Relation of electrical activity to luminal transport in the cauda epididymidis of the rat *in vitro*

Ulla-Marjut Jaakkola and A. Talo

*Laboratory of Animal Physiology, Department of Biology, University of Turku,
20500 Turku 50, Finland*

Summary. Droplets of stained paraffin oil were injected into the lumen of the rat cauda epididymidis through a microcapillary *in vitro* at a mean distance of 36 ± 8 cm from the beginning of the ductus deferens. The location of a droplet was marked on a 6-channel recorder graph after each event of electrical activity of the wall musculature. Transport of the droplets consisted of pendular movements. The droplet moved in the direction of the spread of electrical activity then returned almost to its original position during reflux. The net distance for transport was dependent on the direction of the spread of activity and on the percentage of the non-propagating activity. The mean probability for the spread of the electrical activity towards the ductus deferens between the pairs of the electrodes was 0.54 and the mean net transport was 6.2 ± 1.8 (s.e.m.) mm/h, towards the ductus deferens in 7 out of 10 ducts.

Introduction

Contractions of the smooth muscle lining the epididymal duct appear to transport the spermatozoa through the epididymis. The transport takes place even if fluid flow from the testis is prevented by ligation of the ductuli efferentes (Macmillan & Aukland, 1960) and it takes place against an increasing hydrostatic pressure gradient from the testis to the caput and from the caput to the cauda (Johnson & Howards, 1976). The luminal epithelium is lined with immotile stereocilia (Hamilton, 1975).

The frequency of the electrical activity of the smooth muscle declines from the caput to corpus and further in the cauda towards the ductus deferens. Electrical activity spreads over long distances in the distal cauda epididymidis and ductus deferens, but not in the caput, corpus and proximal cauda epididymidis. The conduction velocity of the electrical activity declines from the ductus deferens (3.4 mm/sec) to the middle of the cauda (1.1 mm/sec) (Talo, Jaakkola & Markkula-Viitanen, 1979). The transport rate of spermatozoa is not directly proportional to the conduction velocity of the electrical activity because the length of the rat ductus epididymidis is about 3.6–4 m (Pragliani & Pinca, 1955; Talo *et al.*, 1979) and spermatozoa are transported through the epididymis in 8 days, taking 3 days through the caput and corpus, which is about two-thirds of the epididymal length, and 5 days through the cauda (Amann, Johnson, Thompson & Pickett, 1976).

This study examines *in vitro* the relation of various characteristics of the electrical activity of the cauda epididymidis of the rat to the net transport of the epididymal contents.

Materials and Methods

Ten Sprague–Dawley male rats were killed by decapitation under a light ether anaesthesia and the testis, epididymis and a short portion of the ductus deferens were removed quickly and kept in modified Ringer solution (see below) at room temperature (22°C) and oxygenated (95% O₂, 5% CO₂). Fat and connective tissue covering the epididymis were removed with a pair of forceps under a dissecting microscope and a portion of the epididymal duct a few centimetres long was uncoiled from the cauda epididymidis at a mean distance of 36 ± 8 cm from the junction with the ductus deferens. Paraffin oil droplets stained with Sudan black were injected into the uncoiled region through a bevelled microcapillary (Hinton & Setchell, 1978). The droplets moved with the intraluminal contents and were easily seen under the microscope. The tissue was placed in a water-jacketed chamber containing modified Ringer solution (154 mM-NaCl, 5.6 mM-KCl, 0.12 mM-MgCl₂, 2.2 mM-CaCl₂, 5.9 mM-NaHCO₃, 2.5 mM-glucose; pH 7.2) kept with a thermostat at a temperature of $34 \pm 0.5^\circ\text{C}$ and continuously oxygenated. The electrical activity of the smooth muscle of the epididymis in the uncoiled region was recorded with six suction electrodes (E₁–E₆) (Talo *et al.*, 1979), which had an outer tip diameter of 100–250 µm, connected to a Grass-6-channel polygraph.

