Selective Luminal Absorption of L-Carnitine from the Proximal Regions of the Rat Epididymis

Possible Relationships to Development of Sperm Motility

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The absorption of L-carnitine from the duct of the proximal regions of the rat epididymis was investigated using a stopped-flow, split-droplet microperfusion technique. L-carnitine was absorbed from the duct of the proximal caput epididymidis by a time-dependent and saturable transport system (K_m = 25 μm; V_{max} = 0.65 pmoles absorbed/min/mm^3 tubular volume). Furthermore, absorption appeared to be primarily sodium-independent, although the existence of a minor sodium-dependent pathway cannot be ruled out. A similar transport system was not evident along the distal caput epididymidis, where absorption of L-carnitine was attributable to passive diffusion only. The inward and outward movement of L-carnitine across the epithelium of the proximal and distal caput epididymidis appears to be regulated so that the spermatozoa come into contact with high levels of L-carnitine in the distal caput region.

Key words: L-carnitine, rat epididymis, sperm motility, microperfusion

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It has been well established that one of the main functions of the mammalian epididymis is absorption (Shaver, 1954; Burgos, 1964; Nicander, 1965; Barker and Amann, 1971; Hamilton, 1972, 1975; Setchell and Hinton, 1981). The proximal regions of the epididymis have been shown to be extremely effective in the absorption of water, with up to 50% being absorbed by the rat (Levine and Marsh, 1971), and 90% by the boar and bull epididymis (Crabo, 1965). Larger molecules such as proteins, eg androgen binding protein, are also removed from the lumen by the epididymal cells (Pelliniemi et al, 1981). The cells of the distal region of the epididymis also appear to be involved in absorption of both large and small molecular weight compounds (Friend and Farquar, 1967; Moore and Bedford, 1979; Hinton and Setchell, 1980a; Setchell and Hinton, 1981; Cooper, 1982; Yeung and Cooper, 1982). Furthermore, absorption by the epididymis may be important in phagocytosis of spermatozoa (Simeone and Young, 1931; Flickinger, 1972; Hoffer et al, 1975), as well as the production of a specialized microenvironment (Hinton, 1980). In the present study, we have used a stopped-flow, split-droplet microperfusion technique to determine whether L-carnitine, a constituent of epididymal luminal fluid, is readily absorbed from the epididymal duct. We chose to study the proximal regions of the epididymis, since important sperm maturational changes occur there (Orgebin-Crist et al, 1975; Bedford, 1975).

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Materials and Methods

Rats

Adult male Sprague-Dawley rats (400–470 g; Hilltop, Philadelphia, PA) were housed in the University vivarium under a 12-hour light:12-hour dark (lights on at 0600 hours) cycle with free access to food and water.

Isotopes

The following isotopes were purchased from either New England Nuclear (Boston, MA) or Amersham (Arlington Heights, IL) [^{3}H]-L-glucose (10.7 Ci/mmol), [^{3}H]-L-carnitine (87 Ci/mmol), [^{14}C]-polyethylene glycol (1 mCi/g).

Preparation of Animals for Micropuncture

Rats were prepared for micropuncture as previously described (Hinton et al, 1979a). Microperfusion of the epididymal lumen was performed at either the proximal caput or distal caput regions (sites 2 and 3, respectively, Hinton et al, 1979a) at 34 to 35 C as described below.

Stopped-flow Split-droplet Microperfusion

A stopped-flow, split-droplet technique was used to assess the outward movement of L-carnitine from the epididymal lumen (Fig. 1). A micropipette was inserted into the epididymal duct and approximately 50 to 100 nl of Sudan black-stained mineral oil was introduced into the lumen. A second micropipette containing L-carnitine and tracer amounts of [^{3}H]-L-carnitine together with a water-space marker ([^{14}C]-polyethylene glycol) dissolved in artificial luminal fluid (Table 1) was then inserted into the duct containing the oil. To visualize the perfusion, the artificial luminal fluid contained 0.1% dialysed Lissamine green; it was important to dialyse the dye against double distilled water to remove the sodium ions that are found in high concentrations in commercial preparations of the dye. The solution was perfused (Microject perfusion pump, Biovent Hb, Sweden) into the lumen at a rate of 0.22 μl/min for approximately 20 to 30 minutes until the oil droplet was split. The total volume perfused was then calculated. This flow rate was chosen because the duct did not distend during the perfusion and a good length of duct was completely cleared of luminal contents. The microperfusion pump was then switched off for a period of time (times varied for individual experiments), after which another micropipette was inserted downstream from the perfusion pipette and a luminal fluid sample was collected (approximately 0.5–1.0 μl). Although there were few, if any, spermatozoa in the collected sample, the sample was centrifuged and the radioactivity determined in the fluid as described by Hinton and Howards (1982). In preliminary experiments, it was found that absorption of different organic solutes (glucose, inositol and L-carnitine) was not significantly different if either the artificial luminal fluid (AF; Table 1) or the modified luminal fluid (MAF; Table 1) was used. Consequently, the modified luminal fluid was used in all experiments involving L-carnitine.

