Segmental distribution of α -glucosidase, ornithine decarboxylase and polyamines in the human epididymis

K. Purvis and I. Egdetveit

Andrology Laboratory, Institute of Pathology, The National Hospital, Oslo, Norway

Regional differences in the total activities of the epididymal secretory parameter, α -glucosidase, were demonstrated in the 20 000 g supernatants of human epididymal homogenates. A comparison of the enzymic activities in the supernatants and the washings from 12 one centimetre segments of human epididymides indicated an activity peak in segments 3–4 which appeared to be largely intracellular and which presumably reflects the acidic isoenzyme. A second peak in the caudal region, the segmental localization of which was more variable and differed in *post-mortem* and operation specimens, appeared to be primarily intraluminal. The activities of ornithine decarboxylase (ODC), the rate-limiting enzyme in the polyamine pathway, also exhibited regional differences with higher activities in relatively short segments in both caput and caudal portions. Corresponding alterations were also found in the tissue concentrations of the enzyme products, spermidine and spermine. The increased intracellular activities of ODC and α -glucosidase in the distal caput segments presumably reflect the transition of epithelium from the efferent to the epididymal ducts.

Introduction

Specific alterations occur in the biochemistry and function of spermatozoa during epididymal transit. These include changes in the intracellular concentrations of enzymes such as protein methyltransferase (Gagnon *et al.*, 1984), protein carboxyl-methylase, and cAMP and cGMP phosphodiesterases (Purvis *et al.*, 1982) and alterations in various phospholipids (Voglmayr, 1975) and membrane proteins (Olson and Danzo, 1981). Some of these alterations are likely to be related to the gradual acquisition of progressive motility which occurs as spermatozoa pass from the efferent ducts to the cauda epididymis (for review see Bedford, 1975). At least some of these alterations in sperm biochemistry may reflect an interaction between the spermatozoon and components of epididymal secretion, secretory products which may be produced in discrete regions of the organ. It is significant, in this respect, that specific proteins are present and absent from the luminal fluid of different epididymal segments (Sylvester *et al.*, 1984; Ross *et al.*, 1990) and that histological studies imply regional differences in cellular function (Holstein, 1980).

The polyamines, spermidine and spermine, are believed to be general mediators of RNA and protein synthesis in somatic cells (Russell, 1980) and ornithine decarboxylase (ODC; EC 3.5.3.1) is a rate-limiting intracellular enzyme in their biosynthesis (Russell, 1980). This enzyme can be rapidly activated by testosterone in androgen target tissues (Pegg *et al.*, 1970; Purvis *et al.*, 1986). Seminal α -glucosidase (EC 3.2.1.20) is acknowledged to be a marker of human epididymal secretory function (Guérin *et al.*, 1986; Casano *et al.*, 1987; Cooper *et al.*, 1988). Although the role of α -glucosidase in sperm function is still unknown, it has

been suggested that it may be involved in the modification of surface glycoproteins during epididymal transit, thus facilitating sperm–ovum interaction (Fournier-Delpech and Courot, 1987).

The aim of the present study was to ascertain whether regional changes in general metabolic activity, reflected in the tissue concentrations of ODC and the polyamines, could be related to the epididymal distribution of one of its secretory products, α -glucosidase. Studies on regional alterations in epididymal biochemistry have obvious relevance to our understanding of the processes involved in sperm maturation.

Materials and Methods

Source of epididymides and initial preparation

For the measurements of ODC, epididymides were obtained from men undergoing orchidectomy because of prostatic cancer (four cases; median age 62 years; range 58–67 years) and from men after necropsy (two cases; 26 and 36 years). The organs were first dissected free from fat and connective tissue and divided into 1 cm long segments from the testicular end (12 segments in all; Fig. 1). Segments 1–4, 5–6, 7–10 and 11–12 corresponded approximately to the caput, corpus and caudal regions of the epididymis and to the vas deferens, respectively. Epididymides with any observable pathology, including atrophy, were excluded from the study. For the α -glucosidase and polyamine measurements, organs were obtained after necropsy from men involved in traffic accidents (seven cases; mean 23 years; range 21–40 years). In all cases, the epididymides were removed within 48 h of death. In the interim period, the cadavers were maintained at 4°C. The epididymal segments

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Fig. 1. (a) The distribution of α -glucosidase in 20 000 *g* supernatants prepared from human epididymal segments (geometric means and 95% confidence limits; $n = 7$) from organs obtained at necropsy. (b) The epididymides were divided into 12 segments of 1 cm lengths. Enzymic activity was expressed in terms of the conversion of substrate $\text{min}^{-1} \text{mg}^{-1}$ protein in the epididymal supernatants.

were homogenized for 45 s in 10–20 volumes (depending on the size of the tissue fragments) of Tris-HCl buffer (50 mmol l^{-1} , pH 7.2) containing 5 mmol dithiothreitol l^{-1} (DTT), 0.1 mmol EDTA l^{-1} and 0.2 mmol pyridoxal phosphate l^{-1} (Sigma Chemical Co., MO) followed by centrifugation at 20 000 *g* for 30 min. The resulting supernatants were then stored frozen at -70°C .

