Secretion of inositol and glucose by the perfused rat cauda epididymidis

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Summary. Inositol was detected by gas–liquid chromatography in fluid perfused at 1.3 $\mu\text{l}/\text{min}$ through the lumen of the distal cauda epididymidis of anaesthetized rats. The concentration (247 μM) exceeded that in blood (48 μM) and the secretion rate was constant for 5 h. D-[^3H]Myo-inositol infused for 3 h into the general circulation of rats (1 $\mu\text{Ci}/\text{min}$) also appeared in fluid perfusing the lumen, whether or not 50 mM-inositol was present in the perfusing solution. No plateau of radioactivity was reached during infusion, and by the end of 3 h perfusate activity was 26% of that in blood. Calculation of the specific activity of inositol in perfusates and blood plasma suggested that blood was not the immediate source of luminal inositol, and that any endogenous pool of cyclitol has a turnover time of greater than 3 h. [^3H]Myo-inositol perfused through the lumen was not absorbed by the tissue. These data suggest that the high concentration of inositol in epididymal fluid (49 mM) is derived in part by epididymal secretion from a pool that is only slowly replenished from blood, and maintained by the impermeability of the epithelium. Glucose also appeared in fluid perfused through the epididymal lumen, but its concentration (461 μM) was much less than in blood (8.5 mM), so this sugar may diffuse down a concentration gradient.

Introduction

Spermatozoa stored in the cauda epididymidis are bathed in inositol in all species studied, although its concentration varies from <6 mM (ram, bull, boar, rabbit) to 30–90 mM (rat, hamster: see Hinton, White & Setchell, 1980). In all species studied the inositol concentration in fluid entering the epididymis is similar (2–8 mM), and calculations reveal that this source alone could provide more than that leaving the epididymis each day even in the rat (Hinton *et al.*, 1980) but more so in domestic species. While removal of luminal inositol in proximal parts of the epididymis must explain its lower concentration in the fluid of the cauda epididymidis of domestic species, the higher concentration in rodents could be explained, solely or together, by: (a) selective resorption of fluid without inositol (Hinton *et al.*, 1980), (b) secretion of inositol synthesized in the epithelium (Robinson & Fritz, 1979), or (c) uptake from blood (Lewin & Sulimovici, 1975; Lewin, Yannai, Sulimovici & Kraicer, 1976).

The present study investigated the relative importance of the transfer of circulating inositol into the epididymis by means of luminal perfusion.

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Materials and Methods

Animals and perfusion technique

Mature male rats (CD strain, Charles River Breeding Colony, Manston, Kent) weighing 327–420 g were anaesthetized with urethane (ethyl carbamate, 25 mg/kg, i.p.) and the left carotid artery, right jugular vein and the distal cauda epididymidis on each side were cannulated as previously described (Cooper & Waites, 1979). Epididymides were perfused through the lumen at a mean rate of 1.3 $\mu\text{l}/\text{min}$ (range 1.2–1.5 $\mu\text{l}/\text{min}$) with a solution containing physiological concentrations of sodium and potassium ions as established by Levine & Marsh (1971), namely: 50 mM-KCl, 7 mM-NaCl, 88.6 mM-choline chloride, 33 mM-(*N*-morpholino)-propanesulphonic acid, buffered with 13 mM-NaOH to epididymal pH (6.9). This solution has been shown to maintain a fairly normal epithelial morphology (Cooper & Yeung, 1980). In some cases *myo*-inositol (50 mM) was added to the solution and an osmotic equivalent of choline chloride omitted.

In 4 rats D-[2-³H]*myo*-inositol (Radiochemical Centre, Amersham, U.K.) in 154 mM-NaCl (sp. act. 3.9 Ci/mmol; 111 $\mu\text{Ci}/\text{ml}$) was infused into the general circulation at 9 $\mu\text{l}/\text{min}$ for 3 h. Sequential samples of perfusates were collected over 30 min and carotid arterial blood samples were collected every 30 min starting 15 min from the start of the infusion. Samples of undiluted epididymal plasma were centrifuged for 5 min, and whole blood for 1 min, at 13 000 g_{max} at room temperature, and the resulting cell-free fluid was stored at -20°C .

