KINETICS OF CARNITINE UPTAKE BY RAT EPIDIDYMAL CELLS

Androgen-dependence and lack of stereospecificity

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1. Introduction

Carnitine, a vital cofactor in lipid metabolism, is derived from the diet and is also synthesized in the liver. Other tissues of the body acquire carnitine by uptake from the bloodstream [1]. The process of carnitine uptake into tissues is of considerable importance since one of the factors contributing to human carnitine deficiency is impaired active-uptake into tissues such as muscle [2,3]. There is also good evidence that carnitine plays an important role in the maturation and maintenance of spermatozoa within the epididymal duct [4–6]. It is in this region of the male reproductive tract that immature spermatozoa from the testis develop the potential to fertilize eggs.

It was shown in [7] that the epididymis contains the greatest concentration of carnitine of any tissue in the body. In [8,9] this carnitine was shown to be transported from the blood into the epididymal lumen against a gradient of 2000:1 [6] to establish an intraluminal concentration as high as 60 mM [4,10]. However, little is known of the actual mechanism of carnitine uptake in the epididymis. In vivo the process is androgen-dependent [7,10] and is likely to involve two vectorial pumping sites [6].

Here, we report that dispersed epididymal cells take up carnitine by a saturable process which is apparently not stereospecific since D- and L-isomers display similar kinetics. After castration the rate of carnitine uptake decreased by ~90% and was no longer a saturable process, indicating that androgens are necessary to maintain the normal carnitine-transport system in the epididymis.

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2. Materials and methods

2.1. *Resolution of D-[^{3}H]carnitine and L-[^{3}H]carnitine*

D,L-[^{3}H]Carnitine (1 mCi/μmol from Amersham) was resolved into its isomers by a procedure based on that in [11]. Briefly, 9.6 mM D,L-[^{3}H]carnitine was incubated with 7.7 mM acetyl-CoA, 1.2 mM 4,4'-dithio-bis-pyridine and 10 μg carnitine acetyltransferase in 52 μl total vol. of 0.1 M potassium phosphate (pH 7.0) at 30°C for 1 h. The reaction mixture was then loaded onto pre-coated silica gel aluminium sheets (E. Merck) and developed with methanol/chloroform/water conc. ammonia/formic acid (55:50:10:7.5:2.5 by vol.) [12]. Acetyl-L-[^{3}H]carnitine and D-[^{3}H]carnitine were located using a radiochromatogram scanner; the radioactivity was eluted from these areas (4 ×) with water. Acetyl-L-[^{3}H]carnitine was hydrolysed with 4.4 M ammonia (30°C for 1 h). The samples containing L-[^{3}H]carnitine and D-[^{3}H]carnitine were dried with a stream of nitrogen and the residues were dissolved in 0.1 M potassium phosphate buffer (pH 7.4). The D-[^{3}H]carnitine was taken through the complete procedure again to check that it was free of L-[^{3}H]carnitine. The specific radioactivity of the labelled D-carnitine was assumed to be equal to that of the labelled L-carnitine.

2.2. *Dispersion of epididymal cells*

Isolated cells were obtained after digestion of rat epididymis with collagenase and protease based on the

method in [13]. Rats were decapitated and the epididymides were perfused with saline administered into the aorta [14] until they were visibly clear of blood. That region of the epididymis known to be active in carnitine uptake [8] was dissected free of fat, chopped finely with a razor blade and depleted of sperm by washing twice (10 min at 33°C) with culture medium (1 ml contained 11.1 mg powdered tissue culture medium (TC 199), 4.8 mg Hepes, 1 mg BSA) (pH 7.4). Weighed tissue mince from 4 epididymides was incubated (2 h at 33°C) with 15 ml TC 199 containing 15 mg protease (Sigma, type VI), 15 mg collagenase (Sigma, type I), and 2 mg DNase (Sigma, DN-100). The mixture was agitated by suction with a Pasteur pipette at 20 min intervals. After straining the mixture through nylon mesh, the dispersed cells were gently pelleted by centrifugation and resuspended in TC 199 containing DNase (150 μg/ml). Viability determined by trypan blue exclusion was usually ~90%.

