoforms and α_1 -acid glycoprotein receptors separates the spermatocytes and the early spermatids from the late spermatids. The difference in receptor composition implies a difference in the effect of different steroid hormones. The Leydig cells contained α_1 -acid glycoprotein and lectin-like receptors for one of the glycoforms of α_1 -acid glycoprotein from birth. The interaction between α_1 -acid glycoprotein glycoforms and their receptors may modulate the actions of testosterone and other steroids in the testis.

Introduction

As differentiating cells integrate into complex tissues, their changing interactions with neighbouring cells and extracellular matrix are determined by the expression of specific extracellular matrix components and receptors. Lectins participate in this process by recognizing complementary glycoconjugate saccharides expressed on cells or in matrix (Barondes, 1978; Lis and Sharon, 1993).

The appearance and cellular distribution of many animal lectins are developmentally regulated and coordinated with changes in the distribution of complementary carbohydrate structures (Barondes, 1978; Arya and Vanha-Perttula, 1986; Malmi *et al.*, 1990). Glycoconjugates and lectins may play a role in directing the cellular kinetics in cell-to-cell adhesion during differentiation.

The glycoforms of orosomucoid or α_1 -acid glycoprotein (AGP) are well known (Bierhuizen *et al.*, 1988; Schmid, 1989). A lectin-like receptor for glycoform C was described on macrophages (Carpentier *et al.*, 1993). We have described receptors for glycoform A, B, and C in mouse testis (Andersen *et al.*, in press, a) and rat prostate and seminal vesicle (Andersen *et al.*, in press, b). These receptors are glycoform specific. They are not related to the hepatic Ashwell receptor (Ashwell and Morell, 1974), because the presence of sialic acid does not influence the binding (Andersen *et al.*, in press, a, b).

The aim of this study is to follow histochemically the cellular distribution of the lectin-like receptors for AGP during postnatal development of rat testes until sexual maturity.

Materials and Methods

AGP was purified using the methods described by Chan and Yu (1991). The three AGP glycoforms were separated by Con A (concanavalin A) chromatography (Bøg-Hansen, 1973) and thereafter biotinylated separately.

Histochemistry

Tissues. Groups of three rats were killed by cervical dislocation at 1, 4, 5, 7 and 12 weeks. Testes were fixed in Bouin's fixative for 24 h at 4°C.

The fixed tissue was dehydrated, embedded in paraffin wax, and sections of 4 μ m were dried at 37°C overnight and the paraffin wax was removed by xylene and ethanol. The sections were then washed three times for 5 min in Tris-buffered saline (TBS) (pH 7.2, 0.1 mol l⁻¹).

The cells in the developing rat testes were identified according to the results of Hagenäs *et al.* (1981) and Ekwall *et al.* (1984). The stages of spermatogenesis were identified according to the criteria set up by Leblond and Clermont (1952) and Parvinen (1982).

Incubation. Nonspecific reactions were blocked by incubating the sections with 1% (w/v) BSA in TBS (pH 7.2, containing 0.1 mmol CaCl₂, MnCl₂ and MgCl₂ l⁻¹) for 30 min at room temperature and then washing in TBS (pH 7.2, containing 0.1 mmol CaCl₂, MnCl₂ and MgCl₂ l⁻¹) three times for 5 min. The sections were then incubated with biotinylated AGP at a final concentration of 0.5 μ g ml⁻¹ or biotinylated anti-AGP overnight at 4°C, washed as above and incubated with alkaline

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phosphatase-containing avidin (Dako, Glostrup, Denmark) in TBS (pH 7.6, 0.1 mol l^{-1}) for 30 min. Sections were then washed three times for 5 min in TBS (pH 7.2, 0.1 mol l^{-1}) containing 2.5 mg per 10 ml levamisole (Sigma, St Louis, MO) and incubated for 30 min in 18 ml enzyme substrate media containing TBS (pH 9.5, 0.2 mol l^{-1}), 10 mmol $\text{MgCl}_2 \text{ l}^{-1}$, 3 mg 5-bromo-4-chloro-indoxyl phosphate (Sigma) in 0.2 ml *N,N*-dimethylformamide, 6 mg nitroblue tetrazolium (Sigma) in 0.2 ml *N,N*-dimethylformamide and 5 mg levamisole, and then immersed for 15 min in 10% (v/v) formaldehyde in phosphate buffer (pH 7.4, 0.1 mol l^{-1}) (Kirkeby *et al.*, 1991). Sections were rinsed in water and mounted in Aquatex (Merck, Darmstadt). Positive reactions with alkaline phosphatase were identified as blue reaction products.