α -Glucosidase activity in epididymal tissue and secretory compartments

In one study, single epididymides from three individuals were used to assess the relative proportion of α -glucosidase activity in the epididymal tissue and secretion. All three epididymides were obtained after orchidectomy of patients with prostatic cancer. The ages of the donors were 55, 59 and 63 years. No form of chemical castration had been initiated prior to the operation. The tissue was transported on ice and processed within 30 min of removal. After dissection the 12 individual segments were cut into thin slices and washed in 2 ml medium (Dulbecco's medium without calcium and magnesium, Flow Laboratories, Irvine, UK) in Petri dishes (4.5 cm diameter) under gentle mixing for 30 min. The washings were aspirated, filtered through nylon mesh (100 μm) and centrifuged at 1500 *g* for 10 min. The resulting supernatants were then frozen at -70°C until the day of assay. The tissue fragments remaining

were pooled and also stored at -70°C . They were later homogenized in the same buffer.

Determination of α -glucosidase

The assay used was that described by Chapdelaine *et al.* (1978) with minor modifications and is based on the colorimetric determination of *p*-nitrophenol- α -D-glucopyranoside (Sigma Chemical Co.). No attempt was made to differentiate between acid and neutral forms of the enzyme as described by Cooper *et al.* (1988), so that all measurements reflected total enzymic activity in each segment. For the analysis of 100 samples (50 samples in duplicate), a stock solution of the substance (500 mg in 70 ml potassium phosphate buffer, 0.067 mol l^{-1} , pH 6.8) was first preincubated at 37°C for 10 min with 2.5 mg of reduced glutathione (Aldrich-Chemie, Germany) dissolved in distilled water. Aliquots of the substrate solution (725 μl) were then added to tubes containing aliquots (25 μl) either of the cytosols or of the washings from the minced segments before homogenization. After mixing, the tubes were incubated for 1.5 h at 37°C ; distilled water blanks were included in each assay. The reaction was terminated by the addition of 0.1 $\text{mol Na}_2\text{CO}_3 \text{l}^{-1}$ (4250 μl). After mixing, the resulting yellow colour was quantitated colorimetrically at 400 nm, relative to a standard curve prepared with increasing concentrations of *p*-nitrophenol in Na_2CO_3 buffer (6–120 nmol ml^{-1}). Units of enzymic activity were defined in terms of the quantity of *p*-nitrophenol (in nmoles) produced per min by 1 ml of 20 000 *g* supernatant or washings (Fig. 2) or per min per mg protein in the 20 000 *g* supernatants (Fig. 1).

The rate of hydrolysis of the substrate was linear between 5 and 100 μl for the cytosols and for the washings of the minced segments. Linearity was also obtained between 30 and 120 min of incubation when 25 μl was used. The coefficients of inter-assay variation never exceeded 10% and intra-assay variation of duplicates was never greater than 6%.

Determination of ornithine decarboxylase

The assay was based on the measurement of $^{14}\text{CO}_2$ liberated from [^{14}C]ornithine as described by Kobayashi *et al.* (1971). This technique has been applied to the measurement of the enzyme in prostatic tissue by Rui *et al.* (1987). Briefly 1 ml of the 20 000 *g* supernatant was transferred to 25 ml flasks for the enzyme assay. The reaction was started by the addition of 1 ml buffer containing 25 nmol of L-ornithine and 6 nmol (400 000 c.p.m.) of [^{14}C](DL)-ornithine (58 mCi mmol^{-1} ; Amersham, UK). The flasks were closed with rubber stoppers and incubated in a shaking water bath at 37°C for 2 h. The reaction was terminated by injecting 1 ml citric acid (2 mol l^{-1}) through the rubber stopper. It was allowed to stand for a further 60 min to ensure the complete release of CO_2 , which was absorbed by a Whatman 17 filter strip containing 50 μl of ethanolamine (Sigma) diluted to 33% in ethylene glycol. The filter strip was placed in a plastic well held by the rubber stopper. After incubation, the paper was transferred to vials containing 10 ml scintillation fluid (Hydroluma, Lumac Systems, USA) and counted. The protein concentrations and the incubation times used in the assay were derived from the linear

Fig. 2. The distribution of α -glucosidase in 12 epididymal segments from three different individuals (a–c) after orchidectomy. Enzymic activity was expressed as nmol of substrate converted ml⁻¹ and measured in the washings from the minced segments (■) and in the 20 000 g supernatants of the remaining tissue fragments (□).

portion of the activity curves, which were linear between 0.5 and 5 mg protein and 0 and 4 h (data not shown).

