Permeability characteristics of the epithelium in the rat caput epididymidis

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Summary. Micropuncture techniques were used to study the in-vivo transfer of radioactive compounds of different molecular weights across the epithelium of the rat caput epididymidis. Small molecular weight compounds such as $^3\text{H}_2\text{O}$ and ^{14}C urea equilibrated in less than 20 min and 2 h respectively after intravenous injection. Compounds of larger molecular weight (^3H polyethylene glycol, ^3H inulin and ^{125}I -labelled bovine serum albumin) entered the lumen more slowly and reached less than 5% of blood plasma concentrations 3 h after injection. The luminal concentration of ^3H inulin remained below 7% of the blood level for 18 h. These results demonstrate that a permeability barrier exists in the epithelium of the rat caput epididymidis.

Introduction

Developing spermatozoa in the seminiferous tubules are protected by a specialized barrier. In several species, the anatomical (Dym & Fawcett, 1970; Fawcett, Leak & Heidger, 1970) and physiological (see Setchell & Waites, 1975; Setchell, 1980) roles of the blood–testis barrier are well documented. However, very few data are available for the blood–epididymis barrier. Howards, Jessee & Johnson (1976) examined the transfer of substances of different molecular weight across the epithelium of the hamster cauda epididymidis and found that radioactive small molecular weight compounds equilibrated with epididymal luminal fluid within a short time; larger molecular weight compounds entered the lumen more slowly. Cooper & Waites (1979) using an in-vivo luminal perfusion technique demonstrated that the epithelium of the rat cauda epididymidis was also relatively impermeable to high molecular weight compounds. Neither of these studies included observations on the caput region of the epididymis. Since the epithelial morphology changes along the length of the rat epididymis (Reid & Cleland, 1957), it cannot be assumed that the permeability properties of the cauda epithelium are similar to those of the rest of the epididymal epithelium. Similarly, it cannot be assumed that the permeability properties of the testicular and epididymal epithelia are identical for each species. This latter point has been clearly demonstrated by Howards *et al.* (1976) who showed that hamster seminiferous tubules were not freely permeable to ^{14}C urea; which is in direct contrast to results of studies on rams and rats (see Setchell & Waites, 1975). This study was therefore undertaken to determine whether the permeability properties of the epithelium of the rat caput epididymidis are similar to those of the cauda epididymidis. Study of the rat caput epididymidis is important because several organic compounds are secreted into luminal fluid in this area (Hinton, Snoswell & Setchell, 1979b; Hinton & Setchell, 1980b; see Hinton, 1980).

Materials and Methods

Rats

Adult male Sprague–Dawley rats (372–547 g; Hilltop, Philadelphia, U.S.A.) were housed in the University Vivarium under a 12 h light/12 h dark cycle (lights on 06:00 h) and had free access to food and water.

Isotopes

The following isotopes were purchased from New England Nuclear (Boston, Massachusetts, U.S.A.) or Amersham (Arlington Heights, Illinois, U.S.A.): $^3\text{H}_2\text{O}$ (sp. act. 1 mCi/g); [^{14}C]urea (sp. act. 59 mCi/mmol); methoxy [^3H]inulin (sp. act. 488.4 mCi/g); [$1,2\text{-}^3\text{H}$]polyethylene glycol (sp. act. 2 mCi/g); and ^{125}I -labelled bovine serum albumin (sp. act. 0.6 mCi/g.).

Surgery and micropuncture

Rats were anaesthetized with an intraperitoneal injection of urethane (ethyl carbamate, 1.2 mg/g), and cannulae (PE 50) were inserted into the jugular vein and the carotid artery; the renal blood vessels were ligated close to each kidney with a 4/0 suture to prevent the excretion of radioactive material. A testis and epididymis were exposed through a scrotal incision and prepared for micropuncture as previously described (Hinton, Dott & Setchell, 1979a). Samples of luminal contents were collected from the middle of the caput epididymidis (Site 2, see Hinton *et al.*, 1979a) at 34–35°C (Brooks, 1973). The luminal contents were centrifuged and aliquots of the supernatant were transferred into Bio-vials (Beckman, Somerset, New Jersey, U.S.A.) containing 3 ml scintillation fluid (“Baker Analysed” Reagent; LSC Cocktail 1:2 Triton X-100/toluene-based: J. T. Baker, Phillipsburg, New Jersey, U.S.A.). Blood plasma was treated in a similar manner. For experiments that involved ^{125}I -labelled bovine serum albumin, aliquots of supernatant were transferred into plastic test-tubes (12 × 75 mm; Fisher Scientific Co., Pittsburg, Pennsylvania, U.S.A.) containing 1 ml distilled water and counted for radioactivity (Beckman Biogamma 11).

Injection and collection procedure

Each isotope (see above) was prepared as 1 mCi radioactivity in 1 ml saline (9 g NaCl/l) and injected into the jugular vein of each animal over a period of 2–3 min. Blood samples were collected from the carotid cannula at various times thereafter and epididymal luminal contents were collected by micropuncture over a period of 10–20 min. The blood sample was taken at the mid-point of luminal collection.

In one series of experiments, 3 rats were anaesthetized with pentobarbitone sodium (Nembutal; 50 mg/kg), the renal blood vessels were ligated and 1 mCi [^3H]inulin was injected (in 1 ml) into the jugular vein through a 30-gauge needle. The incisions were sutured and the animals allowed to recover. Micropuncture and blood plasma samples were collected 18 h later as described above.

Calculations

For each collection time, the results were expressed as c.p.m. in luminal fluid/c.p.m. in blood plasma × 100 = % plasma isotope appearing in luminal fluid (see Howards *et al.*, 1976).

