Changes in Lectin Binding Patterns in Rat Testis and Epididymis in Association with Experimentally-Induced Cryptorchidism

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Abstract. Changes in lectin binding in rat testis and epididymis in association with experimental cryptorchidism, in which adult male Wistar rats were subjected to unilateral cryptorchid surgery, were examined by light and electron microscopy with 9 biotinylated-lectins (ConA, WGA, RCA, SBA, DBA, PNA, UEA-I, GS-I and GS-II). In the control testis, ConA, WGA, RCA and GS-I intensely stained every stage of germ cells. PNA, SBA and GS-II bound to all germ cells except spermatogonia. DBA and UEA-I reactions were identified only in spermatocytes and spermatids. Electron microscopically, reaction products were observed mainly along the plasma membrane and in the Golgi apparatus of each germ cell. In addition, the acrosome and/or acrosomal cap were strongly stained with PNA, SBA, WGA, and RCA. In the epididymis, lectin stainings were positive as follows: ConA, WGA, RCA and UEA-I; in all segments examined; PNA; in segments I and II; SBA, DBA, and GS-II; mainly in segments I, II, and V; GS-I; in segments II, III, and V. Electron microscopically, lectin binding sites were distributed mostly on the apical plasma membrane, microvilli, Golgi apparatus, and granules of the principal cells. Two weeks after the cryptorchid operation, stainings of all the lectins used were decreased in intensity and sometimes negative in the spermatogenic cells of the testis. In the epididymis, lectin staining was decreased primarily in segments I, II, and V. The present results indicate that certain glycoconjugates containing Gal, GalNAc, and GlcNAc residues are expressed in the acrosome. In cryptorchidism, the elevated environmental temperature caused the decrease in lectin stainings in testis, reflecting temperature-sensitive changes in cell-associated glycoconjugates during spermatogenesis. The decrease in lectin staining of segments I, II, and V in the epididymis under cryptorchid condition suggests that these segments may play a role for maturation of spermatozoa and that glycoconjugates in these segments are easily affected by an elevated environmental temperature.

Key words: Lectin, Cryptorchidism, Testis, Epididymis, Histochemistry.

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It has been known that naturally occurring and experimentally-induced cryptorchidism results in degenerative changes in the seminiferous epithelium in many mammalian species including

humans [1–3]. Experimental cryptorchid animals have been used as a model system to investigate the histological and hormonal changes in the process of cryptorchidism [4], although the histochemical changes in seminiferous epithelium has not fully been understood in these animals. This was mainly due to a technical limitation, since only the PAS reaction has been used to describe responses of the epididymal duct to temporal

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cryptorchidism [5]. Recently, lectin histochemical techniques have become applied for the cytochemical demonstration of carbohydrate moieties of glycoconjugates on the cell surface [6–8]. It allows researchers to describe changes in germ cell- and principal cell-associated glycoconjugates [9–13]. The present study describes on the light and electron microscopic changes in the distribution pattern of glycoconjugates in the spermatogenic cell of testis and in the principal cell of epididymis in early experimental cryptorchidism in rats by means of conjugated-lectins.

Materials and Methods

Preparation of cryptorchid rats

Ten adult male Wistar rats weighing 250–350 g were used in this study. The rats were subjected to unilateral cryptorchid surgery. The rats were anesthetized with intraperitoneally injected pentobarbital sodium (Nembutal) (25 mg/kg body weight). Left lateral scrotal compartments including left testis and epididymis were moved into the abdomen via the inguinal canal and then the canal was sealed by silk thread. The right testis and epididymis were used as the control.

Tissue preparation and fixation

Animals were killed and simultaneously fixed at two-weeks interval after the operation by perfusion via the heart for 30 min with 2.5% glutaraldehyde-2% formaldehyde-0.1M phosphate buffer (PB). Then both right and left testes and epididymides were removed, and each epididymis was longitudinally cut into 5 segments (I~V) [14]. All the tissues were divided into 2 parts and used for light and electron microscopy.