The electrode from which each activity originated and the direction and distance of its spread were determined by manual analysis for 1 h and in one experiment 6 successive periods each lasting 10 min were analysed separately. The criterion for propagation was a propagation speed of 1–5 mm/sec (Jaakkola & Talo, 1980). If activity began at two adjacent electrodes almost simultaneously and then propagated in one or both directions, activity was assumed to have begun at the electrode which first registered it, although it had probably begun between electrodes. When the electrical activity spread simultaneously to an area from both directions, the direction registered depended on previous activity and on conduction velocity. The net distance for the movements of the oil droplets from the injection point was measured. The probability for the direction of the spread to the ductus deferens was calculated from the total number of propagation activities ($E_{1-2}/E_{1-2} + E_{2-1}$) between each electrode pairs. The percentage of non-propagating activity (E₁, E₂) was also calculated for each pair of electrodes ($E_1 + E_2/E_1 + E_2 + E_{1-2} + E_{2-1}$) (Talo & Hodgson, 1978). The net distance and the direction of the transport of the oil droplets were compared to different factors affecting the electrical activity, and the results were tested with regression analysis. The mean frequency and the frequencies of the non-propagating and local activities were determined from a total of 14 h 3 min recording time. The mean probability for spread to the ductus deferens was also determined.

Results

The oil droplets injected into the epididymal lumen moved back and forth with luminal contents. The movement forward started when the spread of electrical activity approached the droplet, and the direction of its movement changed when the activity ceased in the vicinity of the droplet or passed it (Text-fig. 1). The droplet returned almost to its original position. Thus the net distance moved during one pendular movement of 5–10 mm magnitude was minimal. Since the direction of spread of electrical activity varied, pendular movements in both directions were observed. Spread of activity simultaneously from both directions towards the droplet resulted in a small movement or no movement at all. The timing of the movements of the droplet and of the spread of electrical activity (Text-fig. 1) was only approximate, since the movements were followed visually.

As shown in Text-fig. 2(a) the direction of net transport was towards the ductus deferens, the direction in which the frequency of electrical activity declined. In Text-figs 2(b) and 2(c) this decline was not as clear in the segment containing the droplet. In 7 out of 10 ducts the net

Text-fig. 1. Movements of an oil droplet in relation to the spread of electrical activity in the cauda epididymidis. Two events spreading in opposite directions are marked. The first one (1) spreading toward the testis from electrode E6 to E3 results in a pendular swing with a small net movement of the droplet (open circle) as indicated by the curved arrow. The second one (2) spreading from E1 to E4 passes the droplet and results in a pendular movement of larger magnitude toward the ductus deferens. The droplet remains motionless between the activities. The distances between electrodes are 0.5–1 cm. Pre-amplifier time constant 0.3 Hz.

Text-fig. 2. (a, b, c) Total frequency (●), frequency of pacemaker activity (○) and local activity (▲) in 6 electrode locations (1–6 from left to right) in 3 different epididymal ducts and (d, e, f) the probability for spread of activity towards the ductus deferens (●) and the percentage of non-propagating activity (■) between electrode pairs in the same 3 ducts. The arrows in the diagrams drawn in scale with the ordinates, indicate the direction and distance of the net transport of the droplets in 1 h. The distance of the recording area from the beginning of the ductus deferens is indicated in centimetres.

transport was in the general direction of the decreasing frequency gradient. The direction of the net movement of the droplet was, in all cases, as shown in Text-fig. 2, away from the highest pacemaker area. The higher pacemaker activity in electrode 1 in Text-figs 2(a) and 2(b) probably includes events initiated outside the recording area.

The relative incidence of spread of activity in the direction of the ductus deferens was expressed as a probability. In the duct where the distance of net movement towards the ductus deferens was short (Text-fig. 2d), the transport of the droplet in the direction of the ductus deferens was antagonized by the majority of spreads being in the testicular direction between electrodes 4 and 5. A high percentage of spreading activities terminated in the area of the droplet, which may in part explain the short net distance moved. When the transport was over a longer distance towards the ductus deferens, the probability of spread in that direction was much higher than in the opposite direction and the percentage of events ceasing was low throughout the segment (Text-fig. 2e). In Text-fig. 2(f), all probability values were less than 0.5, indicating that the majority of spread occurred in the same direction in which the droplet moved. The higher percentage of non propagation between electrodes 4 and 5 was outside the transport area and without effect.

The activity of the ductus epididymidis did not remain constant (Text-fig. 3). Initially the dominant pacemaker was located under electrode 3, then under 5, and in the last period both areas had approximately similar pacemaker activity. The highest frequency of electrical activity changed from electrode 3 in Text-fig. 3(a) to electrode 4 in Text-figs 3(b) and 3(c).

