In-vitro spontaneous electrical activity of rat efferent ductules

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Summary. In rats, the branching pattern of uncoiled ductuli efferentes varied; mostly 5–8 ductuli joined in pairs and formed a single terminal ductule which joined the ductus epididymidis inside the epididymal capsule. Ciliary beat and contractions moved the luminal contents in both directions. Myoelectrical activity of the smooth muscle layer consisted of slow waves of about 4 sec duration and their frequency declined distally.

Introduction

The ductuli efferentes transport spermatozoa from the rete testis to the epididymis. In the rat, they are lined with a simple columnar epithelium consisting of ciliated and absorptive cells (Hoffer, 1972). A thin layer of smooth muscle surrounds each ductule. Although the histological structure of the efferent ductules has been described for many species (see Ramos & Dym, 1977), there is disagreement concerning their branching pattern in the rat (Macmillan & Harrison, 1955; Reid & Cleland, 1957; Hamilton, 1975).

Little is known about the transport of spermatozoa through the ductuli efferentes. The presence of a ciliated epithelium suggests that cilia may play a role. Contractions have been observed (Macmillan & Harrison, 1955; Risley, 1958) and medium injected into the ductuli is transported even when the fluid flow from the testis is prevented by a ligature (Macmillan & Harrison, 1955).

In this study the myoelectrical activity of the smooth muscle in the ductuli efferentes of the rat was recorded. The anatomy of the uncoiled ductules and the movement of the luminal contents were also observed.

Materials and Methods

The Sprague–Dawley rats used were housed in groups of 3–6 males and were 3–6 months old when killed by cervical dislocation. The epididymis was removed with the testis and immersed in oxygenated (95% O₂ + 5% CO₂) Ringer solution (9 g NaCl, 0.42 g KCl, 0.6 g NaHCO₃, 0.24 g CaCl₂, 0.025 g MgCl₂ and 0.5 g glucose in 1000 ml) at about 20°C. Starting from the constricted region near the epididymis, the isthmus by Hamilton (1975), and using watchmakers forceps, the ductuli were trimmed free of fat and uncoiled, together with a short portion of the initial segment of the epididymis. The dissection took about 1 h but complete straightening was not attempted because this can result in damage to the duct wall. The measurements of length are therefore underestimations.
Small suction electrodes (Talo & Hodgson, 1978) were used to record electrical activity in a tissue bath at 36.5 ± 0.5°C. This temperature was used to obtain data comparable to those by Talo, Markkula-Viitanen & Jaakkola (1979) in the epididymal duct. The Ringer solution was slowly changed and continuously oxygenated. The inner diameter of the electrodes was 100–150 μm and inside them was a 50 μm thick chloridized silver wire. One common reference electrode was placed into the bath. Recordings were made on a 6-channel Grass 7P Polygraph. Most of the recordings were made using high-pass filter positions of 0.15 or 0.08 Hz, at which frequencies the signals are attenuated by 50%. Some d.c. recordings were also made.

Movements of the luminal cells were observed under a dissection microscope or a Wild M 20 microscope with phase optics.

**Results**

Considerable variation of the branching pattern of the ductuli efferentes was observed. In 7 out of 10 dissected ducts the branching pattern resembled that shown in Text-fig. 1(a) and in the other 3 that shown in Text-fig. 1(b). In two of the latter group loops located at the bulbous area were long and formed a separate extension covered by a connective tissue sheath. These extensions were loops and not diverticula. In all cases the ductuli merged to form a single terminal duct which joined the epididymis. This transition took place inside the epididymal capsule and the portion of the terminal efferent ductule inside the epididymis was several millimetres in length. The ductuli were filled by a clear fluid and were transparent. A few scattered spermatozoa and round cells or groups of round cells were visible in all parts of the ductules, although a clump of densely packed spermatozoa was found in the terminal ductule or in the preterminal branches but never near the rete testis. Contractions moved the clump back and forth but its net movement was negligible. At the junction between the terminal ductule and the ductus epididymidis there was a sudden increase in sperm density which made the duct opaque. Contraction were seen to spread from the terminal ductule towards the ductus epididymidis. The single spermatozoa and round cells were moved by cilia. The ciliary beat was not exclusively in an epididymal direction because when the movement was in that direction on one side of the lumen it was abe epididymal on the opposite side of the lumen. That the contractions were effective in moving luminal contents was shown by movement of fluid several millimetres distant from the point of contraction. This suggests that the pressure inside the ductuli keeps the walls firm.