Staining with lectins for light microscopy

Samples were further fixed overnight in Bouin's fluid, and then embedded in paraffin. Four-micron-thick sections were made, deparaffinized with xylene, and rehydrated through a graded series of ethanol. The sections were first treated with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS), pH 7.2, and then incubated with biotinylated lectins (25 µg/ml, Vector Lab., CA, USA) in 0.1% BSA-PBS for 30 min at room temperature. Lectins used were concanavalin A (ConA), wheat germ agglutinin

(WGA), *Ricinus communis* agglutinin (RCA), peanut agglutinin (PNA), soybean agglutinin (SBA), *Dolichos biflorus* agglutinin (DBA), *Ulex europeus* agglutinin-I (UEA-I), *Griffonia simplicifolia* agglutinin-I (GS-I), and *Griffonia simplicifolia* agglutinin-II (GS-II). After a rinse with PBS, the sections were incubated with ABC reagent (Vector Lab., CA, USA) for 30 min. The sections were washed again with PBS, immersed in 3,3'-diaminobenzidine (DAB, 0.2 mg/ml PBS)-H₂O₂ (0.005%) for 10 min, rinsed with distilled water, dehydrated, and mounted.

Staining with lectins for electron microscopy

The specimens were further fixed with 2.5% glutaraldehyde-2% formaldehyde-0.1 M PB for 30 min, and then embedded in Lowicryl K4M [15]. Ultrathin sections were made, mounted on nickel grids, treated with 1% BSA-PBS for 10 min, and incubated with biotinylated lectins shown above (25 µg/ml) in 0.1% BSA-PBS for 1 h. The sections were rinsed with PBS, incubated with streptavidin-colloidal gold conjugates (15 nm in diameter, prepared by us [13]) in 0.1% BSA-PBS for 30 min, rinsed again with PBS, post-stained with uranyl acetate and lead citrate, and then observed with a transmission electron microscope (JEM-1200EX) at 80 kV.

Control experiments

To confirm lectin binding specificity, sections were preincubated with appropriate hapten sugars (0.2 M) for each lectin: α -methyl-D-mannoside for ConA; N-acetyl-D-glucosamine for WGA and GS-II; lactose for RCA, PNA, and GS-I; L-fucose for UEA-I; and N-acetyl-D-galactosamine for SBA and DBA. Then the sections were incubated with biotinylated lectins in the presence of the hapten sugars both for light and electron microscopy. Non-specific staining was also checked by the incubation with the ABC reagent and DAB-H₂O₂ solution alone for light microscopy, and with the streptavidin-gold conjugate alone for electron microscopy.

Results

Light microscopic observations

Testis: Specific binding pattern for each lectin in the seminiferous epithelium in both control and

cryptorchid testes at 2 weeks after the operation are summarized in Table 1.

In the control testis, ConA, WGA (Fig. 1a), RCA, and GS-I stained the plasma membrane of spermatogenic cells of all stages. On the other hand, these reactivities in cryptorchid samples were significantly decreased (Fig. 1b).

PNA (Fig. 1c), SBA, and GS-II stained the cell surface of every types of spermatogenic cell except the spermatogonium. The acrosomes of spermatids and spermatozoa were strongly positive for PNA and SBA. In the cryptorchid samples, the spermatogenic cell showed a lower reactivity with all three of these lectins, especially with PNA and GS-II (Fig. 1d).

DBA and UEA-I diffusely stained the plasma membrane and cytoplasm of secondary spermatocytes and spermatids in the control testis. In the cryptorchid testis, these reactivities were also decreased.

Epididymis: Specific binding pattern for each lectin observed in principal cells of both control and cryptorchid epididymides at 2 weeks after the operation are summarized in Table 2.

In the control epididymis, most of the lectins used variably stained the luminal surface, including microvilli, from segment I to segment V. At the Golgi zone, the reactivity of WGA, RCA, UEA-I, and ConA was observed in all segments examined. PNA binding was limited mainly to segment I and segment II (Fig. 2a). SBA stained segments I, II, III, and V; and DBA, mainly segments I, II, and V. The GS-I reaction was positive in segments II, III, and V. GS-II stained segments I, II, IV, and V, and the stainability in segment V was especially obvious.

In the cryptorchid epididymis, the binding of all the lectins used was generally decreased, especially in segments I, II, and V. For example, the reactivity of PNA in segments I and II (Fig. 2b), and of GS-II in segment V, was significantly less.