Determination of spermidine and spermine

The method was based on the fluorescent detection of dansylated amine derivatives after HPLC separation and has been described in detail elsewhere (Rui *et al.*, 1985). Epididymal segments were homogenized in 5 volumes of Tris–HCl buffer (see above) and, after centrifugation at 20 000 g for 30 min, duplicate aliquots of the supernatant (25 μ l) were mixed with 375 μ l of internal standard (1,6-diaminohexane, 2.5 nmol l⁻¹ in water) and deproteinized with 100 μ l 10% trichloroacetic acid (TCA) on ice for 20 min, followed by centrifugation at 2000 g for 30 min. The TCA was then removed from the supernatant by extraction twice with 2.5 volumes of diethyl ether. Duplicate samples (100 μ l) of the supernatant were mixed with 100 mg NaHCO₃ and 100 μ l dansyl chloride (Sigma; 30 mg in 10 ml acetone). The reaction mixture was allowed to stand overnight in the dark at room temperature. Excess dansyl chloride was removed by the addition of 100 μ l L-proline (1.3 mol l⁻¹ in water) followed by a further 30 min incubation in the dark. The contents of the tubes were then dried at 65°C under a stream of air dissolved in 100 μ l water, and extracted with 500 μ l toluene. An aliquot (300 μ l) of the toluene phase was evaporated to

dryness and dissolved in a final volume of 600 μ l methanol. A 20 μ l sample was applied to the column (250 $\times$ 4.6 mm i.d., Supelcosil LC-18, particle size 5 μ m, Supelco Inc., Pasadena, CA) by an autosampler, and separation of the dansyl derivatives was obtained using a linear methanol/water gradient changing from 65 to 100% methanol (Ratburn Chemicals Ltd, Walkersburn; HPLC grade) in 23 min at a flow rate of 1 ml min⁻¹. Elution times for the internal standard and the dansyl derivatives of spermidine and spermine were 15.1, 18.9 and 22.5 min, respectively. External standards were run after every seventh sample and contained 3.75, 11.25 and 3.75 pmol of standard, spermidine and spermine, respectively. The assay sensitivity was 10 pmol for the polyamines and the intra-assay coefficient of variation was <4%. The fluorescence detector was equipped with an excitation filter of 340 nm and an emission filter of 460 nm.

Determination of protein

Protein was determined by the method of Lowry *et al.* (1951).

Results

The average segmental distribution of α -glucosidase in epididymides obtained at necropsy is shown (Fig. 1). A general

Fig. 3. The distribution of ornithine decarboxylase activity in the 20 000 g supernatants of 12 epididymal segments from organs obtained (a) at necropsy (range; $n = 2$) and (b) after orchidectomy (geometric means and 95% confidence limits; $n = 4$).

increase in enzymic activity was apparent between segments 2 and 4 with a second increment in segments 6 and 7. Thereafter, there was a gradual decrease towards the vas deferens. These peaks in activity were more discernible in the fresh preparations removed after orchidectomy (Fig. 2). In subjects (a) and (b), the initial peak in the caput epididymis between segments 2 and 4 was localized primarily in the 20 000 g supernatant. After a decline in the corpus area, a second increase in enzyme activity could be detected between segments 7 and 10 both in the tissue and the diluted epididymal secretion. The second peak in these fresh preparations was associated with more distal segments (7–10) than in the organs removed at autopsy (segments 6–9). In subject (c) (Fig. 2), although a caput enzyme peak could be detected, there was only a minor increase in activity in the caudal area in both the tissue and fluid compartments.