In 5 rats D-[2-³H]*myo*-inositol (sp. act. 3.9 Ci/mmol; 1.4 Ci/ml), D-[2-³H]glucose (sp. act. 10 Ci/mmol; 0.8 $\mu\text{Ci}/\text{ml}$), 3-*O*-methyl-D-[1-³H]glucose (sp. act. 7.7 Ci/mmol; 0.97 $\mu\text{Ci}/\text{ml}$) or [³H-methoxy]inulin (sp. act. 1.1 Ci/mmol; 127 nCi/ml), all from the Radiochemical Centre, Amersham, U.K., was added to the solution perfused through the lumen. The concentration of isotope leaving the epididymis (outflowing perfusate) was expressed as a percentage of that initially entering it, to provide information on the possible removal of tracer from the epididymal lumen. Samples (10 μl) of perfusates and blood plasma were prepared for liquid scintillation counting as previously described (Cooper & Waites, 1979).

Assay of inositol and glucose

Inositol and glucose were quantified by gas-liquid chromatography (g.l.c.) on a Pye Series 204 Gas Chromatograph. Separation was effected in glass columns (2 m $\times$ 4 mm) containing 3% SE-30 on Gas Chrom Q (80–120 mesh) run isothermally at 190°C with carrier gas flow at 40 ml/min and flame ionization detection. This adequately separated inositol, with a retention time relative to the internal standard (RRT) of 0.341, from α - and β -D-glucopyranoside (RRT 0.423 and 0.711 respectively). Blood plasma (100 μl) was deproteinized with 2 volumes ethanol containing 20 μM -methyl-4,6-*O*-benzylidene- α -D-glucopyranoside (MBG: Koch Light) as internal standard. Epididymal plasma was first diluted 30–40 times with water and 100 μl were deproteinized with 2 volumes ethanol containing 1 mM-MBG. In each case 200 μl supernatant, after centrifugation of the precipitated proteins, were dried overnight *in vacuo* over P_2O_5 . Perfusates (20 μl) were added directly to vials containing 20 nmol MBG and dried as above. Mixed standards of glucose and inositol in the expected range were run in each assay. Dried samples were taken up in 25 μl silylating reagent (trimethylsilylimidazole:dry toluene 1:1, v/v), mixed and allowed to stand at room temperature for at least 5 min before injection. Peak heights were measured relative to the internal standard.

Validation of assay

For inositol the coefficient of correlation between that estimated and that added at 5 concentrations in the range 0.25–5.0 mM was 0.998. The mean recovery was 103% and the

mean coefficient of variation over this range was 14%. For glucose the coefficient of correlation between that estimated and that added at 5 concentrations in the range 0.0625–2.0 mM was 0.998. The mean recovery was 99% and the mean coefficient of variation over the range was 11%.

Thin-layer chromatography

Dried samples of infusates, perfusates and extracts of deproteinized blood plasma were taken up in 30 μ l saturated aqueous *myo*-inositol, spotted on to thin layers (250 μ m) of unactivated silica (SilicAR TLC 7GF, Mallinckrodt) and developed for 15 cm in saturated tanks of isopropanol:ethyl acetate:water:ammonia (50:10:30:10 by vol.). Plates were scanned for radioactivity (Panax Nucleonics, Mitcham, Surrey) and authentic inositol standards were visualized as described by Trevelyan, Proctor & Harrison (1952).

Results

Concentrations of carbohydrates in body fluids

Inositol concentrations were $48.3 \pm 6.8 \mu\text{M}$ ($N = 13$) in blood plasma and $49.3 \pm 5.7 \text{ mM}$ ($N = 10$) in epididymal fluid from the distal cauda epididymidis. Glucose concentration in blood plasma taken at the end of the perfusion experiments was $8.5 \pm 1.5 \text{ mM}$ ($N = 6$), but was undetectable in epididymal fluid. The values are the means $\pm$ s.e.m.

Concentrations of carbohydrates in perfused fluids

Both inositol and glucose appeared in epididymal perfusates at the concentrations and rates of secretion given in Table 1 (see Text-figs 1 and 2). The mean $\pm$ s.e.m. length of perfused tubules was $19.8 \pm 1.2 \text{ cm}$ (range 15–28.5 cm, $N = 16$). Although there was no definite trend towards increased inositol secretion with longer lengths of perfused tubules, the coefficient of variation of the secretion rates when expressed per cm perfused length (24.8%) was smaller than that for total secretion (29.2%) or concentration (35.5%) (see Table 1). The secretion of inositol was constant for 5 h of perfusion (9 epididymides, 6 rats: see Text-fig. 2). Perfusion solutions were confirmed to be initially free of inositol and glucose.