Electron microscopic observations

Testis: The binding sites of lectins in both control and cryptorchid testes were demonstrated ultrastructurally in accordance with the light microscopic observations. In the control, gold particles representing the WGA binding sites, for example, were demonstrated on the acrosome, Golgi apparatus, and cell membrane of spermatids (Fig. 3a). SBA and PNA (Fig. 3c) binding sites were densely distributed on the acrosome and the trans region of Golgi cisternae. The bindings of PNA and SBA were not seen on the cell membrane of spermatids.

In the cryptorchid testis, the acrosomal area of the spermatid had become flattened and the nuclear membrane revealed a characteristic invagination (Fig. 3b). The binding of lectins was still observed on the reduced acrosomal area and in the Golgi apparatus (Fig. 3b and d).

Epididymis: The binding sites of lectins in both control and cryptorchid epididymides were also demonstrated ultrastructurally in accordance with the light microscopic observations. Gold particles representing PNA binding sites, for example, were densely distributed in secretory granules, Golgi apparatus, as well as on the cell membrane of principal cells of segment II in normal epididymis (Fig. 4a). In contrast, gold particles indicating PNA binding sites were significantly decreased in the cytoplasm of the principal cells in experimentally induced cryptorchidism (Fig. 4b).

Table 1. Lectin bindings in germ cells of normal (N) and cryptorchid (C) testes

	ConA		WGA		RCA		PNA		SBA		DBA		UEA-I		GS-I		GS-II	
	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C
spermatogonia	++	+	++	+	+	-	-	-	-	-	-	-	-	-	++	+	-	-
primary																		
spermatocytes	++	+	++	+	+	+	++	+	+	-	-	-	-	-	+	+	+	-
secondary																		
spermatocytes	++	+	++	+	+	±	++	±	+	-	+	-	+	-	+	-	++	-
spermatids	++	-	++	+	+	±	++	±	+	-	+	-	+	-	+	-	++	-
spermatozoa	++	-	++	-	+	-	++	-	+	-	-	-	-	-	±	-	++	-

Staining intensities are scored as follows: - (negative), ± (faint), + (moderate), ++ (intensely positive).

Fig. 1. Light micrographs showing WGA and PNA binding in rat testis. In each micrograph arrowheads point out the basement membrane of seminiferous tubule. (a) Normal testis. WGA stains the plasma membrane of spermatogenic cells of every stage. $\times 510$. (b) Cryptorchid testis. The degenerative changes of seminiferous epithelium are obvious. The staining of WGA on the plasma membrane of germ cells is decreased. $\times 420$. (c) Normal testis. PNA stains every stages of spermatogenic cell except the spermatogonium (arrow). $\times 830$. (d) Cryptorchid testis. PNA reaction is very faint. $\times 890$.

Table 2. Lectin bindings in principal cells of normal (N) and cryptorchid (C) epididymis

	ConA		WGA		RCA		PNA		SBA		DBA		UEA-I		GS-I		GS-II	
	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C
segment I	+	+	+	+	+	+	+	+	+	+	+	+	±	—	—	+	—	
segment II	+	+	+	+	+	+	+	+	+	±	+	+	±	+	+	+	—	
segment III	+	+	+	+	±	±	—	—	±	±	±	±	+	+	+	—	—	
segment IV	+	+	+	±	±	±	—	—	—	—	±	±	+	+	—	—	±	—
segment V	+	+	+	+	+	+	—	—	+	+	+	+	+	+	—	+	±	

Staining intensities are scored as follows: - (negative), ± (faint), + (moderate), + (intensely positive).

Fig. 2. Light micrographs showing PNA binding in rat epididymis. (a) Normal epididymis. Supranuclear zone (arrowheads) and luminal surface (arrows) of principal cells in segment II are positive. S: sperm in the lumen. $\times 780$. (b) Cryptorchid epididymis. Only the supranuclear zone (arrows) is weakly positive. Lectin-positive material, probably a secreted substance, has accumulated in the lumen (*). $\times 840$.

Cytochemical controls

At both light and electron microscopic levels, the lectin binding was inhibited by the addition of the appropriate hapten sugars. Non-specific binding of avidin conjugates and endogenous peroxidase activity were mostly undetectable.