2.3. *Measurement of carnitine uptake*

Radiolabelled carnitine was prepared at various concentrations by dilution with non-radioactive carnitine in TC 199 and added to microfuge tubes (final vol. 50 μl). In each case the radioactive isomer was diluted with the appropriate steric form of the non-radioactive isomer. For L-carnitine, the concentration of the non-radioactive stock solution was checked by enzymatic assay [15]; the D-carnitine stock solution contained 0.7% L-carnitine. Incubations (25 min at 33 °C) began with the addition of 50 μl cell suspension. Carnitine uptake was linear during this period and the radioactivity taken up was decreased with increasing amounts of non-radioactive carnitine in the medium. Incubations were terminated by loading the cell suspension onto 150 μl silicone oil (Dow Corning grades 500 and 200 mixed in proportion 8:2 to give a final specific gravity of 1.025) and rapidly pelleting the cells through the oil by centrifugation at 10 000 × *g* for 10 s in a Beckman microfuge. The silicone oil and incubation medium were decanted and the bottom of the tube containing the cells was cut off with a razor blade and transferred to a scintillation vial for the determination of radioactivity. The amount of extracellular radioactivity adhering to the cells was <0.5% of the volume of the cells. This was accounted for by separating cells and radioactive supernatant at 'zero' time and subtracting these counts from those at the end of the incubation. Typically, the cells accumulated 2–3-times the radioactivity measured at 'zero' time.

Radioactivity was determined by adding the cut-off tube to 1 ml toluene:Triton X-100 (2:1, v/v) containing 5 g PPO/l. Samples were left overnight to allow full extraction of radioactivity into the scintillation fluid. Final counting efficiency was 27%.

3. Results

Dispersed epididymal cells accumulated both L-carnitine and its biologically inactive isomer, D-carnitine, by an apparently saturable mechanism (fig.1). The apparent K_m and V_{max} values for each isomer were calculated from several experiments (table 1). There was no significant difference between the two isomers in either K_m or V_{max} when the results were analysed by Student's *t*-test. Because the results indicated that the carnitine-transport system was not stereospecific, we used a racemic mixture of carnitine for the remainder of this study.

Since epididymal carnitine uptake in vivo and epididymal lipid oxidation is decreased in androgen-deprived animals [7,10,16], we measured the effect of castration on the apparent K_m and V_{max} of D,L-carnitine uptake into dispersed epididymal cells. Prior castration not only decreased the velocity of carnitine uptake (e.g., 10% of the rate in cells from intact ani-

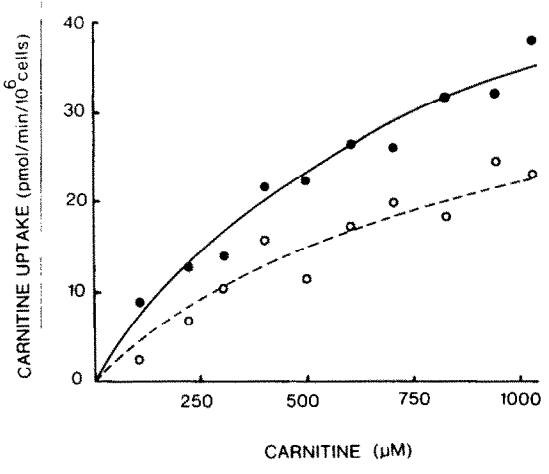

Fig.1. The effect of carnitine concentration on its uptake by dispersed epididymal cells. Each incubation tube contained 0.15 μCi L-[^{3}H]carnitine or 0.24 μCi D-[^{3}H]carnitine at the indicated concentrations and 7.9 × 10^5 cells. Incubation conditions are described in the text and the data represent the means of duplicate determinations. (•) L-carnitine; (○) D-carnitine.

Table 1
K_m and V_{max} values for carnitine uptake

Isomer	App. K_m (μM)	V_{max} (pmol . 10^6 cells . min^{-1})
L-Carnitine	927 ± 174	62 ± 14
D-Carnitine	995 ± 164	49 ± 7

Kinetic parameters were obtained by a direct fit to the hyperbolic plot by the procedure in [27]. The values represent the means and their standard errors from 4 determinations for L-carnitine, and 3 determinations for D-carnitine. In these experiments, the cell no./incubation tube was $5.5 - 7.9 \times 10^5$

mals at 50 μM), but the data further indicate that carnitine uptake is not a saturable process in cells from androgen-deprived rats (fig.2). This was confirmed in other experiments in which the concentration range was extended up to 10 mM.

