Ultrastructural Studies on the Development of the Blood-Epididymis Barrier in Immature Rats

ASHOK AGARWAL AND ANITA P. HOFFER

The development of the blood-epididymis barrier in immature rats (8, 11, 14, 18, and 21 days old) was examined with an electron microscope using lanthanum nitrate as an electron dense tracer. A gradual increase in the development of the blood-epididymis barrier was noted with age. On Day 8, lanthanum was frequently detected in both the intercellular spaces and the lumen. On day 14, no lanthanum penetration into the lumen was observed in 75% of the junctions in the caput, 40.3% in corpus, and 30% in cauda epididymidis. On Day 18, only 7.5%, 9%, and 15%, of the junctions in the caput, corpus, and cauda epididymidis, respectively, remained permeable to lanthanum. No lanthanum was observed in the lumen of any tubules in the 21-day-old rat epididymis. These findings indicate that the postnatal development of the blood-epididymis barrier is gradual, and that its formation is virtually completed by Day 21. As with adult rats, the zonula occludens is the ultimate structural component of the blood-epididymis barrier in immature rats (Agarwal and Hoffer, 1985).

Key words: Blood-epididymis barrier, ultrastructure, immature.


From the Harvard Program in Urology and Brigham Andrology Laboratory, Harvard Medical School and Brigham & Women's Hospital, Boston, Massachusetts

The presence of a blood-tissue barrier restricting movement of certain substances (e.g. protein, amino acids, ions) in some body organs has been known for many years (Waites and Setchell, 1969). The most important examples are the blood-brain (Reese and Karnovsky, 1967), blood-thymus (Raviola and Karnovsky, 1972) and blood-testis (Fawcett et al, 1970; Dym and Fawcett, 1970) barriers. Few ultrastructural studies on the blood-epididymis barrier have been published to date (Howards et al, 1976; Hoffer and Hinton, 1984). In the rat epididymis, peritubular myoid cell layers, elaborate tight junctions (occluding junction of zonula occludens), and desmosomes (zonula adherens) serve as a barrier between luminal fluid and blood capillaries. The epididymal-tight junctions are highly developed among other epithelial cell contacts studied. They form a continuous zonule, or gasket, around the cell, sealing the spaces between the epithelial cells so that the luminal space and the intercellular spaces become separate physiological
compartment (Friend and Gilula, 1972). Zonula occludens at the apicolateral surface of the epididymal epithelium serve as the ultimate structural component of the rat blood-epididymis barrier. The flow of intravascularly perfused lanthanum is not significantly impeded by the vascular endothelium, peritubular myoid layer, or other lateral cell surface specializations (Hoffer and Hinton, 1984).

The present investigation studied for the first time, the development of the blood-epididymis barrier in immature rats to determine the exact morphological location of the structural component of the blood-epididymis barrier in the head, body, and tail of the developing epididymis by using an electron-dense tracer, lanthanum nitrate.

Materials and Methods

Immature Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) 8, 11, 14, 18, and 21 days old were used. The animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, revised 1978). Immature rat testes were fixed by antegrade perfusion through the thoracic aorta using 5\% glutaraldehyde with 0.16 M collidine (pH 7.4) containing 2\% lanthanum nitrate according to previous methods (Hoffer and Hinton, 1984); except that 2.5 g/100 ml of polyvinylpyrrolidone (PVP) were dissolved in the collidine buffer containing lanthanum nitrate by stirring. The pH of the final fixative (5\% glutaraldehyde buffered with 0.16 M collidine containing a final concentration of 2\% lanthanum nitrate and 2.5\% PVP) was 6.9. This solution
was routinely filtered through a Buchner funnel (Whatman No. 1) before use. Following a brief rinse in collidine buffer, tissue blocks from the caput, corpus, and cauda were postosmicated and stained en bloc with uranyl acetate (Terzakis, 1968) to enhance the contrast of membranes and to reveal their trilaminar structure. After en bloc staining, the blocks were dehydrated in increasing concentrations of acetone and embedded in araldite by routine methods. In some cases, the same lanthanum concentration was maintained in all subsequent sections up to and including the osmium tetroxide; in others, lanthanum was omitted from the immersion solutions to confirm that the observed path of tracer was caused exclusively by extravasated lanthanum and not by random diffusion of the tracer from the immersion solutions into the interstitium.

