Intraluminal pressures in the seminiferous tubules and in different regions of the epididymis in the rat

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Summary. Intraluminal pressures in the seminiferous tubules and in various regions along the epididymis of the rat were measured by micropuncture using a servo-nulling pressure transducer system. The intraluminal hydrostatic pressure in the testis was significantly lower than that in all regions of the epididymis. There was an increasing pressure gradient from the caput to the cauda epididymidis. Spontaneous contractions were observed in all parts of the epididymis but not in the seminiferous tubules. The amplitude of contraction in the epididymis increased from the caput to the proximal cauda. However, an appreciable decrease in the amplitude occurred in the distal cauda, although the frequency of contraction declined from the proximal caput to the distal cauda with substantial reductions in the mid-caput and the proximal cauda.

Introduction

At spermiation, spermatozoa are released from the Sertoli cells and transported through the seminiferous tubules to the rete testis. This process may be assisted by contraction of myoid elements around the seminiferous tubules and by contraction of the testicular capsule (Hargrove, MacIndoe & Ellis, 1977). Sperm transport in the epididymis is also believed to be aided mainly by contraction of the epididymal duct since spermatozoa are immotile in their native fluid and spontaneous contraction of the epididymis has been demonstrated *in vivo* and *in vitro* (Bedford, 1975). Studies of tubular contraction *in vivo* include visual observations (Risley & Turbyfill, 1957; Macmillan & Auckland, 1960) and measurements of intraluminal pressure via an open-ended catheter located at the epididymal–vas deferens junctions (Melin, 1970; Knight, 1974; Hib, 1976; Hib & Ponzio, 1977; Hib, Ponzio & Vilar, 1982). Although the latter method yields quantitative data regarding the contractility of the duct it is limited to the distal portion of the epididymis. In addition, the hydrostatic pressure registered by this method may not represent the actual intraluminal pressure at rest since the open end of the epididymis is connected to the closed system of the pressure transducer. A micropuncture technique has been described to measure intratubular pressure in the testis and the epididymis of hamsters (Johnson & Howards, 1975) and guinea-pigs (Johnson & Howards, 1976). However, only static pressure was recorded in these studies. Changes in intraluminal pressure during contractions of the seminiferous tubules and the epididymis have not been demonstrated.

The present investigation was undertaken to determine intraluminal hydrostatic pressure and contractility in different regions of the male reproductive tract in the rat under free-flow conditions by micropuncture and by using a servo-nulling measuring system, which has been successfully used

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to measure intracapillary pressures of frog mesentery (Wiederhielm, Woodbury, Kirk & Rushmer, 1964) and skeletal muscles (Richardson & Zweifach, 1970), and intraluminal pressure of the renal tubule (Falchuk & Berliner, 1971).

Materials and Methods

Animals. Sexually mature male Fischer rats weighing 250–330 g were housed in groups of 6–8, separate from females, with 14 h light/24 h and with free access to food and water. They were fasted overnight and were anaesthetized with an intraperitoneal injection of sodium 5-ethyl-5-(1-methylpropyl)-2-thiobarbiturate (Inactin: Byk Gulden Konstanz, West Germany) at a dose of 100 mg/kg body weight and then were placed on a warm operating board. Tracheostomy was performed and a short (2.5 cm) polyethylene cannula (PE 240, Clay-Adams) was inserted and fixed in place.

Micropuncture. The testis and the epididymis on one side were exposed through a scrotal incision and placed in a Perspex cup holder. The tissue was secured in position with 2% agar in saline. Small slits (1–2 mm) were made through the tunica albuginea (about 4 mm from the rete testis) and the epididymal capsule at different regions to reveal seminiferous tubules and the epididymal duct, respectively. Dehydration was prevented by allowing a 0.9% (w/v) NaCl solution kept at 32°C to flow slowly over the surface of the tissue.

Micropipettes were made from thin-walled glass capillary tubing (0.7–1.0 mm o.d., 0.1–0.2 mm wall thickness: Kimble, Illinois, U.S.A.) by using a horizontal microelectrode puller (Industrial Science Associates, Ridgewood, U.S.A.). The tapered end was cut and sharpened to a tip diameter of 15–20 μm with an air-driven grinding stone (Vurek, Bennett, Jamison & Troy, 1967).

