Carnitine/organic cation transporter OCTN2-mediated transport of carnitine in primary-cultured epididymal epithelial cells

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Abstract

Carnitine is essential for the acquisition of motility and maturation of spermatozoa in the epididymis, and is accumulated in epididymal fluid. In this study, carnitine transport into primary-cultured rat epididymal epithelial cells was characterized to clarify the nature of the transporter molecules involved. Uptake of carnitine by primary-cultured epididymal epithelial cells was time, Na⁺ and concentration dependent. Kinetic analysis of carnitine uptake by the cells revealed the involvement of high- and low-affinity transport systems with *K_m* values of 21 μ M and 2.2 mM respectively. The uptake of carnitine by the cells was significantly reduced by inhibitors of carnitine/organic cation transporter (OCTN2), such as carnitine analogues and cationic compounds. In RT-PCR analysis, OCTN2 expression was detected. These results demonstrated that the high-affinity carnitine transporter OCTN2, which is localized at the basolateral membrane of epididymal epithelial cells, mediates carnitine supply into those cells from the systemic circulation as the first step of permeation from blood to spermatozoa.

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Introduction

Spermatozoa produced in the testis travel along the epididymis to the vas deferens, and acquire motility and fertilizing ability during their passage through the epididymis. The maturing spermatozoa are protected by the blood–epididymis barrier, which is constituted from epididymal epithelial cells (Hinton *et al.* 1995). This barrier controls the luminal fluid environment, which is different in composition from the blood plasma (Brooks *et al.* 1974, Hinton & Palladino 1995). Carnitine, which is an essential cofactor for fatty acid metabolism, is present in epididymal plasma and spermatozoa at a concentration of 1–63 mM (Marquis & Fritz 1965, Casillas 1972, Hinton *et al.* 1979, Jeulin & Lewin 1996), while the blood plasma concentration is only about 50 μ M, and carnitine is believed to play an important role(s) in sperm maturation (Casillas & Chaipayungpan 1979) and motility (Hinton *et al.* 1981) in the epididymis. Carnitine concentration is reduced in the seminal fluid of infertile patients (Matalliotakis *et al.* 2000), and improvements in sperm motility and viability of spermatozoa can be obtained by treatment with carnitine and acetylcarnitine (Vicari & Calogero 2001). Since the carnitine concentration in the epididymal fluid can reach more than 1000 times that in the blood, active transport mechanisms through epididymal epithelial cells into the lumen are assumed to operate (Bremer

1983). The presence of systems promoting carnitine permeation across epididymal epithelial cells has been reported (Yeung *et al.* 1980, Cooper *et al.* 1986, Radigue *et al.* 1996). In addition, it was found that carnitine uptake in dispersed epididymal cells was saturable and the Michaelis constant (*K_m*) value for carnitine was 927 μ M (James *et al.* 1981).

In mammals, several carnitine transporters have been isolated and characterized. We have reported that carnitine/organic cation transporter (OCTN) 1 and OCTN2 in humans and mice and OCTN3 in mice transport carnitine (Tamai *et al.* 1998, 2000, Yabuuchi *et al.* 1999). OCTN2 is an Na⁺-dependent, high-affinity (Michaelis constant (*K_m*) = 4–25 μ M) carnitine transporter (Sekine *et al.* 1998, Tamai *et al.* 1998, 2000, Wu *et al.* 1999), which serves to maintain the concentration of carnitine in serum by functioning as a reabsorption transporter of carnitine that is eliminated from the blood stream by glomerular filtration (Nezu *et al.* 1999, Yokogawa *et al.* 1999, Tamai *et al.* 2001). It has been reported that human and mouse OCTN1 transport carnitine (Yabuuchi *et al.* 1999, Tamai *et al.* 2000), while rat OCTN1 does not exhibit carnitine transport activity (Wu *et al.* 2000). Human carnitine transporter CT2 and mouse OCTN3, which are present selectively in male reproductive tissues (Tamai *et al.* 2000, Enomoto *et al.* 2002), transport carnitine with high affinity (*K_m* = 20 μ M and 3 μ M respectively) in a sodium-independent manner.