Sections showing silver to pale-gold interference colors were cut with a diamond knife on a Sorval MT 6000 microtome. The sections were stained twice with uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965). A Philips 200 or a Jeol 100s electron microscope was used to examine the sections. The percentage of patent junctions was calculated as follows:

\[
\text{% Patent Junctions} = \frac{\text{tight junction with lanthanum leaks}}{\text{total number of junctions}} \times 100
\]

A total of 1500 junctions from sections of immature rat epididymis were counted under a transmission electron microscope. In each group, 300 junctions were observed. Chi-square tests were used to compare the percentage of patency between different age groups (8, 11, 14, 18, and 21 days) and regions of the epididymis (caput, corpus, and cauda). The null hypothesis was rejected if the chi-square value was \( \geq 6.6349 \) (DF = 1). \( P \) values less than 0.05 were considered to be significantly different.

**Results**

All intercellular contacts between epididymal epithelial cells, capillary endothelial cells, and peritubular myoid cells were considered as possible sites of the blood-epididymis barrier. Each site was analyzed in the caput, corpus, and cauda epididymidis to determine whether any variations in the permeability of the barrier could be detected along the epididymal duct.

**Days 8 and 11**

On Day 8, lanthanum was detected in the intercellular spaces leading to the lumen. The presence of lanthanum in the basal lamina and in the intercellular spaces near the apical and the basal surfaces of the principal cells was observed (Figs. 1 and 2). A paucity of tight junctions, at this time, allowed the lanthanum to penetrate into the tubular lumen. Sixty-three percent of the junctions were patent in the caput, 46% in the corpus, and 32% in the cauda. The most significant decrease in the percentage of patent junctions was in the caput region, where it fell from 65% on Day 8 to 3% on Day 21.

There were no histological differences between the epididymis of 8 and 11-day-old rats. On day 11, 60% of the caput junctions, 36% of the corpus junctions, and 43% of the cauda junctions were patent and allowed the passage of lanthanum particles in the lumen (Fig. 3). Except in the corpus, there was no significant difference in the percentage of patency between 8 and 11-day-old rats.

**Day 14**

In the epididymis of 14-day-old rats, light microscopic observations of sections from the three regions revealed an increase in tubular diameter, as well as an increase in epithelial cell height. Mitotic division was observed in some of the epithelial cell
nuclei. The density of cells and fibers in the intertubular connective tissue had increased, and tubules were more compactly arranged than in 8- and 11-day-old rats.

In 14-day-old rats, a significant decrease in the number of patent junctions was observed in the caput; the corpus and cauda did not reveal any change. The lanthanum particles were prevented from reaching the lumen by multiple points of fusion between the outer leaflets of opposing cell membranes (Figs. 4 and 5).

Halo cells were first observed at this time. A few round mitochondria, vesicles, and ribosomes were present in the cytoplasm. Cytoplasmic processes extended between the adjacent epithelial cells, but no junctional complexes were seen between halo cells and columnar cells.