Table 1. Concentrations of *myo*-inositol and glucose in outflowing perfusates and their secretion rates into the perfused distal cauda epididymidis of the rat

	Inositol	Glucose
Concentration (μM)	274.0 ± 32.4	461.4 ± 107.6
Secretion rate (pmol/min)	353.7 ± 34.4	604.0 ± 138.3
($\text{pmol}/\text{min}/\text{cm}$)	18.2 ± 1.5	31.5 ± 6.2

Values are mean $\pm$ s.e.m. from 9 epididymides from 6 rats. For each epididymis the mean of 10 sequential samples throughout the 5-h perfusion period was calculated.

Entry of circulating [^3H]inositol

Radioactivity entered the epididymal lumen within the first 30 min of infusion, rose steadily throughout the infusion and did not reach a plateau. At no point throughout the infusion was the extent of entry of tracer significantly lower in the presence of luminal inositol (50 mM). At the end

Text-fig. 1. A g.l.c. trace following injection (Inj.) of an extract of the fluid perfused for 30 min through the lumen of the distal cauda epididymidis of an anaesthetized rat. It reveals 3 major peaks with retention times of α - and β -D-glucopyranose and D-myoinositol, in addition to the added internal standard (I.S.).

Text-fig. 2. The secretion of glucose and inositol as quantified by g.l.c. in fluid perfused through the lumen of the distal cauda epididymidis of anaesthetized rats. The rate is expressed per unit length of the perfused tubules (ordinate) during and after a 3-h i.v. infusion of [^3H]inositol (horizontal bar). Values are mean $\pm$ s.e.m. of 9 samples (during infusion) and 4 samples (after infusion) from 4 rats.

of the infusion (3 h) perfusate activity had reached a mean of 26% (range 15–43%) of plasma concentrations (8 epididymides, 4 rats). Radioactivity continued to rise, but less steeply, in the perfusates after the end of the infusion, whether inositol was present or not, despite the initial rapid decline in circulating tracer (Text-fig. 3).

Text-fig. 3. The specific activity of inositol in carotid arterial blood plasma and fluid perfused through the lumen of the distal cauda epididymidis, during and after a 3-h i.v. infusion of [^3H]inositol into anaesthetized rats (horizontal bar). Because inositol concentrations in blood and perfusates were constant throughout the experiments, the raw data (d.p.m./ μl) from which the specific activity was calculated followed a similar time course. Values are mean $\pm$ s.e.m. for 4 (plasma) and 5 (perfusate) samples.

Specific activity of secreted inositol

Single peaks of radioactivity co-migrating with authentic *myo*-inositol were found in both blood plasma and perfusates during infusion (Text-fig. 4). Because the amount of unlabelled inositol infused over 3 h was $<5\%$ of the calculated total plasma pool, and no change was noted in inositol concentration in plasma sampled every 30 min for 3 h during infusion (2 rats), estimates of the specific activity of inositol in blood plasma and perfusates were made assuming that the total radioactivity in both fluids was solely associated with inositol. Equilibrium of the

Text-fig. 4. A radio-t.l.c. trace of fluid perfused through the lumen of the distal cauda epididymidis during a 3-h i.v. infusion of [^3H]inositol into anaesthetized rats. The dried sample (see text) was spotted onto silica plates (at origin, O) and run for 15 cm (to solvent front, S_f) together with authentic inositol (horizontal bar beneath radiopeak). A similar trace was obtained from blood plasma.

perfusing fluid with blood plasma was never achieved (Text-fig. 3) because of the high secretion of unlabelled cyclitol. By the end of the infusion the specific activity of inositol in the perfusate was 3.3% of that in plasma.

Uptake of inositol, glucose, 3-O-methylglucose and inulin by the epididymis

When [^3H]inositol was perfused through the lumen of the epididymis there was little loss of the compound, the ratio [outflow]/[inflow] being 1.04 and 0.92 (2 expts). Values for [^3H]glucose (0.72) and 3-O-methyl-[^3H]glucose (0.89) were lower, indicating partial loss of the sugars, since the inulin ratios (1.03, 1.07, 1.09; 3 expts) indicated (Wong & Yeung, 1978) that water was not secreted with this perfusing solution.