**Text-fig. 1.** Schematic drawing of the branching patterns of the ductuli efferentes of the rat. Loops like those shown in (b) could be long. The point where the ductuli efferentes and the ductus epididymidis join is arrowed.
Electrical activity of the ductuli efferentes could not be recorded near the rete testis even when contractions were visible, probably due to the thinness of the walls in this region. In other parts the activity consisted of slow waves of somewhat variable shape and lasting 3–4 sec. In no case were spikes recorded on the top of the slow waves. Text-figure 2 illustrates activity recorded from different parts of the ductuli and at the beginning of the ductus epididymidis. Recordings of myoelectrical activity with two closely spaced electrodes in the terminal ductule (Text-fig. 3) commonly showed a gradual phase shift with time. Waxing and waning of the slow wave

**Text-fig. 2.** Recording of the myoelectrical activity of the ductuli efferentes at the sites indicated. Amplitude calibration marks represent 100 µV except for 6 which is 50 µV. Time signals are at 1 sec intervals.

**Text-fig. 3.** Recording with two electrodes 1·3 mm apart showing a wave form different from that in Text-fig. 2 and a gradual phase shift between waves at this short distance.

**Text-fig. 4.** Waxing and waning of wave amplitude. The period of waxing and waning is different in electrodes 3 and 4. The insert indicates the point of recording. Calibrations 100 µV. 10 sec.
amplitude was also a common feature (Text-fig. 4). The mean frequency per minute declined slightly towards the epididymis. It was 13.8 ± 1.17 (mean ± s.d., n = 6) where the ductules join in pairs, 12.8 ± 1.65 at the preterminal ductules, 11.5 ± 1.08 at the distal end of the terminal ductule and 10.1 ± 1.60 at the beginning of the ductus epididymidis.

Discussion

The ductuli efferentes of the rat join to form a single terminal ductule which continues as a ductus epididymidis as described by Reid & Cleland (1957) and Macmillan & Harrison (1955); in the rats of this study they did not join individually to the ductus epididymidis as suggested by Hamilton (1975) and their number was 5–8 and not 2 as reported by Cooper & Jackson (1972).

Spontaneous electrical activity of the ductuli efferentes consists of slow waves which wax and wane in amplitude like those in the small intestine (Prosser & Bortoff, 1968). The waxing and waning suggests that the recorded activity consisted of the sum of two loosely coupled oscillators with slightly different frequencies.

The presence and minimal net movement of the clump of spermatozoa at the preterminal or terminal ductules is surprising because an increase of speed of flow distally would be expected on a hydrodynamic basis. When 5–8 ductules converge to join a single ductule the cross-sectional area decreases proportionally and the speed of flow should be 5–8 times higher. Effective fluid reabsorption by the ductuli efferentes (Holbrugger, 1980) may compensate the decrease in cross-sectional area and balance the speed of flow.

The sharp increase in sperm density at the beginning of the ductus epididymidis suggests that the speed of transport of spermatozoa is much higher in the ductuli efferentes than in the initial segment of the ductus epididymidis since it is unlikely that the fluid uptake alone could explain such a dramatic increase taking place in a segment about 1 mm long. Further studies are needed to determine regional variation of transport speed of fluid and spermatozoa in this region of the efferent ducts of the male.

References


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