Text-fig. 3. The fluctuations of the total frequency (●), frequency of pacemaker activity (○) and local activity (▲) in one experiment during three consecutive periods (a, b, c) of 10 min each.

When the results of all experiments were pooled, the mean frequency of electrical activity was 136 ± 8.5 (s.e.m.)/h, and the mean of the net transport speeds of the oil droplets was 6.2 ± 1.8 (s.e.m.) mm/h, occurring in 7 out of 10 ducts towards the ductus deferens. In 8 out of 10 ducts, the direction of the net transport was that in which the majority of contractions spread in the vicinity of the oil droplet, and there was a linear correlation ($r = 0.938$, $P < 0.001$) between the probability for the spread towards the ductus deferens and the net transport distance ($y = 45.4x - 23.1$). This indicates that the transport speed increased very steeply when the probability for spread of activity differed from 0.5. The mean of probability for the spread towards the ductus deferens included only the propagating activities and was 0.54 for all pairs of electrodes. The relationship between the percentage of non-propagating activities in the vicinity of the oil droplet and the distance of the net transport was further analysed (Text-fig. 4). Regression analysis with inverse values gave $b_{yx} 0.007$ and $a_{yx} 0.098$ with a correlation coefficient of $r = 0.933$ ($P < 0.001$), indicating that when the relative frequency of local,

non-propagating activities increased, the transport speed declined. The mean of non-propagating activity between all pairs of electrodes was $21.5 \pm 3.1\%$. This value also includes the local activity appearing in one electrode only, the mean percentage of which was $4.3 \pm 1\%$.

Text-fig. 4. Relationship between the net transport distances to percentage of the non-propagating activity in 10 experiments. The curve indicates the relation $y = 1/0.007x + 0.098$.

Discussion

The total time for transport of spermatozoa through the epididymal duct has been estimated (Amann *et al.*, 1976). The present study is the first examination of the relation of the electrical activity of the epididymal muscles to the transport of the epididymal contents. The frequency of the electrical activity in this study agrees well with earlier measurements from the cauda epididymidis (Talo *et al.*, 1979) if the temperature of the present study, about 2.5°C lower, is taken into account (Jaakkola & Talo, 1980). The frequency of the movements of the oil droplets injected into the lumen of the duct was the same as that of electrical activity, agreeing well with earlier visual observations (Cross, 1955; Risley, 1963; Melin, 1970).

The results suggest that the transport of spermatozoa in the distal cauda is a stochastic process like the transport of ova in the oviduct (Verdugo, Blandau, Tam & Halbert, 1976; Talo & Hodgson, 1978). This conclusion is partly based on observations of the movements of the oil droplets, and partly on the properties of electrical activity; the pendular movements of the oil droplets occur in both directions and take place in irregular sequences. If several droplets are injected simultaneously their movements may follow different courses, indicating not only the stochastic nature of transport but also that the luminal contents are mixed effectively. The electrical activity initiates in many pacemakers, without any fixed location, and spreads for variable distances in both directions, resulting in large spatial variation of the probability of spread in the direction of the ductus deferens. Due to temporal variation in the pacemaker activity, the spatial variations of the probability even out, and the mean overall activity is significantly more towards the ductus deferens than the testis, indicating that the process is not random (mean of probabilities 0.54). Because the activity spreads from pacemakers in both directions, it is likely that the activity from the high frequency region spreads more often towards the region of lower frequency than in the opposite direction. The decreasing frequency gradient in the distal cauda (Talo *et al.*, 1979) may therefore be the factor which determines the directional effect towards the ductus deferens and thus maintains the slow net transport in that direction.

No direct conclusion concerning the transport of the luminal contents can, however, be drawn from the recordings of electrical activity. The magnitude of pendular movements was less than the distance of spread of electrical activity, and the net movement during the pendular

movement was only a small fraction of the distance of spread. This poor relationship is a result of the small change in the luminal diameter during the contraction, since theoretical and experimental studies made of the ureter have shown that incomplete closure of the lumen results in ineffective transport of fluid (Fung, 1971).