Discussion

Degenerative changes in the germinal epithelium and the response of the epididymal duct to the disappearance and reappearance of spermatozoa induced by artificial cryptorchidism have been described by many authors [1, 4, 5]. And it is generally assumed that the extent of germinal epithelium disorganization or disruption is time dependent. According to the investigation of Clegg [1], artificial cryptorchidism in the rat resulted in degenerative changes in the seminiferous tubule, which were maximal at about the 15th day after the operation. Jones *et al.* [4] also found that prompt disruption of spermatogenesis became more extensive with time. In accordance with their results, we observed in our preliminary studies that spermatogenic cell degeneration induced by experimental cryptorchidism was especially significant around 2 weeks after the operation. Therefore, in the present study, we noticed the changes at the 2 weeks period after the

cryptorchid operation.

It is generally accepted that the elevation of testicular temperature in cryptorchidism would directly harm the seminiferous epithelium histologically. Some authors [16, 17] reported that the activities of certain enzymes, e.g. aminopeptidase, correlated with spermatogenesis were altered by cryptorchidism, and indirect evidence was presented showing the release of enzymes by thermally sensitive lysosomes in the testis following artificial cryptorchidism [16, 17].

The present study showed that lectin staining in the spermatogenic cells of the cryptorchid testis at 2 weeks after the operation was significantly decreased compared with that of the control. The results indicate the occurrence, in cryptorchidism, by which the environmental temperature of the testis is raised, of not only histological degenerative changes in the seminiferous tubules but also a decrease in lectin stainings reflecting changes in cell-associated glycoconjugates of the seminiferous epithelium. For example, in the intact testis, acrosomes of spermatids and spermatocytes were strongly stained with PNA, SBA, WGA, and RCA. In cryptorchidism, such reactions, which represent the presence of certain glycoconjugates containing Gal, GalNAc, and GlcNAc residues, were strikingly affected.

As for the epididymal duct, the epithelial cells are considered to produce a glycoprotein that binds to spermatozoa and takes part in their

Fig. 3. Electron micrographs of normal (a, c) and cryptorchid (b, d) spermatids showing WGA (a, b) and PNA (c, d) binding in Lowicryl K4M-embedded specimens as revealed by the colloidal gold labeling method. (a) Gold particles are localized in the acrosome (arrowheads), Golgi apparatus (G), and the plasma membrane (arrows). $\times 13,500$. (b) The acrosome has a flattened appearance and the nuclear membrane is often invaginated (arrowheads). WGA binding is similar to that observed in (a). $\times 21,000$. (c) Gold particles are localized in the acrosome (arrowheads) and the trans region of the Golgi apparatus (G). $\times 24,000$. (d) PNA binding is observed along the flattened acrosomal area (arrowheads). $\times 20,000$.

Fig. 4. Electron micrographs of normal (a) and cryptorchid (b) principal cells of the epididymis at segment II. PNA binding is shown in Lowicryl K4M-embedded specimens. (a) Gold particles are densely distributed in the Golgi apparatus (G), secretory granules (arrowheads), and the plasma membrane (arrows). $\times 14,000$. (b) Few gold particles are found in the cytoplasm. G: Golgi apparatus. $\times 26,000$

maturation [18]. Previous studies also indicated that the epididymal duct is histologically and cytologically divided into several segments, each of which seems to be functionally different [19, 20]. Takano [14] morphologically divided the mouse epididymal duct into five segments. Some researchers [5, 21] reported that a PAS (periodic acid-Schiff) -positive material, which was believed to bind to spermatozoa, was secreted in the distal portion of segments I and II. In our study, the results (Table 2) showed that in the cryptorchidism, the binding of all the lectins used was generally decreased in the epididymis, especially in segments I, II, and V. We may speculate that, similar to the case for spermatogenesis, the activities of certain enzymes correlated with glycoprotein production and/or degradation are influenced at the elevated environmental temperature. Alternatively, the glycoprotein status of principal cells in each segment may be influenced by the accumulation of testicular fluid or aspermia that occurs

during cryptorchidism. The decrease in lectin staining, especially in segments I, II, and V, suggests that these segments are possibly important for maturation of spermatozoa and also are easily affected by the elevated environmental temperature in cryptorchidism.

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