Day 18 and 21

In the principal cells, the chromatin appeared diffused as euchromatin in the nucleus. Endoplasmic reticulum was smooth in the apical cytoplasm, but granular in the basal region of the cell. Lysosomes appeared as dense membrane bound sacs. The most significant change during the third postnatal week was the appearance of narrow cells in all the three segments of the epididymis. Basal cells were not observed. The narrow cells had various features that set them apart from the rest of the epididymal epithelium. These cells were clearly identifiable in light microscopic preparations by their shape and intense toluidine blue staining. Other components of the narrow cells included endoplasmic reticulum consisting of short elements, multi-vesicular bodies, dense lysosomes, and a small golgi apparatus in the

Figs. 4 and 5. In the caput epididymidis, a significant decrease in the number of patent junctions is first observed at 14 days after birth. These electron micrographs of the epididymal epithelium at Day 14 show a tight junction (Fig. 4) and a patent junction (Fig. 5) in the caput and corpus respectively. Fig. 4: In the junction between the caput epithelial cells, the lanthanum is prevented from reaching the lumen by multiple points of fusion (arrows) between the outer leaflets of opposing cell membranes. Fig. 5: The junction between corpus principal cells is still patent and lanthanum can be observed as it extends all the way to the lumen.
Fig. 6. Electron micrograph of the peritubular myoid layer and basal lamina of the caput epididymidis of the 21-day-old rat. Extravasated particles of tracer are observed adjacent to the basal lamina of the epididymal epithelium and appear to encounter little resistance along the basal and mid portions of the lateral cell surfaces.

Fig. 7. Electron micrograph of the corpus epididymidis of 21-day-old rat intravascularly perfused with lanthanum. The passage of tracer particles in the intercellular spaces is obstructed by the zonula occludentes; 97% of the occluding junctions are impermeable to lanthanum at this time.

supranuclear cytoplasm. The nucleus was long, and the chromatin appeared dense compared to that of surrounding cells.

There was a significant decrease in the percentage of patent junctions between 8- and 18-day-old rats, as well as between 8- and 21-day-old rats. In the caput and corpus of 18-day-old rats, about 92% of the junctions became impermeable to lanthanum passage (Figs. 6 and 7). In 21-day-old rats, 97% of the junctions in the caput and corpus, and 93% in the cauda became impermeable to lanthanum passage. In the caput, corpus, and cauda of 21-day-old rats, the percentage of patency in tight junctions did not differ. There was, however, a significant difference in the percentage of patency between the caput, corpus, and cauda of 18 and 21-day-old rats. The development of the blood-epididymis barrier was virtually completed by Day 21 (Fig. 8).

Extravasated particles of tracers were observed adjacent to the basal lamina of the epididymal epithelium. The particles appeared to encounter little resistance along the basal and mid portions of the lateral cell surfaces.

Discussion

The junctional complex is the most elaborate contact zone between mammalian epithelial cells. It includes the zonula occludens (tight junction), zonula adhaerens (intermediate junction), and macula adhaerens (desmosomes). The junctional complex, in particular, zonula occludens, is found in epithelia, which restrict interchange of large molecules between extracellular and intercellular space (Friend and Gilula, 1972; Howards et al, 1976; Turner et al, 1980). The zonula occludens of the epididymis is one of the best developed tight junctions anywhere.
in mammalian tissues (Friend and Gilula, 1972). The ultimate site of the blood-epididymis barrier differs from the blood-tissue barriers of other organs, such as the thymus (Raviola and Karnovsky, 1972), the brain (Reese and Karnovsky, 1967), and the testis (Fawcett et al, 1970; Dym and Fawcett, 1970). In the epididymis, the zonula occludens near the luminal surface of the epididymal epithelium are exclusively responsible for the maintenance of the blood-epididymis barrier, since neither the capillary endothelium nor the peritubular myoid layer significantly impedes the flow of tracers towards the epididymal lumen (Hoffer and Hinton, 1984). Although ultrastructural studies of the blood-epididymis barrier in adult rats have been reported earlier from this laboratory (Hoffer and Hinton, 1984), the development of the blood-epididymis barrier in the mammalian epididymis is described here for the first time.