Controls consisted of incubations without AGP glycoforms.

Inhibition. Binding was inhibited by pretreating sections for 2 h at room temperature with buffers containing simple carbohydrates (mannose (D(+)-mannose), galactose (D(+)-galactose), GalNAc (*N*-acetyl-D-galactosamine) and GlcNAc (*N*-acetyl-D-glucosamine) (Sigma)) at concentrations of 0.1, 0.05 or 0.01 mol l^{-1} in $0.1 \text{ mol TBS l}^{-1}$ (pH 7.2, containing 0.1 mmol CaCl_2 , MnCl_2 and $\text{MgCl}_2 \text{ l}^{-1}$) and incubated overnight at 4°C with biotinylated AGP glycoforms in the inhibitor-containing buffer.

Another series of inhibition experiments was performed with buffers containing steroid hormones (testosterone, cortisone, aldosterone, oestradiol and progesterone (Fluka, Buchs)) at concentrations of 50 or 100 nmol l^{-1} in $0.1 \text{ mol TBS l}^{-1}$ (pH 7.2, containing 0.1 mmol CaCl_2 , MnCl_2 and $\text{MgCl}_2 \text{ l}^{-1}$). The hormones were dissolved in methanol at a final solution of 10% (v/v) methanol in the buffer. Control experiments were performed with AGP in 10% (v/v) methanol/TBS without hormones.

Incubation with anti-AGP. IgG antiserum to human AGP (Dako, Glostrup) was added to the incubation buffer at a final dilution of 1:1000. Control slides were reacted with the same antiserum that had been preincubated for 2 h at room temperature with purified AGP at a concentration of $10^{-5} \text{ mol l}^{-1}$.

Results

Localization of binding in germ cells and Sertoli cells

α_1 -Acid glycoprotein. No germ cell in 1-week-old rats was stained. In 4-week-old rats the cytoplasm of spermatocytes and

the basal part of the Sertoli cells stained positively for AGP glycoform A (AGP-A) (Fig. 1a). At this age a few positively stained spermatids were the most advanced germ cell stage

Fig. 1. Testes from (a) 4-, (b) 5-, and (c) 12-week-old rats are stained with biotinylated α_1 -acid glycoprotein (AGP) glycoform A. (a) Seminiferous epithelium consists of spermatogonia, spermatocytes and Sertoli cells. No spermatids can be identified in this photomicrograph. Receptor activity for AGP-A is expressed in the cytoplasm of all spermatocytes, spermatids and Sertoli cells. The Leydig cells are negatively stained. (b) Spermatocytes, spermatids and the Sertoli cells are positively stained. (c) Positive staining for AGP-A receptors in the cytoplasm of spermatocytes. All spermatids in this tubule at stage XII–XIII are elongated and are negatively stained. Scale bars represent 50 μm .

Table 1. The appearance and cellular distribution of α_1 -acid glycoprotein glycoform A lectin receptors in rat spermatogonia, spermatocytes and spermatids

Age (weeks)	Spermatogonia	Spermatocytes	Spermatids Steps 1–10	Spermatids Steps 11–19
1	0			
4	0	+	+	
5	0	+	+	0
7	0	+	+	0
12	0	+	+	0

0: no reaction; +: lectin receptor present.

present. The cellular distribution of the AGP-A receptors in the 5-week-old rats was identical to the cellular distribution in the adult rats: in the basal part of the Sertoli cells, the spermatocytes and the early spermatids (steps 1–10) (Fig. 1b, c; Table 1). The later stages of elongated spermatids did not react after incubation with AGP-A.