To determine whether the microperfusions were adversely affecting the cellular integrity of the caput epithelium an ultrastructural study was undertaken. The ducts of the proximal and distal caput epididymidis were microperfused with artificial luminal fluid alone (n = 2 animals for AF and MAF perfusions) for 60 minutes, after which the rat was perfused with Karnovsky's fixative by retrograde cannulation of the aorta (Forsmann et al, 1977). The portion of epididymis perfused was then removed and the tissue fixed for a further 2 to 3 hours, and then processed for observation by light and electron microscopy. Adjacent, non-perfused epididymal sections were used as controls. The results from this study demonstrated that the artificial luminal fluids did not adversely affect the ultrastructure of the epithelium; coated and non-coated vesicles, mitochondria, plasma and luminal

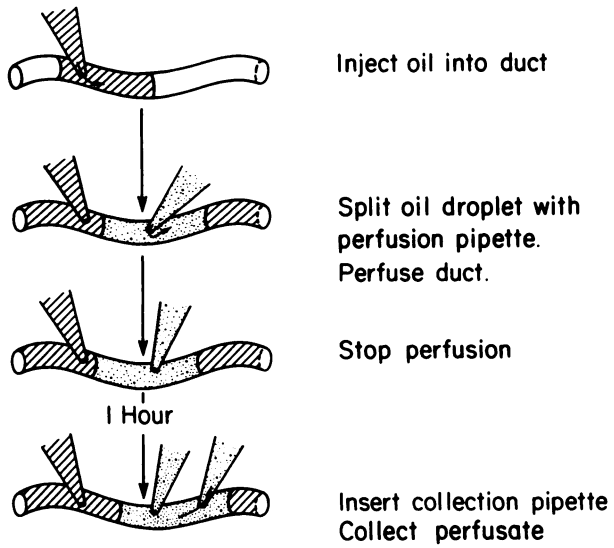

Fig. 1. Schematic diagram illustrating the stopped-flow, split-droplet technique used to study the absorption of L-carnitine from the proximal regions of the rat epididymis. Sudan black stained mineral oil was first introduced into the lumen, then the microperfusion pipette was inserted and the oil column was split as the duct was perfused with the solution under investigation. The perfusion was stopped, time allowed to elapse, and then another micropipette was inserted into the duct downstream from the perfusion pipette and approximately 0.2–0.5 μl of luminal fluid was collected.

TABLE 1. Constituents of Artificial Luminal Fluids (AF) and Modified Artificial Luminal Fluid (MAF) (mM)*

	AF	MAF	MAF-Na^+-Free†
NaCl	55	100	—
Na_2SO_4	10	—	—
K_2HPO_4	10	—	—
$NaHCO_3$	10	—	—
$CaCl_2$	0.5	—	—
$Mg_2SO_4.7H_2O$	1	—	—
Na_2HPO_4	10	—	—
$KHCO_3$	—	25	25
Tris-HCl or KCl	—	—	100
Pipes buffer	10	20	20
Inositol	8	—	—
Raffinose	70	70	70

*pH 6.5 (1 M Tris buffer was used to bring the artificial luminal fluids to their physiologic pH); measured osmolarity = 300–320 mOsm/kg water (Wescor Vapour Pressure Osmometer; Logan, UT).

†MAF-Na^+-free = modified artificial luminal fluid-sodium-free. Glycerylphospholcholine was not added because of the difficulty in removing cadmium from commercially available preparations. Any deficit in osmolarity was made up with raffinose (Samaržija et al, 1982).

membranes, intercellular spaces, etc, appeared normal (Hamilton, 1975). Experiments, therefore, could be conducted with the knowledge that the microperfusion solutions did not alter cellular integrity at the ultrastructural level.