4. Discussion

The kinetics of L-carnitine uptake have been examined in several rat tissues such as liver, muscle and kidney cortex [12,17–19] and in cultured human heart cells [20]. In these studies estimates of K_m have ranged from 5 μM for human heart cells to 5.6 mM for rat liver cells. The apparent K_m obtained for epididymal cells in this study was ~1 mM, being lower than liver [12] but greater than muscle and kidney [17–19]. Since the apparent K_m is significantly above the normal serum concentration of carnitine (50–90 μM) [1,21] it is expected that the transport system would not be saturated with substrate in vivo.

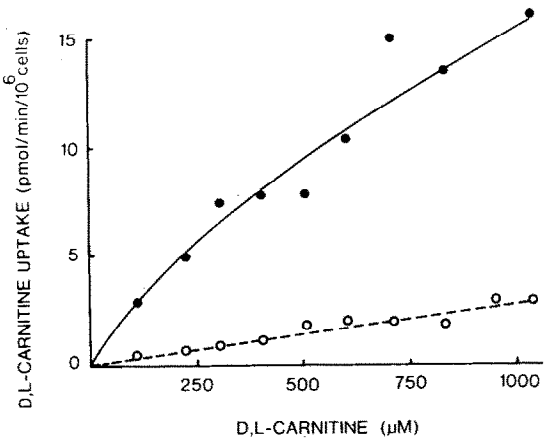

Fig.2. The effect of prior castration on D,L-[^{3}H]carnitine uptake into dispersed epididymal cells. Each incubation tube contained 0.25 μCi D,L-[^{3}H]carnitine and 9.6×10^5 cells from intact rats, or 0.49 μCi D,L-[^{3}H]carnitine and 1.3×10^6 cells from rats castrated for at least 8 weeks. Incubation conditions are described in the text and the data represent the means of duplicate determinations. (•) Cells from intact rats; (o) cells from castrated rats.

D-Carnitine was transported with the same kinetics as L-carnitine and hence the transporting system shows no stereospecificity between the two isomers. In [22] the overall carnitine transport process in the perfused caudal region of the epididymis was stereospecific. However, overall transport is likely to involve both an inwardly-directed basal pump and an outwardly-directed apical pump located in the epithelial cells [6]. It is probable that only the basal pump was measured in our cell preparations and hence the combined results may suggest that the basal pump is not stereospecific whereas the apical pump is.

In a direct study of D-carnitine transport with liver cells a lack of stereospecificity was also found [12]. On the other hand, competition studies suggest that the transport system may be stereospecific in muscle and cultured heart cells [17,23]. Since butyrobetaine is apparently transported by the same carrier as carnitine in liver, heart and muscle [12,18,20] it would seem that the carrier primarily recognizes the quaternary ammonium portion of the molecule. This would explain the ability of other compounds containing this grouping to reduce carnitine transport [17,23]. However, a negatively charged moeity within the molecule may also be important since choline, which contains the quaternary ammonium but lacks a carboxyl group, is a poor inhibitor of carnitine transport [17,22].

By using the protein content of epididymal cells (0.28 mg/10^6 cells) and the total protein content of the epididymis (122 mg/g wet wt tissue [24]), it is possible to convert our results for V_{max} into units equivalent to those used for liver [12] and for muscle [17]. When this is done the maximum velocity of uptake in the epididymis is ~10-times slower than liver but 100-times faster than muscle.

In contrast to the saturable process of carnitine uptake by epididymal cells from normal animals, uptake was slower and non-saturable in cells from castrated animals. The non-saturable uptake by castrate cells may reflect a carrier-independent mode of uptake, in which case it could be postulated that castration results in a loss from the cell membrane of specific carrier molecules. Whatever the mechanism for carni-

tine uptake, the results (fig.2) clearly show that at the concentration of carnitine normally present in plasma (50–90 μM), uptake by cells from normal rats is 10-times greater than that of cells from castrated rats. The difference in uptake kinetics for epididymal cells from the two endocrine states is fully consistent with the observed effect of androgens on epididymal carnitine accumulation in vivo [7,10].

The control of carnitine transport by sex hormones may be a feature of most body tissues since carnitine in blood plasma is raised by androgens and reduced by oestrogens [25] and marked sex differences are noted in carnitine content of liver and muscle and in the rate of tubular excretion in the kidney [21]. Moreover, there is some evidence that the concentration of plasma carnitine may, in itself, regulate the number of membrane carriers [26].

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