The activities of the ODC in the various segments of the epididymis are summarized (Fig. 3). Of the six epididymides examined, the two removed at necropsy (Fig. 3a) had markedly higher ODC activity than those removed at operation (Fig. 3b). However, the positions of the enzyme peaks were relatively constant and not influenced by the conditions of organ removal. Two peaks in activity could be demonstrated, in segments 3 and segments 7–8. The first peak corresponded closely to

Fig. 4. The distribution of (a) spermine and (b) spermidine in the 20 000 g supernatants of 12 epididymal segments (geometric means and 95% confidence limits; $n = 7$) from organs obtained at necropsy.

the rise in tissue concentrations of spermidine and spermine measured in a different study using exclusively necropsy organs (Fig. 4). The second ODC peak, which in the majority of cases was higher than the first, was also associated with a secondary, albeit minor, increase in the tissue concentrations of both polyamines.

Correlation analyses of α -glucosidase and polyamines in the same epididymal segments revealed highly significant positive associations between all three parameters (α -glucosidase versus spermine: $r = 0.41$, $P < 0.001$; α -glucosidase versus spermidine: $r = 0.43$, $P < 0.001$; spermine versus spermidine: $r = 0.78$, $P < 0.001$).

Discussion

Studies on the human epididymis suggest that histologically it can be subdivided into discrete segments (Holstein, 1980; Vendrely and Dadoune, 1988). The study reported here suggests that these histological divisions reflect regional differences in biochemistry. The finding that discrete regions in the caput and caudal segments of the epididymis exhibit increased ODC activities suggests a high degree of metabolic activity in

these regions. This is also confirmed by corresponding alterations in the tissue concentrations of its metabolic products, spermidine and spermine. Previous unpublished studies failed to detect ODC activity in seminal plasma or in washed human spermatozoa, implying that the enzyme is intra-epithelial and not secreted into the epididymal lumen. In the study reported here it is not possible to conclude whether the polyamines are components of the epididymal secretion. Anatomical studies indicate that a major portion of the human caput epididymidis is composed of efferent ducts (Saitoh *et al.*, 1990; Yeung *et al.*, 1991). This increase in ODC activity can, therefore, reflect the transition from ductal cells to the secretory epithelium of the epididymal ducts. Results from a primate model indicate that there are significant regional differences in the epididymis with regard to 5 α -reductase and the concentration of androgen receptors, with the highest amounts recorded in the distal caput and corpus segments (Roselli *et al.*, 1991). This initial peak in polyamine synthesis may therefore reflect increased androgen action in these proximal segments of the organ. Indeed, the higher activities of ODC in the necropsy specimens can be related to the relatively young age of the men and the androgen dependency of ODC. The secondary rise in activity of ODC in the caudal region suggests that, in addition to its function as a storage reservoir for spermatozoa, the caudal epithelium may also be actively involved in RNA and protein synthesis.

α -Glucosidase, an acknowledged secretory product of the human epididymis (Guérin *et al.*, 1986; Casano *et al.*, 1987; Cooper *et al.*, 1988), appears to be present in higher concentrations in the same distal caput segments as ODC (segments 3 and 4) and based on the washing studies is, apparently, predominantly intracellular. This supports the conclusions of Guérin and Czyba (1987), whose studies suggest that enzymic activity in the proximal segments of the epididymis reflects the acidic iso-enzyme. On the basis of histochemical studies and selective inhibition of the neutral form, the acid form appears to be primarily intracellular (Yeung *et al.*, 1990). In contrast, the high activity in the caudal regions (segments 7–9) appears to be largely intraluminal since it appears in greater quantities in the tissue washings and as suggested by Yeung *et al.* (1990) presumably constitutes the neutral, secreted form of the enzyme. These neutral and acidic isoforms may represent unrelated biochemical entities with different functions or may simply reflect structural differences of the same enzyme associated with intracellular storage and secretion.

The reason why the second peak in α -glucosidase activity is more proximal to the testis in the necropsy specimens than in the operated organs is unclear. It may be explained by a *post-mortem* diffusion of the epididymal luminal contents or may reflect the age difference of the subjects providing the operation and *post-mortem* specimens. In a previous study, the concentrations of carnitine in the human epididymis also appeared to be highest in a region closely corresponding to the segments 3–4 of the present study (designated Caput II; Bøhmer *et al.*, 1978). Carnitine is also an acknowledged secretory product of the epididymis and has previously been used as a functional parameter (Wetterauer and Heite, 1978). The implication is that this area of the human epididymis may be of particular importance with regard to secretory processes and that any pathological lesions in these proximal segments may have detrimental consequences for sperm maturation. It may also explain

recent reports of pregnancies after epididymovasostomies involving caput segments, where most of the epididymis is bypassed (Silber, 1989) and yet where spermatozoa can still acquire an adequate fertilizing ability.

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