Measurement of intraluminal pressure. Intraluminal pressures in the seminiferous tubules and along the length of the ductus epididymidis were measured by a servo-nulling pressure transducer system (Model 3, Instrumentation for Physiology and Medicine Inc., San Diego, U.S.A.). This device, which has been described by Wiederhielm *et al.* (1964), comprises a micropipette filled with a high conductivity salt solution (0.5–2 M-NaCl) as an arm of a Wheatstone bridge. An interface is formed between the salt solution at the tip of the pipette and isotonic saline by immersion of the pipette tip into the layer of saline covering the tissue surface. The effective pipette resistance is a function of the length of the column of isotonic saline contained from the tip to the interface. The pipette is ready for use when the resistance of the pipette balances the bridge and a stable interface is formed. Entry of the pipette into a tubule causes movement of the interface further into the pipette as a consequence of pressure in the tubule. This results in an increase in the resistance of the pipette which is detected by a sensing device as an imbalance of the bridge. The output signal from the sensing device serves as a feedback message to a motor-driven hydraulic pump which, in turn, drives fluid out of the pipette until the pipette resistance once again balances the bridge. The pressure generated by the pump is simultaneously monitored by a pressure transducer (P23Db, Statham, Puerto Rico) and is equivalent to the hydrostatic pressure inside the tubule. The output from the pressure transducer was continuously recorded on a Grass polygraph (Model 79D, Grass Instruments, Quincy, U.S.A.). Since the response of the system to increments in pressure is linear and very rapid, both hydrostatic and dynamic changes in pressure can be measured. The frequency response is linear from 0 to 30 Hz.

In this study the micropipettes were filled with 2 M-choline chloride solution (containing 3% lissamine green) to avoid induction of sperm motility in the epididymal tubule which leads to invasion by and clogging of spermatozoa inside the pipettes. The system was calibrated daily before the experiment using an air-tight Perspex chamber containing 0.9% (w/v) NaCl and a water manometer. The calibration curve of the system, which is independent of the pipette tip diameter in the range of 15–20 μm , is shown in Text-fig. 1. The sensitivity of the system, was not changed

Text-fig. 1. A calibration curve of a servo-nulling pressure transducer using micropipettes with an external tip diameter of 15–20 μm . Each value is the mean of triplicate measurements in a calibrating chamber containing physiological saline ($\circ$), artificial caput fluid (Δ) or artificial cauda fluid ($\square$).

when it was calibrated in artificial solutions similar to those from the caput or the cauda epididymidis with respect to sodium, potassium and sperm concentrations (caput fluid, 112 mM-Na, 16 mM-K, 24% spermatocrit; cauda fluid, 20 mM-Na, 55 mM-K, 40% spermatocrit).

The micropipette was inserted using a micromanipulator (Leitz, Wetzlar, West Germany) into different sites of the tubule in a random sequence. A free-flow column of dyed solution in the tubule could be seen when the tip of the pipette entered the lumen and a slight pressure was applied. Immediately after insertion of the micropipette a sudden and transient increase in pressure was recorded. The pressure recording then declined to a steady level (basal pressure). Superimposed on the basal pressure were monophasic pressure waves with a more or less regular pattern and frequency resulting from tubular contraction (Text-fig. 2). The recording could mostly be maintained and followed for more than 15–20 min. Withdrawal of the pipette caused a sudden drop in pressure to the original baseline. Occasionally, the micropipette was clogged during insertion which was evident by a drift in the recording during measurement or a change in baseline after withdrawal of the pipette. A drift in basal pressure was also seen when the tip of the pipette touched the luminal wall. Only pressure recordings showing no evidence of drifts in basal pressure and baseline were included in the present study. Since intraluminal pressures in 3 adjacent tubules in each area of the epididymis were not different in 10 rats, only one measurement was made at each site in the later experiments. Preliminary measurements of intraluminal pressure in the testis and the epididymis of hamsters confirmed those previously reported by Johnson & Howards (1975).