L-carnitine Absorption

Utilizing the stopped-flow, split-droplet microperfusion technique, a series of experiments were designed to determine the extent of absorption of L-carnitine from the lumen of the proximal and distal caput epididymidis. Results from the following experiments were expressed as pmoles absorbed/min/mm^3 tubular volume, except where stated.

Saturation Experiments. Different concentrations of L-carnitine (0.01–0.1 mM) together with 1 μCi/15 μl [^{3}H]-L-carnitine and 0.1 μCi/15 μl [^{14}C]-polyethylene glycol were microperfused into the lumen of the proximal caput epididymidis. Absorption of L-carnitine was determined after one hour, as described above, in 3 to 5 animals at each concentration.

Time-course Experiment. Experiments were performed as described above, except that 0.01 mM L-carnitine, together with each tracer, was perfused into the lumen of the proximal caput epididymidis. Absorption of L-carnitine was estimated at 0, 15, 30, 45, and 60 minutes, and the results expressed as pmoles absorbed/mm^3 tubular volume in 4 to 5 animals at each time point.

Sodium Dependency. Experiments were performed as described above, expect that the sodium in the artificial luminal fluid was replaced by Tris-HCl (Mann and Yudilevich, 1984) or KCl (Table 1) and absorption was estimated after 60 minutes in four animals.

Proximal vs. Distal. The extent of L-carnitine absorption from the lumen of the distal caput epididymidis was determined as described above in four animals.

Passive Diffusion Control. 0.01 mM [^{3}H]-L-glucose was perfused into the lumen of the proximal caput epididymidis of five animals to determine the extent of passive diffusion of an organic solute with a molecular weight similar to that of L-carnitine.

Statistical Analysis

Where appropriate, the data were analyzed by analysis of variance, followed by Duncan's multiple range test ($P < 0.05$).

Results

The absorption of L-carnitine from the lumen of the proximal caput epididymidis took place through a time-dependent and saturable carrier system having an approximate K_m of 25 μM and V_{max} of 0.65 pmoles absorbed/min/mm^3 tubular volume (Figs. 2 and 3).

Absorption of L-carnitine was reduced by approximately 25% when sodium was replaced by either Tris-HCl (n = 3 animals; 0.097 ± 0.004 pmoles absorbed/min/mm^3 tubular volume) or KCl (n = 3 animals; 0.097 ± 0.14 pmoles absorbed/min/mm^3 tubular volume). In Fig. 4, the data from the Tris-HCl and potassium studies have been combined into one category and are designated sodium-free.

Facilitated absorption of L-carnitine seemed to be limited to the proximal caput epididymidis (Fig. 4), since absorption of 0.01 mM L-carnitine by the distal caput epididymidis (0.006 ± 0.003 pmoles absorbed/min/mm^3 tubular volume) was not significantly dif-

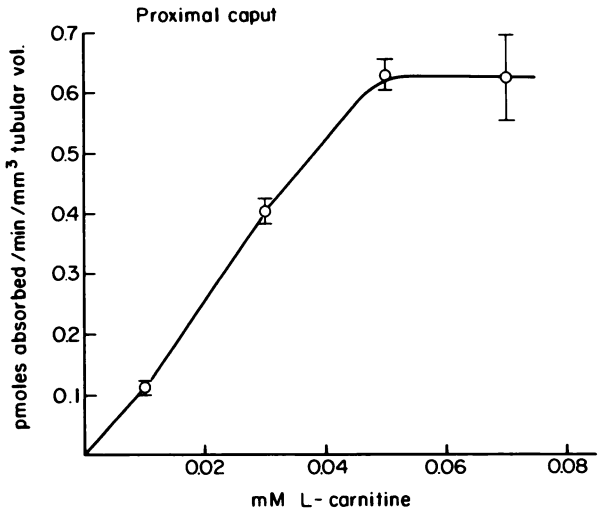

Fig. 2. Saturation curve. Utilizing the stopped-flow, split-droplet microperfusion technique, the absorption of different concentrations of L-carnitine from the lumen of the proximal caput epididymidis was estimated. Values shown are mean ± SEM for n = 3–5 animals at each concentration.