In addition, Nakanishi *et al.* (2001) reported that the Na⁺- and Cl⁻-coupled neutral and cationic amino acid transporter ATB^{0,+} can transport carnitine with low affinity ($K_m = 0.83$ mM). OCTN2 may be the prime candidate for the major transporter, since it is localized at the basolateral membrane of epididymal epithelial cells (Rodríguez *et al.* 2002) and OCTN2-deficient juvenile visceral steatosis (jvs) mice show male infertility with epididymal dysfunction (Toshimori *et al.* 1999).

In the present study, we examined the mechanism of carnitine transport in epididymal epithelial cells by using primary-cultured, rat epididymal epithelial cells, focusing on the involvement of OCTN2 as the major transporter.

Materials and Methods

Materials

L-[³H]carnitine (80.0 Ci/mmol) was purchased from Amersham Biosciences Corp. Collagenase and trypsin were obtained from Sigma Chemical Co. and Becton Dickinson Microbiology Systems (Sparks, MD, USA) respectively. All other reagents, unless otherwise noted, were purchased from Sigma Chemical Co. or Wako Pure Chemical Industries Co. (Osaka, Japan).

Preparation and primary culture of rat epididymal epithelial cells

Epididymal epithelial cells were isolated from 5-week-old Sprague–Dawley rats (Saitama Experimental Animal Supply Co. Ltd, Saitama, Japan) according to the method reported previously (Kierszenbaum *et al.* 1981, Leung *et al.* 2001). To minimize contamination of non-epithelial cells and inhibition of attachment of epithelial cells to culture dishes by spermatozoa, we used immature rats, which do not contain spermatozoa (Kierszenbaum *et al.* 1981, Leung *et al.* 2001). Briefly, epididymides were dissected out and minced into small fragments. These fragments were transferred into 0.25% trypsin (Type II; Sigma Chemical Co.) in Hank's balanced salt solution (HBSS, pH 7.6) and incubated at 32.5 °C for 30 min with shaking (60 cycles/min). The sample was centrifuged (800 *g* for 5 min). The resultant pellet was suspended in 0.1% collagenase I in HBSS (pH 7.6) and incubated at 32 °C for 60 min with shaking (60 cycles/min). The sample was allowed to settle for 5 min, then the supernatant was removed and the sediment was suspended in Eagle's minimal essential medium (EMEM) supplemented with 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 4 mM glutamine, 1 nM 5 α -dihydrotestosterone, 10% fetal bovine serum, 100 units/ml benzylpenicillin and 100 μ g/ml streptomycin. The resultant cell suspension was filtered through four sheets of gauze. Isolated epididymal cells were plated in tissue culture dishes at 5×10^5 cells/ml and incubated at 32.5 °C for 10 h. Contaminating fibroblasts and smooth muscle cells became attached to the dishes within 10 h, so that

epididymal epithelial cells could be separated from non-epithelial cells (Kierszenbaum *et al.* 1981). The supernatant containing epididymal epithelial cells was collected and transferred to new dishes at 1×10^5 cells/cm². Epididymal epithelial cells were cultured at 32.5 °C in supplemented EMEM for 4–5 days and supplemented EMEM was renewed daily. Cultured epididymal epithelial cells were used for RT-PCR analysis and for transport studies. More than 80% of the cells were epithelial cells as judged from immunocytochemistry with anti-pan cytokeratin antibody (AE1/AE3; BIOCARTA, San Diego, CA, USA).

Carnitine transport experiments

Uptake of carnitine in suspended primary-cultured rat epididymal epithelial cells was examined using the same method as in a previous study with primary-cultured Sertoli cells (Kato *et al.* 2005, Kobayashi *et al.* 2005). Briefly, primary-cultured epididymal epithelial cells were harvested with a cell scraper and suspended in transport medium containing 137 mM NaCl, 5 mM KCl, 0.39 mM NaHCO₃, 0.44 mM KH₂PO₄, 0.95 mM CaCl₂, 0.8 mM MgSO₄, 25 mM D-glucose and 10 mM HEPES, adjusted to pH 7.4. The cell suspension was preincubated at 32.5 °C for 20 min in the transport medium, then centrifuged, and the resultant cell pellets were re-suspended in 200 μ l transport medium containing [³H]carnitine to initiate the uptake. At an appropriate time, the cell suspension was diluted with 800 μ l ice-cold transport medium and centrifuged immediately (7000 *g* for 1 min) to terminate the uptake reaction. The cells were then resuspended in ice-cold transport medium and obtained as the pellet after centrifugation. The resultant cell pellets were solubilized in 1 M NaOH and the cell-associated radioactivity was measured with a liquid scintillation counter (Aloka, Tokyo, Japan) using Cleasol-1 (Nacalai tesque, Kyoto, Japan) as a liquid scintillation fluid. Na⁺-free transport medium was prepared by replacing 137 mM NaCl and 0.39 mM NaHCO₃ in the standard transport medium with 137 mM lithium Cl, 137 mM KCl or 137 mM choline Cl and 0.39 mM KHCO₃ respectively, and was used to assess the uptake in the absence of sodium ion.