Each value for measurements taken in this study, i.e. basal pressure, amplitude and frequency of contraction, was an average of the pressure recording over at least 10 min. Data are presented as mean $\pm$ s.e.m. and comparisons between different regions along the tubule were made by using analysis of variance and Student's *t* test.

Results

Seminiferous tubules

Measurements of intraluminal pressure in 29 seminiferous tubules showed a relatively low pressure of 3.0 ± 0.2 cmH₂O. There were no rhythmic changes in pressure recordings (Text-fig. 2).

Text-fig. 2. Typical records of intraluminal pressure in (a) the seminiferous tubules; (b) the mid-caput (site 2); (c) the mid-corpus (site 4); (d) the proximal cauda (site 6) (see Text-fig. 3 for sites referred to). Different patterns of spontaneous contraction of the epididymis are shown: peaks of contractions are single (b, c and d, left tracing) or double (b and d, right tracing); in (c) (right tracing) there is an oscillation in the basal pressure and amplitude of contractions in the corpus.

Visual observations simultaneously with pressure recordings also failed to reveal any tubular contraction.

Caput epididymidis

Intratubular pressure in the caput epididymidis was higher ($P < 0.05$) than that in the seminiferous tubules. The basal pressures in all three sites (sites 1, 2 and 3) were not significantly different (Text-fig. 3a). The mean $\pm$ s.e.m. pressure in the caput was 3.6 ± 0.2 ($n = 29$) cmH₂O. A pulsatile pressure related to spontaneous contraction was always recorded in all areas in the caput. The recordings revealed a single or double peak of contraction with a more or less regular amplitude and frequency (Text-fig. 2b). There was no apparent difference in the amplitude of contractions in the 3 sites (Text-fig. 3a), but the frequency of contraction in the proximal caput (site 1), 9.3 ± 0.8 per min, was higher ($P < 0.01$) than that in the mid-caput (site 2), 5.7 ± 0.5 per min, and the distal caput (site 3), 6.1 ± 0.5 per min. The frequency of contractions in sites 2 and 3 were not different.

Corpus epididymidis

The pressure recordings were qualitatively similar to those of the caput, except that an oscillation of the basal pressure and amplitude of contractions was sometimes observed (Text-fig. 2c). The basal pressure and the amplitude of contractions in the mid-corpus (site 4) were 4.4 ± 0.3

Text-fig. 3. Mean $\pm$ s.e.m. intraluminal pressures and frequency of contraction in different regions of the rat epididymis; (a) basal pressures (hatched bars) and amplitude of contractions (open bars); (b) frequency of contraction. Insert shows the sites of pressure measurements. Figures in parentheses indicate the number of measurements at each site.

and 3.3 ± 0.4 cmH₂O, and those in the distal corpus (site 5) were 4.1 ± 0.1 and 6.4 ± 0.7 cmH₂O, respectively. These values were higher ($P < 0.05$) than those in all regions of the caput, but the frequency of contraction in the distal caput was not different from that in the mid-corpus, 4.9 ± 0.4 per min, and the distal corpus, 5.0 ± 0.7 per min. There was no basal pressure gradient in the corpus but the amplitude of contraction in the mid-corpus was lower ($P < 0.001$) than that in the distal corpus (Text-fig. 3a).

Cauda epididymidis

The basal pressure in the proximal cauda (site 6) was almost 2-fold greater than that in the proximal segment of the epididymis (Text-fig. 3a) and the amplitude of contraction was 7.3, 4.2 and 2.5 times those in the caput, the mid-corpus and the distal corpus, respectively. However, the frequency of contraction, 1.2 ± 0.2 per min, was appreciably lower ($P < 0.001$) than that in the caput and the corpus (Text-fig. 3b). The tubule in the distal cauda (site 7) was less contractile than that in the proximal cauda, but basal pressures in these two sites, 7.0 ± 0.5 and 7.2 ± 0.5 cmH₂O, were not different. There were more quiescent tubules in the distal cauda than in the proximal cauda, at least during the 10–30-min period in this study. This accounts for an apparent decrease in

the frequency of contraction from the proximal to the distal cauda (Text-fig. 3b). The amplitude of contraction in the active tubule of the distal cauda, 5.6 ± 0.9 cmH₂O, was smaller ($P < 0.01$) than that in the proximal cauda, 15.3 ± 1.3 cmH₂O (Text-fig. 3a).