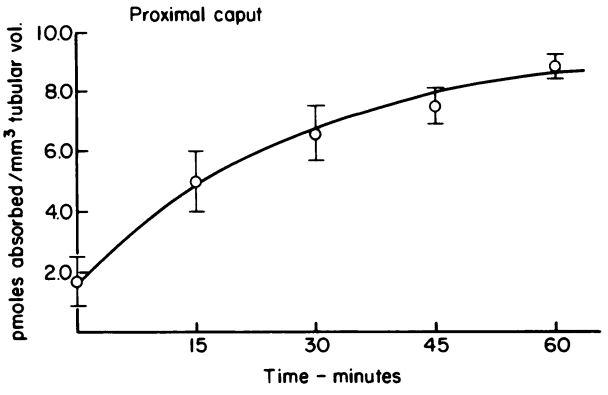

Fig. 3. Time course. Utilizing the stopped-flow, split-droplet microperfusion technique, the absorption of 0.01 mM L-carnitine from the lumen of the proximal caput epididymidis over the period of one hour was estimated. Values shown are mean ± SEM for n = 4–5 animals at each time point.

ferent ($P < 0.05$) from the absorption of the passive-diffusion control, L-glucose (Fig. 4; 0.011 ± 0.007 pmoles absorbed/min/mm³ tubular volume).

Discussion

The secretion and absorption of water, ions, organic solutes and macromolecules, such as proteins, into and out of the epididymis contribute to the formation of a specialized microenvironment. This microenvironment has been considered to play an important role in sperm maturation (Hinton, 1980). The secretory role played by the epididymis is being pursued extensively by several investigators, but the absorptive role has not been elucidated fully.

Our study has shown that there was considerably more outward movement of L-carnitine when it was microperfused into the lumen of the proximal caput epididymidis than when it was perfused into the distal caput. Our results suggest that there is a carrier mechanism located on the luminal membrane of the proximal caput epididymidis which is involved in the outward movement of L-carnitine. This carrier, however, is not seen in the more distal regions of the epididymis, though carriers may be present but have lower affinities and V_{max}. In view of the earlier studies by Brooks et al (1973), Johansen and Bøhmer (1979), Hinton and Setchell (1980b) and James et al (1981), and together with the results of the present study, it would appear that there is considerable control of the movement of L-carnitine into and out of the proximal regions of the rat epididymis. That is, when the epididymis requires L-carnitine, it rapidly transports it into the distal caput lumen (Brooks et al, 1973; Hinton and Setchell, 1980b) and there is very little efflux (see Fig. 4). If, however, the epididymis does not require L-carnitine, there is very little movement into the proximal caput (Brooks et al, 1973; Hinton and Setchell, 1980b), but if some does enter, facilitated efflux occurs (Fig. 3, Fig. 4). The control of the movement of L-carnitine in this region of the epididymis may be very important for sperm maturation in light of the studies performed by Hinton et al (1979b; 1980). These workers have shown that L-carnitine is secreted into the epididymis in the same region as the spermatozoa begin to develop the capacity for motility, and it has been suggested that L-carnitine is important for the development of sperm motility in the rat epididymis. Therefore, in view of these studies and the results of the present investigation, it would seem feasible that the epididymis would need to control the movements of L-carnitine into and out of its lumen so that the spermatozoa come into contact with high levels of L-carnitine in the distal caput epididymidis (Fig. 5).

Our studies have further shown that absorption of L-carnitine from the lumen of the proximal caput epididymidis was primarily sodium-independent since only a 25% reduction in absorption of L-carnitine was seen when sodium was replaced by either Tris-HCl or potassium. The results also suggest that L-carnitine may be absorbed by a minor sodium-dependent pathway.

In preliminary studies, we have found that the sugars, 3-0-methyl-D-glucose and inositol, do not readily leave the lumen of the proximal caput epididymidis. Similar results for inositol absorption from the lumen of the cauda epididymidis have also been shown by Cooper (1982). It would appear, therefore,