α_1 -Acid glycoprotein glycoform B and glycoform C. The distribution of the coloured reaction product after incubations with AGP glycoform B (AGP-B) and AGP glycoform C (AGP-C) was identical. No staining with AGP-B and AGP-C was seen in the spermatogenic cell line of the 1-week-old rats (Fig. 2a). For as long as they are the most differentiated cells present, the spermatocytes and first spermatids contained AGP-B/C receptors (Fig. 2b). As more mature cells developed, the cellular localization of the AGP-B/C binding changed to the adult pattern: in the late spermatids (steps 9–19) and the luminal part of Sertoli cells (Fig. 2c, d; Table 2).

Binding in Leydig cells

The cytoplasm in Leydig cells from rats at all ages stained strongly after incubation with AGP-B and AGP-C, but not after incubation with AGP-A (Fig. 2a–d).

Inhibition of the binding of AGP glycoforms by carbohydrates and hormones

AGP glycoform A. The binding of AGP-A in the seminiferous tubules of rat testis was inhibited by testosterone (50 nmol l⁻¹), oestradiol (50 nmol l⁻¹), and progesterone (50 nmol l⁻¹) (Table 3). Mannose, galactose, GlcNAc, GalNAc, cortisone or aldosterone did not inhibit the binding of AGP-A (Table 3).

AGP glycoform B and glycoform C. The binding of AGP-B in the rat seminiferous epithelium and Leydig cells was inhibited by mannose (1 mmol l⁻¹), GlcNAc (1 mmol l⁻¹), cortisone (100 nmol l⁻¹), aldosterone (100 nmol l⁻¹), oestradiol (50 nmol l⁻¹) and progesterone (50 nmol l⁻¹) (Table 3). In all sections, mannose was a more effective inhibitor than GlcNAc. Galactose, GalNAc or testosterone did not inhibit the binding of AGP-B (Table 3). The inhibition pattern

of AGP-C was identical to the inhibition pattern of AGP-B (Table 3).

The presence of AGP in the developing testes

As glycoform-specific antibodies are not available, the presence of the three individual glycoforms could not be tested. By performing an immunochemical localization with human anti-AGP, the appearance of AGP was observed from the age of 4 weeks in all spermatocytes and spermatids except the testicular spermatozoa and the latest stages of spermatids (Fig. 3). The Leydig cells were positive from birth. The reaction with anti-AGP did not occur when the antiserum was preincubated with an excess of purified AGP.

Control experiments

No reaction was observed in any control section.

Discussion

Two receptors were identified in developing rat testes. Both receptors are glycoform specific. One recognizes AGP-A and the other recognizes AGP-B and AGP-C. The receptors in mouse testis were identified as lectin-like (Andersen *et al.*, in press, a).

The undifferentiated spermatogenic stem cell contained neither AGP nor AGP glycoform receptors. AGP and its receptors appear in the spermatogenic cell line after the early peak in the concentration of testosterone and 5 α -androstane-3 α ,17 β -diol in the seminiferous tubule at the time of the first meiotic division (Rivarola *et al.*, 1975). Thus the production of AGP glycoform receptors and AGP in Sertoli cells and germ cells, like that of other testicular lectins and proteins, may be initiated and maintained by androgens (Arya and Vanha-Perttula, 1986; Malmi *et al.*, 1990).

The AGP-A and AGP-B/C receptors appear in the spermatocytes and in the first spermatids. A clear developmental pattern was found in the cellular localization of AGP-B/C receptors. Between 4 and 5 weeks of age there is a shift in the cellular localization of AGP-B/C receptors from the spermatocytes and early spermatids to the newly appeared, late spermatids. The present methods appear to be useful in the characterization of the different cells in the stages of spermatogenesis.

The differences in the receptor composition of the differentiating germ cells may reflect their specific function and degree of maturation during spermatogenesis. The AGP glycoforms and their receptors may be some of the cell surface ligands that are involved in the adhesion of germ cells with Sertoli cells (Jégou, 1992). The cell recognition mediated by AGP and its receptors may be necessary for the transfer of germ cells at specific stages of development from the spermatocytes to the final stages of spermatids.