Discussion

To our knowledge, this is the first report of intraluminal pressures of the testis and different regions of the epididymis in the rat. The pressure in the seminiferous tubules of rats in this study is very close to that of the guinea-pig (Johnson & Howards, 1976) but considerably lower than that of the hamster (Johnson & Howards, 1975). The intraluminal pressure of seminiferous tubules at a distance 4 mm from the rete testis in our study is comparable to the hydrostatic pressure in the rete testis of rats measured by cannulation of the efferent ducts (Free & Jaffe, 1979). The failure to record pulsatile pressure in the seminiferous tubules suggests that there were no spontaneous contractions or that contractions were too weak to cause any significant changes in the intraluminal pressure. In fact, Suvanto & Kormano (1970) described a rather localized and weak depression of the tubular wall of the rat testis. This would also account for the failure to observe any tubular contraction since the magnification used in the present study ($\times 80$) may be too low ($\times 400$; Clermont, 1958; Suvanto & Kormano, 1970). However, rhythmic contractions of the epididymal duct were recorded in all regions. The frequency of contraction was highest in the proximal caput and decreased to the lowest value in the distal cauda. This is consistent with results obtained by measuring the electrical activity of the rat epididymis *in vitro* (Talo, Jaakkola & Markkula-Viitanen, 1979). The amplitude of contraction, on the other hand, increased from the caput, reaching a maximum in the proximal cauda, and then decreased in the distal cauda. This result was similar to that for the rat distal cauda measured by cannulation of the proximal vas deferens *in vivo* (Hib & Ponzio, 1977) and *in vitro* (Markkula-Viitanen, Nikkanen & Talo, 1979). The increase in the amplitude of contractions towards the distal segment is in accord with the increase in muscular thickness of the tubular walls from the caput to the cauda (Hamilton, 1975). The significance of this finding is presently not clear. It may reflect the requirement for a higher driving force in the distal segment to propel the sperm suspension through the lumen since the fluid content becomes more viscous and is packed with a large number of spermatozoa. The fact that, at rest, the distal cauda appears to be quiescent or contracts rather weakly compared to the proximal cauda may be of functional importance because this segment of the epididymis serves as a sperm reservoir.

The mechanism by which spermatozoa are transported through the seminiferous tubules is still unclear. Several factors may contribute to the force required to propel the sperm suspension through rete testis. Contractions of the testicular capsule (Davis, Langford & Kirby, 1970) and the seminiferous tubules (Hargrove *et al.*, 1977), the secretion of tubular fluid, the absorption of fluid in the efferent ducts and the caput epididymidis, and ciliary action in the efferent ducts all may be involved to various extents. Although contraction of the seminiferous tubules has been described by many authors, quantitative measurements in terms of force or pressure resulting from tubular contraction have failed to demonstrate spontaneous activity or changes in response to autonomic drugs (Davis & Langford, 1971; Pholpramool & Triphrom, 1984). Fluid flow into the proximal caput, occurring against a pressure gradient, cannot be satisfactorily explained by contraction of the seminiferous tubules and casts some doubt on a major role for tubular contraction in sperm transport through the testis.

Since spontaneous contraction of the epididymis is evident, transport of spermatozoa from the caput towards the cauda even against a resting pressure gradient could be the result of tubular contraction. The findings that basal pressure is low in the caput but high in the cauda and that the reverse is true for the frequency of contraction suggest that passage of the sperm suspension through the epididymal duct occurs only during intermittent rises in pressure resulting from contraction of the epididymis. Therefore, any factors affecting contractility of the epididymal duct

would alter sperm transit time and possibly interfere with sperm maturation processes in the epididymis.

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