[³H]Carnitine uptake was usually calculated as observed uptake minus non-saturable uptake, which was taken to be the uptake of [³H]carnitine in the presence of 20 mM unlabeled carnitine.

RNA isolation and RT-PCR

Total RNA was extracted from cultured cells with the ISOGEN RNA extraction solution (Wako Pure Chemical Industries Co.) according to the manufacturer's protocol. cDNA was prepared from the extracted RNA by means of reverse transcription with Improm-II reverse transcriptase (Promega) and oligo(dT) primers according to the manufacturer's instructions. The cDNA was used for PCR amplification under the following conditions. Different sets of

primers were designed and synthesized for PCR analysis of each gene. The primer pair used for amplifying OCTN2, 5'-TTTCGTGGGTGTGCTGATAGTCGC and 5'-GTGGAAGGCGCAACAATCCCATT generated a 487 bp OCTN2 PCR product. For ATB⁰⁺, 5'-AGGTGTGGGAATC-ACGATG and 5'-GTTCACTGGGAAGTTGTCCT generated a 296 bp ATB⁰⁺ PCR product. PCR products were analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide.

Analytical methods

Cellular protein content was determined according to the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Cellular uptake was usually expressed as cell-to-medium ratio ($\mu\text{L}/\text{mg}$ protein), which was obtained by dividing the uptake amount (pmol/mg protein) by the concentration of test compound in the transport medium ($\mu\text{M} = \text{pmol}/\mu\text{L}$).

The apparent kinetic parameters, K_m and maximal transport rate (V_{max}), of carnitine uptake by primary-cultured epididymal epithelial cells were estimated by non-linear regression curve fitting according to the following Michaelis–Menten type equations, where v and $[S]$ are the velocity of substrate uptake and the substrate concentration respectively. In the case of a single saturable component, $v = V_{\text{max}} \times [S]/(K_m + [S])$ (equation 1) and for a system consisting of two saturable components, $v = V_{\text{max}_1} \times [S]/(K_{m_1} + [S]) + V_{\text{max}_2} \times [S]/(K_{m_2} + [S])$ (equation 2). The data were fitted to equation 2 with two saturable transport components, where the indices 1 and 2 indicate the high- and low-affinity components respectively. Non-linear regression analysis was performed using the MULTI program (Yamaoka *et al.* 1981). All data are expressed as means $\pm$ S.E.M., and statistical analysis was performed with Student's *t*-test. The criterion of significance was taken to be $P < 0.05$.

Results

Time-course of [³H]carnitine uptake by primary-cultured epididymal epithelial cells

[³H]Carnitine uptake by primary-cultured epididymal epithelial cells was measured over 60 min at 32.5 °C. Figure 1 shows that [³H]carnitine uptake increased linearly from 15 min to 60 min. The uptake obtained by extrapolation of the data to time zero at 32.5 °C was similar to the non-specific binding of [³H]carnitine estimated by measuring uptake of [³H]carnitine for 1 min at 4 °C (Fig. 1). To estimate non-saturable uptake of [³H]carnitine, uptake of [³H]carnitine in the presence of 20 mM unlabeled excess carnitine was also examined. Unlabeled carnitine (20 μM) significantly inhibited [³H]carnitine (61 nM) uptake by epididymal epithelial cells to $1.33 \pm 0.13 \mu\text{L}/\text{mg}$ protein (Fig. 1). The observed carnitine uptake in the presence of 20 mM unlabeled carnitine corresponded well with the