The net transport speed we found in this study is lower than would be expected from the transport time through the cauda epididymidis reported by Amann *et al.* (1976), even when net transport in the direction of the testis (3 out of 10 cases) is discounted. The probable explanation for this discrepancy is that our measurements were made in the distal cauda *in vitro*, while Amann *et al.* (1976) used the whole cauda epididymidis *in vivo*, thus maintaining the neural connections. Activation of the adrenergic innervation present in the cauda epididymidis (Baumgarten, Holstein & Rosengren, 1971) would probably result in better coupling between the spread of electrical activity and movements of the luminal contents, by increasing the strength of the contractions (Laitinen & Talo, 1981). Stronger contractions would result in a larger decrease of the inner diameter of the lumen and thus in more effective transport (Fung, 1971).

This study was supported by the Emil Aaltonen Foundation and the Academy of Finland.

References

- Amann, R.P., Johnson, L., Thompson, D.L. & Pickett, B.W. (1976) Daily spermatozoal production, epididymal spermatozoal reserves and transit time of spermatozoa through the epididymis of the rhesus monkey. *Biol. Reprod.* **15**, 586–592.
- Baumgarten, H. G., Holstein, A. F. & Rosengren, E. (1971) Arrangement, ultrastructure and adrenergic innervation of smooth musculature of the ductuli efferentes, ductus epididymis and ductus deferens of man. *Z. Zellforsch. mikrosk. Anat.* **120**, 37–79.
- Cross, B.A. (1955) The posterior pituitary gland in relation to reproduction and lactation. *Br. med. Bull.* **11**, 151–155.
- Fung, Y.-C.B. (1971) Peristaltic pumping: a bioengineering model. In *Urodynamics. Hydrodynamics of the Ureter and Renal Pelvis*, pp. 177–198. Eds S. Boyarsky, C. W. Gottschalk, E. A. Tanagho & P. D. Zimskind. Academic Press, New York.
- Hamilton, D.W. (1975) Structure and function of the epithelium lining the ductuli efferentes, ductus epididymis, and ductus deferens in the rat. In *Handbook of Physiology, V. Endocrinology, Section 7: Male Reproductive System*, pp. 259–301. Eds D. W. Hamilton & R. O. Greep. Am. Physiol. Soc., Washington, D. C.
- Hinton, B.T. & Setchell, B.P. (1978) Fluid movement in the seminiferous tubules and the epididymal duct of the rat. *J. Physiol., Lond.* **284**, 16P–17P.
- Jaakkola, U.-M. & Talo, A. (1980) Effect of temperature on the electrical activity of the rat epididymis *in vitro*. *J. Thermal Biol.* **5**, 207–210.
- Johnson, A. L. & Howards, S. S. (1976) Intratubular hydrostatic pressure in testis and epididymis before and after long term vasectomy in the guinea pig. *Biol. Reprod.* **14**, 371–376.
- Laitinen, L. & Talo, A. (1981) Effects of adrenergic and cholinergic drugs on electrical and mechanical activities of the cauda epididymidis of the rat *in vitro*. *J. Reprod. Fert.* **63**, 205–209.
- Macmillan, E. W. & Aukland, J. (1960) The transport of radio-opaque medium through the initial segment of the rat epididymis. *J. Reprod. Fert.* **1**, 139–145.
- Melin, P. (1970) In vivo recording of contractile activity of male accessory genital organs in rabbits. *Acta physiol. scand.* **79**, 109–113.
- Pragliani, L. & Pinca, A. (1955) Modification of epididymis in albino rat. *Boll. Soc. Ital. Biol. Sper.* **31**, 646–649.
- Risley, P.L. (1963) Physiology of male accessory organs. In *Mechanisms Concerned with Conception*, pp. 73–133. Ed. C. G. Hartman. Pergamon Press, London.
- Talo, A. & Hodgson, J.J. (1978) Spike bursts in rabbit oviduct. I. Effect of ovulation. *Am. J. Physiol.* **234**, E430–E438.
- Talo, A., Jaakkola, U.-M. & Markkula-Viitanen, M. (1979) Spontaneous electrical activity of the rat epididymis *in vitro*. *J. Reprod. Fert.* **57**, 423–429.
- Verdugo, P., Blandau, R.J., Tam, P.Y. & Halbert, S.A. (1976) Stochastic elements in the development of deterministic models of egg transport. In *Ovum Transport and Fertility Regulation*, pp. 126–137. Eds M. J. K. Harper, C. J. Pauerstein, C. E. Adams, E. M. Coutino, H. B. Croxatto & D. M. Paton. Scriptor, Copenhagen.

Received 26 March 1981