The results of the present study indicate that the development of the blood-epididymis barrier is virtually complete by the end of the third post-natal week. Even though factors controlling the overall completion of the blood-epididymis barrier by this time are not completely understood, it is noteworthy that the blood-epididymis barrier is completed at approximately the same time as the blood-testis barrier. Vitale et al (1973) have demonstrated that in the rat testes, the blood-testis barrier is established between post-natal Days 16 and 19 in close temporal correlation with the appearance of junctional complexes between Sertoli cells, the onset of fluid secretion by the seminiferous epithelium, the stratification of germinal epithelium, and the development of a lumen in the seminiferous tubule. Using acridine dyes, Kormano (1967) found that the permeability barrier in testes develops gradually during the first 20 post-natal days. During the third post-natal week there is a steep rise in serum gonadotrophins and testosterone (Miyachi et al, 1973; Swerdloff et al, 1971), epididymal secretion of glycerylphosphorylcholine, sialic acid and alkaline phosphatase (Goyal and Dhingra, 1974; Setty and Jehan, 1977), and ABP in the principal cells of the caput epididymidis (Hansson et al, 1974; White et al, 1982). These changes reflect the early activation of pituitary and Leydig cells, which may play a role in initiating sexual maturation and increased impermeability of most junctions throughout the epididymis by Day 21. Of interest is the observation that many of the intercellular junctions in the corpus and cauda are already tight by post-natal Day 8.

In the rat brain, the blood-brain barrier to protein develops early in fetal life and is well-established by birth (Olsson et al, 1968). The exact time when most (~2/3) intercellular junctions in the cauda epididymidis first became impermeable to lanthanum is not known, since the development of the blood-epididymis barrier in animals less than 8-days-old has not been examined to date.

Another intriguing observation is that differences in barrier development rate between epididymal zones can be noted as early as the first 2 post-natal weeks. In fact, by post-natal Day 8, the proportion of intercellular junctions that are permeable to lanthanum in the caput, corpus, and cauda varies from approximately 2/3 to 1/2 to 1/3, respectively. By week 2, there is a disproportionately large drop in the number of patent junctions in the caput, compared to the more distal regions of the epididymal duct. Declines of comparable magnitude do not take place until Day 18 in the corpus and cauda. Early maturation of the blood-epididymis barrier in the caput is consistent with the observation of Sun and Flickinger (1979) that histological maturation occurs earlier in the proximal regions of the epididymis than in the distal ones. The reasons for the initial differences in maturity and the subsequent differential rate of blood-epididymis barrier development, however, are not clear. In the adult rat, maintenance of the initial segment requires more intraluminal testosterone
than the cauda (Fawcett and Hoffer, 1979), but whether this is relevant to the neonatal epididymis is not known. The greater immaturity of the caput blood-epididymis barrier on Day 8, relative to the distal genital duct, is consistent with this possibility. It is also noteworthy that during post-natal days 0–14, there is a steady decline of all endogenous steroids except testosterone and androstaneol, whereas, during days 10–15, the testicular level of androstaneol increases significantly until it emerges as the predominant endogenous androgen of testes in rats 15 days and older (Tapanainen et al, 1984). It is tempting to speculate that blood-epididymis barrier development in the prepubertal epididymis, especially the caput, has a specific need for androstaneol or some other unknown factor(s), and that it cannot mature until this factor(s) is available around the second post-natal week. An alternate hypothesis is that, since the lumen of the seminiferous tubules is formed more or less concurrently with the establishment of the blood-epididymis barrier, the intraluminal presence of testicular secretory products in the epididymis may be required for completion of blood-epididymis barrier development.

References

Fawcett DW, Hoffer, AP. Failure of exogenous androgen to prevent regression of the initial segments of the rat epididymis after efferent duct ligation or orchectomy. Biol Reprod 1979;20:162-181.

Notice to Copiers

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by J. B. Lippincott Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of $0.80 per copy, plus $0.05 per page is paid directly to CCC, 21 Congress St., Salem, MA 01970. 0196-3635/89 $0.80 + $0.05.