Figure 1 Time-courses of uptake of [³H]carnitine by rat primary-cultured epididymal epithelial cells. The cells were preincubated for 20 min at 32.5 °C in the transport buffer (pH 7.4). Uptake of [³H]carnitine (61 nM) by rat primary-cultured epididymal epithelial cells in suspension was measured over 60 min in transport buffer (pH 7.4) at 32.5 °C (solid circles). Non-specific binding of [³H]carnitine was estimated by measuring the uptake of [³H]carnitine for 1 min at 4 °C (open circle). Non-saturable uptake of [³H]carnitine for 30 min was estimated by measuring the uptake of [³H]carnitine in the presence of 20 mM unlabeled carnitine at 32.5 °C (solid triangle). Uptake is expressed as cell-to-medium ratio. Each result represents the mean $\pm$ S.E.M. ($n = 3$ or 4).

non-specific binding of [³H]carnitine ($1.30 \pm 0.26 \mu\text{L}/\text{mg}$ protein), suggesting that non-saturable uptake of carnitine by epididymal epithelial cells was negligible. Accordingly, saturable uptake of carnitine for 30 min was estimated by subtracting the uptake of [³H]carnitine in the presence of 20 mM unlabeled carnitine from the observed uptake in the following analysis.

Sodium and concentration dependence of [³H]carnitine uptake by primary-cultured epididymal epithelial cells

To characterize the saturable uptake systems for carnitine in epididymal epithelial cells, sodium dependence was examined. When sodium ion was replaced with Li⁺, K⁺ or choline⁺, uptake of carnitine was significantly decreased (Fig. 2). When sodium ion was replaced N-methylglucamine, uptake of carnitine was also decreased from 2.65 to 1.14 $\mu\text{L}/\text{mg}$ protein, which was comparable with the uptake in the presence of 20 mM unlabeled carnitine ($1.33 \mu\text{L}/\text{mg}$ protein), showing that the sodium-independent carnitine transporter is negligible. Secondly, the concentration dependence of carnitine uptake by epididymal epithelial cells was measured in the range from 1 μM to 3 mM (Fig. 3). An Eadie–Hofstee plot for carnitine uptake by epididymal epithelial cells indicated the involvement of two saturable transport systems (Fig. 3B). Therefore, the data were fitted to equation 2, shown in Materials and Methods. The kinetic parameters were as follows: high-affinity transport system ($K_{m_1} = 21.1 \pm 14.9 \mu\text{M}$, $V_{\text{max}_1} = 19.1 \pm 13.7 \text{ pmol}/30 \text{ min per mg protein}$) and low-affinity transport system ($K_{m_2} = 2193 \pm 504 \mu\text{M}$, $V_{\text{max}_2} = 2872 \pm 418 \text{ pmol}/30 \text{ min per mg protein}$).

Figure 2 Sodium dependence of saturable [³H]carnitine uptake by rat primary-cultured epididymal epithelial cells. The cells were preincubated for 20 min at 32.5 °C in the transport buffer (pH 7.4). Results show the saturable uptake of carnitine evaluated as observed uptake minus non-saturable uptake measured as the uptake of [³H]carnitine in the presence of 20 mM unlabeled carnitine at 32.5 °C. Uptake of [³H]carnitine (38 nM) by rat primary-cultured epididymal epithelial cells in suspension was measured for 30 min in transport buffer (pH 7.4) in the presence (open bar) or absence (solid bars) of Na⁺. Na⁺ was replaced with lithium (Li⁺), potassium (K⁺) and choline (Choline⁺). Uptake is expressed as cell-to-medium ratio. Each result represents the mean ± S.E.M. (n = 3 or 4). *P < 0.05 compared with uptake in the presence of Na⁺.

Evaluation of carnitine transporter expression with RT-PCR

To identify the two saturable transport systems for carnitine, we examined the expression of carnitine transporters in epididymal epithelial cells by means of RT-PCR analysis. Based on the Na⁺ dependence and Km values of epididymal carnitine transporters and the characteristics of previously reported carnitine transporters, we considered that the observed high- and low-affinity carnitine transport might be ascribed to OCTN2 and ATB⁰⁺ respectively. As shown in Fig. 4, the OCTN2 and ATB⁰⁺ genes were expressed in all freshly isolated (day 0), pre-confluent (day 3) and confluent cells (days 4 and 5). These results suggested that OCTN2 and ATB⁰⁺ may be stably expressed in the cells during cultivation. OCTN3 and CT2, which are carnitine transporters expressed in male reproductive tissues in mice and human respectively (Tamai *et al.* 2000, Enomoto *et al.* 2002), seem not be important for transport of carnitine in rat epididymal epithelial cells, since no Na⁺-independent carnitine transport was detected, as shown in Fig. 2. The expression of these types of transporters in rat epididymal epithelial cells could not be examined, since the corresponding rat molecules have not yet been identified.