Results

Table 1 shows the results of the transfer of $^3\text{H}_2\text{O}$ and ^{14}C urea from blood plasma to the epididymal lumen. The compounds reached equilibration within 20 min ($^3\text{H}_2\text{O}$) and 2 h (^{14}C urea).

Table 1. The amounts (as % of the plasma isotope concentrations) of different substances appearing in the luminal fluid of the caput epididymidis of the rat at various times after intravenous injection

Time after injection (min)	$^3\text{H}_2\text{O}$	^{14}C Urea	^3H Inulin	^3H Polyethylene glycol	^{125}I -labelled BSA
10	82.1 $\pm$ 8.6 (4)	—	—	—	—
20	96.3 $\pm$ 3.7 (7)	71.3 $\pm$ 4.2 (5)	1.5 $\pm$ 0.5 (8)	—	—
40	100.8 $\pm$ 5.4 (7)	86.9 $\pm$ 1.8 (5)	2.1 $\pm$ 0.3 (8)	—	—
60	106.2 $\pm$ 3.6 (7)	88.3 $\pm$ 3.3 (5)	2.9 $\pm$ 0.4 (9)	1.0 $\pm$ 0.2 (5)	1.1 $\pm$ 0.3 (4)
90	105.4 $\pm$ 1.9 (7)	—	3.2 $\pm$ 0.7 (6)	—	—
120	103.6 $\pm$ 1.4 (7)	97.2 $\pm$ 2.2 (5)	2.6 $\pm$ 0.3 (5)	1.4 $\pm$ 0.2 (5)	2.3 $\pm$ 0.2 (4)
180	—	—	4.3 $\pm$ 0.8 (4)	3.8 $\pm$ 0.5 (5)	4.0 $\pm$ 0.6 (4)
1080	—	—	6.8 $\pm$ 0.4 (3)	—	—

Values are mean $\pm$ s.e.m. for the no. of animals in parentheses.

The compounds ^3H polyethylene glycol, ^3H inulin and ^{125}I -labelled bovine serum albumin in the lumen reached less than 5% of blood plasma over the 3-h period; ^3H inulin transfer was <7% even at 18 h after injection.

Discussion

This study has shown that a permeability barrier exists in the rat caput epididymidis. In the present study, $^3\text{H}_2\text{O}$ equilibrated more quickly between blood plasma and rat caput luminal fluid than it did between the blood and cauda fluid of the hamster as reported by Howards *et al.* (1976). This could be due to differences in blood flow between the caput and cauda regions (see Setchell, Waites & Till, 1964), species differences or subtle differences in the techniques. Whatever the cause, this study and earlier studies by Howards *et al.* (1976) and Cooper & Waites (1979) demonstrate that the epididymal epithelium is freely permeable to compounds of low molecular weight. The larger molecular weight compounds used in this study, ^3H polyethylene glycol (mol. wt 4000), ^3H inulin (mol. wt 5500–6000) and ^{125}I -labelled bovine serum albumin (assumed mol. wt 60 000) did not penetrate the caput epithelium readily. Each of these compounds in the lumen reached less than 5% of blood plasma by 3 h after injection and even after 18 h, ^3H inulin remained less than 7%.

The permeability barrier is probably situated at the tight junctions between the epithelial cells of the epididymis (Suzuki & Nagano, 1978a, b) and the compounds used in this study probably transfer across this epithelium by simple diffusion. The tight junctions in the caput epididymidis of the rat and mouse are numerous and well developed (Suzuki & Nagano, 1978a, b) but the degree of impermeability of the epithelium is not necessarily correlated with the number of tight junctions (Martinez-Palomo & Erlig, 1975). We assume that in this tissue, as in the testes (see Setchell, 1978), the epithelia of the blood vessels do not significantly interfere in the transfer of the compounds across the epithelium of the caput epididymidis.

It is possible that the radioactive compounds may have entered the lumen of the caput epididymidis via the efferent ducts or rete testis. The former site must be considered since there is morphological evidence to suggest that this epithelium is quite permeable (Suzuki & Nagano, 1978a). However, in separate experiments involving ligation of the efferent ducts close to the

testis or close to the epididymis, the entry of radioactive inulin into the caput fluid was not significantly different from that of the normal intact animal (B. T. Hinton & S. S. Howards, unpublished observations). Also, since the transit time of spermatozoa, and presumably fluid from rete testis, to caput and cauda epididymidis is of the order of days (see Hamilton, 1972) and the experiments performed in this study were of the order of minutes, it would seem unlikely that the radioactivity detected in the lumen of the caput epididymidis would originate from the rete testis or efferent ducts. The rete testis of the rat has been shown to be relatively impermeable to large molecular weight compounds (see Setchell & Waites, 1975).

Because of the restricted penetration of many substances across the epididymal epithelium, this tissue must therefore utilize various carrier mechanisms to transport required substances across its epithelia to provide a favourable microenvironment for sperm maturation. These substances may also be required for normal epididymal function. Several carrier mechanisms seem to exist on the membranes of the epididymal cells. The active transport of carnitine in the rat caput and corpus epididymidis is well documented (Brooks, Hamilton & Mallek, 1973; Johansen & Bøhmer, 1979; Hinton & Setchell, 1980a) and the entry of glucose into the epididymis has been shown to be via a facilitated diffusion mechanism (Brooks, 1979; Cooper & Waites, 1979). The epididymis can also control the movement of inorganic ions across its epithelium (Levine & Marsh, 1971; Wong, Au & Ngai, 1978).

Further understanding of the basic properties of the epididymal epithelium will provide information on the maintenance of the specialized microenvironment which seems to be important for sperm maturation.

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