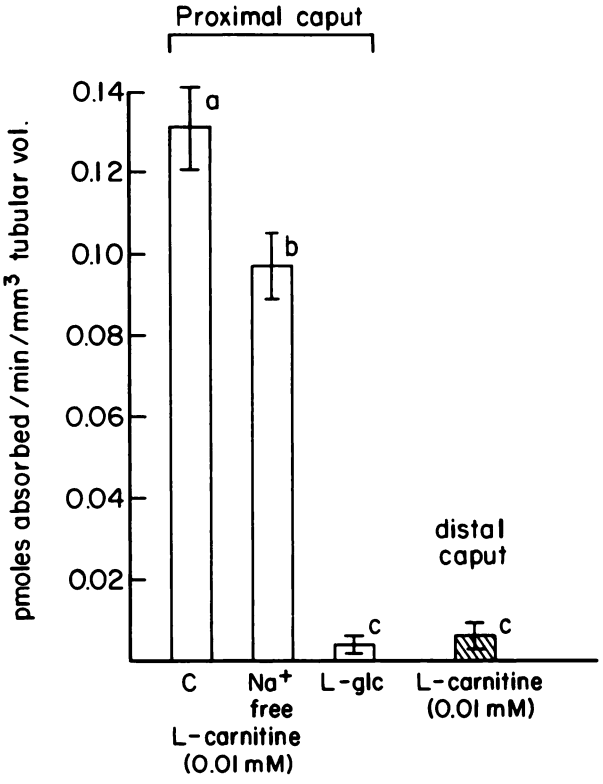

Fig. 4. The absorption of 0.01 mM L-carnitine in Na^+-free luminal fluid (n = 6 animals; combined data from Tris-HCl and potassium data. See results section) and 0.01 mM L-glucose (L-glc; n = 5 animals) from the lumen of the proximal caput epididymidis is compared to the absorption of 0.01 mM L-carnitine (control; the data shown here are the combined data from Figs. 2 and 3, n = 8). Also shown is the absorption of 0.01 mM L-carnitine from the lumen of the distal caput epididymidis. Values shown are mean ± SEM. Mean values with the same lower case letter are not significantly different ($P < 0.05$).

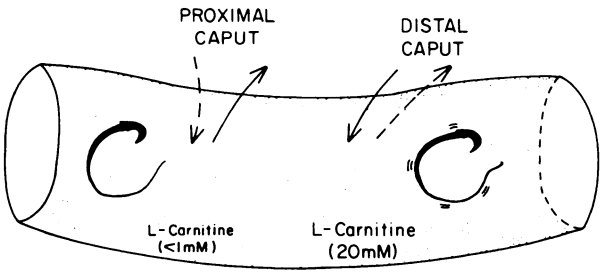

Fig. 5. Schematic representation of the possible interaction between the transepithelial movement of L-carnitine and the development of sperm motility within the proximal regions of the rat epididymis. Spermatozoa enter the proximal caput epididymidis virtually immotile and are bathed in a milieu containing low levels of L-carnitine; the low levels are maintained by (i) limited movement of L-carnitine from the blood to the lumen (broken arrow) and (ii) a substantial absorptive process (arrow). As the spermatozoa progress to the distal caput epididymidis, they become bathed in higher concentrations of L-carnitine and develop the capacity for motility; the higher concentrations are maintained by (i) active transport of L-carnitine from blood to lumen (arrow) and (ii) limited absorption from the lumen (broken arrow). This model is based on data from the present study and from Brooks et al (1973), Hinton et al (1979a, 1979b, 1980), and Johansen and Bøhmer (1979).

that once these sugars are transported into the epididymal lumen, they are kept there and very little transport of them out the epididymal lumen occurs.

In conclusion, the present study has shown that there appears to be a selective permeability barrier to the outward movement of different organic solutes from the proximal regions of the rat epididymis. Furthermore, once compounds that may be necessary for either sperm maturation or epididymal function are transported into the lumen, they do not readily leave.

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International Meeting on Hormonal Therapy of Prostatic Diseases: Basic and Clinical Aspects

An international Symposium on "Hormonal Therapy of Prostatic Diseases: Basic and Clinical Aspects" will be held at the Michelangelo Hotel (Milano, Italy) from May 21 to May 24, 1986. The meeting will be planned by an International Scientific Committee formed by: Bartke A. (USA), Bruchowsky N. (Canada), Geller J. (USA), Motta M. (Italy), Robinson J. (U.K.), Serio M. (Italy), Voigt K.D. (Germany). The program will include invited lecturers as well as sessions of free communications and/or poster presentations on the following topics: The normal prostate: morphological, biochemical and hormonal aspects; The pathological prostate (BPH, Carcinoma, etc.): morphological, biochemical and hormonal aspects; Therapeutic approaches in prostatic diseases (animal and human studies). For further information regarding the program, please contact the Scientific Secretaries. For registration, travel and logistic information, please contact the Organizing Secretariat.

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