Effect of various inhibitors on uptake of [³H]carnitine by primary-cultured epididymal epithelial cells

To clarify whether OCTN2 and ATB⁰⁺ functionally contribute to carnitine uptake by epididymal epithelial cells, an inhibition study was conducted. Leucine and lysine

Figure 3 Concentration dependence of saturable uptake of [³H]carnitine in rat primary-cultured epididymal epithelial cells. Uptake of carnitine by rat primary-cultured epididymal epithelial cells in suspension was measured for 30 min in transport buffer (pH 7.4) at 32.5 °C. (A) Results show saturable uptake of carnitine evaluated as observed uptake minus non-saturable uptake measured as the uptake of [³H]carnitine in the presence of 20 mM unlabeled carnitine at 32.5 °C. (B) An Eadie–Hofstee plot of the results is also shown. The solid line represents the uptake estimated from the kinetic parameters, Km and Vmax, given in the Results. V, uptake rate of carnitine (pmol/mg protein); C, concentration of carnitine (μM); V/C, cell-to-medium ratio (μL/mg protein). Each result represents the mean ± S.E.M. (n = 3 or 4).

Figure 4 RT-PCR analysis for carnitine transporter expression in epididymal epithelial cells before and after cultivation. The specific primers described in Materials and Methods were used for determining expression of carnitine transporters in precultured epididymal epithelial cells (0 day) and primary-cultured epididymal epithelial cells (3, 4, and 5 days). cDNAs of kidney and colon were used as positive controls (C) of OCTN2 and ATB⁰⁺ respectively. No signals were detected in samples without reverse transcription (data not shown) as negative controls.

(500 μM), which are inhibitors of $\text{ATB}^{0,+}$ (Nakanishi *et al.* 2001, Umapathy *et al.* 2004), did not show any significant inhibitory effect (Table 1). In contrast, inhibitors of OCTN2 (Sekine *et al.* 1998, Tamai *et al.* 1998, 2000, Ohashi *et al.* 1999, 2001, Wu *et al.* 2000), cationic compounds (quinidine and pyrilamine) and carnitine analogues (acetylcarnitine, carnitine and γ -butyrobetaine) significantly inhibited carnitine transport by epididymal epithelial cells at 500 μM (Table 1).

Discussion

Carnitine is concentrated in epididymal tissue and luminal fluid compared with plasma, and is a key nutrient for sperm maturation and motility. Furthermore, the presence of carnitine transport systems in epididymal epithelial cells has been reported (Yeung *et al.* 1980, James *et al.* 1981, Cooper *et al.* 1986, Radigue *et al.* 1996), though they have not been identified at the molecular level. Accordingly, in the present study, the carnitine transport system(s) in primary-cultured rat epididymal epithelial cells was characterized to identify the transporters that functionally contribute to carnitine transport in these cells.

Kinetic analysis indicated the presence of two types of carnitine transporters, a high-affinity transporter and a low-affinity transporter (Fig. 3). The K_m value of the high-affinity carnitine transporter (21 μM) corresponds to that of OCTN2 (4–25 μM ; Tamai *et al.* 1998, Sekine *et al.* 1998, Wu *et al.* 1999), CT2 (20 μM ; Enomoto *et al.* 2002) or OCTN3 (3 μM ; Tamai *et al.* 2000). OCTN2 and CT2 were found to be expressed in rat (Fig. 4; Rodríguez *et al.* 2002) and human (Enomoto *et al.* 2002) epididymal epithelial cells. The sodium dependence of the carnitine uptake observed in the present study suggested that OCTN2, which is an Na^+ -dependent carnitine transporter

(Tamai *et al.* 1998), is involved in the transport in epididymal epithelial cells, since OCTN3 and CT2 are not sodium ion dependent (Tamai *et al.* 2000, Enomoto *et al.* 2002). However, some contribution of CT2 and OCTN3 to epididymal carnitine transport from the cells to the lumen cannot be completely excluded, since expression of CT2 is limited to the apical membrane of epididymal epithelial cells (Enomoto *et al.* 2002) and carnitine uptake by epididymal cells was measured in suspension in the present study. It should be noted that rat homologues of CT2 and OCTN3 have not yet been identified.