The binding of AGP glycoforms to lectin-like receptors is inhibited by steroids. The inhibitory mechanism is not known. The Sertoli cells are known to have receptors for testosterone and oestradiol (reviewed in Bardin *et al.*, 1988) and progesterone receptors have been identified on the surface of sperm cells

Fig. 2. Rat testes at the age of (a) 1, (b) 4, (c) 5 and (d) 12 weeks, stained with biotinylated α_1 -acid glycoprotein (AGP) glycoform C. (a) The 1-week-old rat had receptors for AGP-C in the cytoplasm of Leydig cells (arrow). (b) The seminiferous epithelium, consisting of spermatogonia and spermatocytes at 4 weeks of age, shows receptor activity for AGP-C in the cytoplasm of all spermatocytes except the most basally placed. No spermatids can be identified. The Leydig cells are positively stained (arrow). (c) At 5 weeks of age a marked change in the binding pattern of AGP-C has taken place. The spermatocytes and early spermatids are now negatively stained. The late, elongated spermatids and the Leydig cells (arrow) are positively stained. (d) The adult rat stained positively for AGP-C receptors in the late steps of elongated spermatids (stage VI–VII). Leydig cells are positively stained (arrow). (e) Adult rat testis at stage VI–VII stained by haematoxylin. Scale bars represent 50 μ m.

Table 2. The appearance and cellular distribution of α_1 -acid glycoprotein glycoform B/C lectin-receptors in rat spermatogonia, spermatocytes and spermatids

Age (weeks)	Spermatogonia	Spermatocytes	Spermatids Steps 1–8	Spermatids Steps 9–19
1	0			
4	0	+	+	
5	0	0	0	+
7	0	0	0	+
12	0	0	0	+

0: no reaction; +: lectin receptor present.

Table 3. The inhibition pattern by monosaccharides and steroid hormones to the binding of α_1 -acid glycoprotein (AGP) glycoforms

	AGP-A	AGP-B	AGP-C
GalNAc (0.1 mol l ⁻¹)	0	0	0
Galactose (0.1 mol l ⁻¹)	0	0	0
Mannose (0.001 mol l ⁻¹)	0	+	+
GlcNAc (0.001 mol l ⁻¹)	0	+	+
Testosterone (50 nmol l ⁻¹)	+	0	0
Oestradiol (50 nmol l ⁻¹)	+	+	+
Progesterone (50 nmol l ⁻¹)	+	+	+
Aldosterone (100 nmol l ⁻¹)	0	+	+
Cortisone (100 nmol l ⁻¹)	0	+	+

+: Inhibition; 0: no inhibition.

GalNAc: N-acetyl-D-galactosamine; GlcNAc: N-acetyl-D-glucosamine.

(Tesarik and Mendoza, 1993). In addition, steroids other than testosterone have been detected in the seminiferous epithelium (reviewed in Bardin *et al.*, 1988). On the basis of the results of the present study, a difference in the cellular content of AGP glycoforms and AGP receptors separates the spermatocytes and early spermatids from the late spermatids. The difference in receptor composition implies a difference in the effect of different steroid hormones. Testosterone, oestradiol and progesterone inhibited the binding of AGP-A to its receptor. Cortisone, aldosterone, oestradiol and progesterone inhibited the binding of AGP-B and AGP-C to their receptors. This localization of the glycoform receptors and the inhibition by testosterone, aldosterone and cortisone in the adult rat testis are identical to the localization of receptors and the inhibition pattern in the mouse testis (Andersen *et al.*, in press, a).

In contrast to other known lectin activities that do not appear in the Leydig cells until 4 weeks of age (Arya and Vanha-Perttula, 1986), the AGP-B/C receptors and AGP were present in the Leydig cells from 1 week of age. A similar Leydig cell content of AGP glycoform receptors is not seen in mouse testis (Andersen *et al.*, in press, a). The AGP-B/C receptors in rat Leydig cells share the same specificities for carbohydrates and hormones as the AGP-B/C receptors in germ cells. Steroids are present endogenously in the Leydig cells from birth (Tapanainen *et al.*, 1984) and the AGP

Fig. 3. Adult rat testis stained with biotinylated human anti- α_1 -acid glycoprotein. The activity is localized in the cytoplasm of Sertoli cells, all spermatocytes and all spermatids. This photomicrograph shows one generation of negatively stained elongated spermatids. Scale bar represents 50 μ m.

glycoforms and the AGP receptors may modulate the actions of testosterone and other steroids in the testis.

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