Discussion

The concentrations of inositol in blood in the present study are slightly lower than in other reports in which inositol was measured by g.l.c. (60 μM : Lewin & Sulimovici, 1975) or bioassay (82 μM : Hinton *et al.*, 1980), but the value for fluid from the cauda epididymidis was greater than that found in this region by Hinton *et al.* (1980). The present value is closer to their estimate for the vas deferens, and some fluid from the proximal vas deferens would have contaminated epididymal fluid as collected in the present study. The source of the inositol in the epididymis can be traced in the present study in which the appearance of inositol in fluid perfused through the lumen of the cauda epididymidis was documented.

The concentration of inositol in the perfusates exceeded that in blood 6-fold. This clearly indicates that passive movement to the lumen does not occur, and therefore that either a pump must act to concentrate inositol from blood, or movement to the lumen follows local synthesis in the epithelial cells. Evidence that blood was not the immediate source of luminal inositol in these studies came from the infusion data. Here, as in other studies (Lewin *et al.*, 1976), radioactivity in blood and the epididymis was solely associated with inositol, and in the present study it was shown to enter the lumen. The concentration of [^3H]inositol rose much more slowly in the lumen than in blood plasma during the 3-h infusion period, and fell much more slowly after it. The slowly rising concentration of tracer in the lumen relative to blood during the infusion indicates either that the epithelium is poorly permeable to this compound, or that an epithelial pool of inositol was being labelled. The low specific activity of inositol in the lumen relative to blood resulted from continued secretion of unlabelled inositol which diluted tracer entering from blood. This also implies that a source of inositol is present in the epithelium, from which the luminal cyclitol is derived. Because the turnover of the pool was not complete by 3 h, no estimate of the size of the pool could be made from the present data.

Previous studies have shown that the rat epididymis can accumulate labelled inositol from blood, but not as actively as do other accessory sex organs (Lewin & Sulimovici, 1975; Lewin *et al.*, 1976): a single injection of tracer produced a stable blood concentration for up to 24 h, and whole epididymal tissue retained higher activity than blood over this period. These differences from the present report reflect differences in technique. In the present study only the luminal compartment of the epididymis was examined and after removal of epididymal fluid which contained 49 mM inositol; then material entering the lumen was continually removed during short periods of rising blood radioactivity. Under these conditions no equilibration with blood was observed. Together these reports suggest that circulating inositol is taken up by epididymal tissue and gains access to the lumen, but that the impermeability of, or the presence of an endogenous pool within, the epithelium prevents immediate equilibration between luminal fluid and the circulating compound in short-term studies.

Passive movement of infused inositol from the epididymal epithelial cell to the lumen

(rendered inositol-free in the perfusion studies) should be inhibited by luminal application of inositol, yet physiological concentrations did not affect the rate or extent of entry of tracer. These observations could reflect the impermeability of the apical membranes of the epithelial cells towards inositol, and this view receives some support from the retention of [^3H]inositol in the lumen during its perfusion.

It is not known whether secretion of inositol at the rates measured occurs in normal circumstances, although it has been shown that entry of tracer from blood does take place in the face of normal luminal concentrations of inositol. If secretion does occur then inositol entering the epididymis with testicular fluid is not the only source of luminal inositol, and this may explain why inositol has been found in increasing concentrations distally in rat epididymal tissues (Voglmayr, 1974) and luminal fluid (Hinton *et al.*, 1980). Because the secretion did not decline over a 5-h period of perfusion, the data also provide further evidence for the maintenance of physiological function of the preparation, confirming morphological observations with the same perfusing solution (Cooper & Yeung, 1980).

The appearance of glucose in the perfused fluids confirms an earlier report of its enzymic demonstration (Cooper & Waites, 1979). The mean concentrations in perfusates and blood, and thus the transfer of glucose from blood to lumen judged by comparing the relative concentrations in the two fluids (here 5.4%), were similar in the two studies. In contrast to inositol, therefore, glucose is expected to diffuse down a concentration gradient into the lumen. Its absence from epididymal fluid, confirmed here, would reflect its utilization by the epithelium or spermatozoa *in situ*. The possibility that this glucose is a precursor of epididymal inositol *in vivo*, as demonstrated *in vitro* (Robinson & Fritz, 1979), remains to be demonstrated.

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