Furthermore, [^3H]carnitine uptake by epididymal epithelial cells was significantly inhibited by inhibitors of OCTN2 (Table 1). Under the conditions used where the substrate is 46 nM, the contributions of high- and low-affinity carnitine transporters, as estimated from the V_{max} and K_m values for each transporter, were about 40% and 60% of total saturable carnitine transport respectively. The reduction of uptake in the presence of inhibitors of OCTN2 is about 30–60% of that in the absence of inhibitors. It is a similar value to the contributions of the high-affinity carnitine transporter. These results suggested that OCTN2 mediates high-affinity transport of carnitine in rat epididymal epithelial cells.

Carnitine is accumulated in epididymal plasma and sperm. To accumulate carnitine in epididymal plasma, carnitine needs to cross the basolateral and apical membranes of epididymal epithelial cells. As the first step, uptake of carnitine should be mediated by a transporter existing at the basolateral membrane of epithelial cells. OCTN2 is localized at the basolateral membrane of epididymal epithelial cells in rats (Rodríguez *et al.* 2002), and was functional in primary-cultured epididymal epithelial cells as demonstrated in this study. In addition, genetically OCTN2-deficient *jvs* mice are infertile, with epididymal dysfunction (Toshimori *et al.* 1999). These results strongly suggest that OCTN2 is functional and essential for carnitine transport at the basolateral membrane of the epididymis.

$\text{ATB}^{0,+}$, a low-affinity ($K_m = 0.83 \text{ mM}$), Na^+ -dependent carnitine transporter (Nakanishi *et al.* 2001), was also detected in epididymal epithelial cells by RT-PCR (Fig. 4). However, the observed effect of $\text{ATB}^{0,+}$ inhibitors did not support the involvement of $\text{ATB}^{0,+}$, though a low-affinity transporter contributed about 60% of total saturable carnitine uptake. These results suggested that $\text{ATB}^{0,+}$ does not functionally contribute to carnitine transport into epididymal epithelial cells. Therefore, the low-affinity carnitine transporter existing in epididymal epithelial cells seems not to be a known transporter.

Carnitine movement into the caput or corpus epididymis is higher than that into the distal part of the tissue (Bohmer *et al.* 1979, Yeung *et al.* 1980, Setchell & Hinton 1981), suggesting that there is a regional difference in the carnitine transport activity in epididymis. However, in the present study we prepared the cells from whole epididymal tissues. Further studies on the regional difference in

Table 1 Inhibitory effect of various compounds (500 μM) on saturable uptake of [^3H] carnitine by rat epididymal epithelial cells. The cells were preincubated for 20 min at 32.5°C in the transport buffer (pH 7.4). Uptake of [^3H]carnitine (46 nM) by rat primary-cultured epididymal epithelial cells in suspension was measured for 30 min in transport buffer (pH 7.4). Results are shown as a percentage of control uptake measured in the absence of inhibitors after subtracting the non-saturable uptake. Uptake is expressed as cell-to-medium ratio. Each result represents the mean $\pm$ S.E.M. ($n = 3$ or 4).

	Relative uptake (% of control)
Control (no inhibitor)	100 $\pm$ 8.4
Amino acids	
Leucine	83.6 $\pm$ 10.1
Lysine	97.5 $\pm$ 13.5
Cationic compounds	
Pyrilamine	59.2 $\pm$ 7.5*
Quinidine	61.2 $\pm$ 7.2*
Carnitine analogues	
Acetylcarnitine	37.5 $\pm$ 11.1*
Carnitine	45.8 $\pm$ 12.6*
γ -butyrobetaine	71.2 $\pm$ 5.7*

* $P < 0.05$ compared with control uptake.

carnitine transport activity will be important to clarify the roles of carnitine transporters in the maturation of spermatozoa.

In conclusion, the present study shows that OCTN2 is functional as a carnitine transporter in epididymal epithelial cells, and that a molecularly unidentified low-affinity carnitine transporter also exists in epididymal epithelial cells. Molecular identification of carnitine transporters localized at the apical membrane of epididymal epithelial cells, and also of the low-affinity carnitine transporter found in this study, will be important to completely understand the mechanism of control of carnitine concentration in epididymal tissues.

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