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Transport of Organic Cations across the Blood−Testis Barrier

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Abstract: Throughout the mammalian spermatogenic pathway, differentiating spermatogenic cells remain in close contact with somatic Sertoli cells, and this has been considered to be essential for the proliferation, differentiation, and survival of spermatogenic cells. It is thought that Sertoli cells form tight junctions to protect developing spermatogenic cells against harmful agents and provide several nutrients for spermatogenesis from the blood stream. Accordingly, Sertoli cells should regulate the movement of various nutritious and xenobiotic compounds by selective membrane transporters. However, the information on membrane transporters in Sertoli cells is limited. In the present study, we characterized the transport systems of organic cations in Sertoli cells. Uptake of [^{14}C]tetraethylammonium (TEA) was measured by primary-cultured rat Sertoli cells. Initial uptake of TEA was concentration dependent, and an Eadie−Hofstee plot indicated the involvement of two saturable transport systems. The apparent K_m values of high- and low-affinity components were comparable to those of previously known organic cation transporter (OCT) or organic cation/carnitine transporter (OCTN). In addition, OCT1, OCT3, OCTN1, and OCTN2 were expressed in Sertoli cells. In conclusion, multiple organic cation transporters, OCTs and OCTNs are expressed in Sertoli cells and the cells regulate transport of cationic compounds to protect and/or maintain the spermatogenesis in testis as the blood−testis barrier.

Keywords: Testis; transporter; Sertoli cell; organic cation; OCT; OCTN

Introduction

Spermatogenesis takes place within the seminiferous tubules of testis. These tubules are composed of peritubular cells, somatic Sertoli cells, and various stages of germ cells. Throughout the mammalian spermatogenic pathway, differentiating spermatogenic cells remain in close contact with Sertoli cells, and this has been considered to be essential for the proliferation, differentiation, and survival of spermatogenic cells.[1−4] Sertoli cells are attached to each other by tight junctions to protect developing germ cells against harmful agents and provide nutrients for spermatogenesis from blood.[1,5,6] The tight junction is important for polarization of Sertoli cells and forms the blood−testis barrier (BTB) between circulating blood and testicular lumen. So, it is thought that Sertoli cells are major components of the BTB. Since the tight junctions in Sertoli cells minimize the nonspecific permeation, membrane transporters are essential to regulate the apparent permeation of various compounds across the BTB. Accordingly, Sertoli cells should be equipped with those transporters. Previously reported transporters in Sertoli cells include multidrug resistance associated

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protein (Mrp) 1,[7] P-glycoprotein,[8] gonad-specific transporter (GST) 1 and 2,[9] hexose transporter GLUT1, 2, and 3,[10,11] and urea transporter UT-A and B.[12] We have also reported molecular and functional involvement of organic cation/carnitine transporter OCTN2[13] and nucleobase transporters.[14] These transporters include both nutrient and drug transporters, and an accumulation of drugs by such transporters in testis may lead to the toxicity of drugs; therefore, it is important to clarify the transporters present at the BTB.

Previously, propranolol and imipramine were reported to be accumulated in testis.[15] Furthermore, cimetidine, a substrate of OCTs, induces testicular toxicity.[16−18] Since these drugs are cationic at physiological pH, they may not be able to cross the BTB without contribution of membrane transporters.

Organic cation transporters consist of organic cation transporters (OCTs), organic cation/carnitine transporters (OCTNs), and recently found multidrug and toxin extrusion (MATE) transporters. OCTs are a family of polyspecific organic cation transporters responsible for the transport of organic cations, including xenobiotics and endogenous compounds. Among the SLC22A transporter family, cation transporters are OCT1, OCT2, and OCT3[19−21] and are distantly related to OCTN1[22] and OCTN2.[23] In rats, OCTN2 and OCT3 are expressed in whole testis and Sertoli cells.[13,24,25] MATE is a recently found organic cation transporter family and is important for the renal excretion of cationic compounds across the apical membrane of renal tubular epithelial cells.[26] The human MATE family currently consists of four members, MATE1, MATE2, MATE2B, and MATE2K.[27] At present the expression of those transporters in testis is not well-known, while mouse MATE2 has been found in testis.[26]

Although there are reports of the expressions of these transporters in the mRNA level, currently, there is no direct evidence of functional expression of OCTs, OCTNs, and MATEs at the BTB. Therefore, in this study we examined the transport of organic cations especially focusing on OCTs and OCTNs in Sertoli cells. For the evaluation of those transporters, we used a primary culture of rat Sertoli cells and examined the transport of tetraethylammonium (TEA)

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to evaluate organic cation transport at the BTB. When required, we used the rat Sertoli cells primary cultured on the Matrigel invasion chambers to obtain the polarized cell monolayers.[28−30]

Experimental Section

Materials. [^{14}C]Tetraethylammonium ([^{14}C]TEA) (55.6 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Collagenase and trypsin were obtained from Sigma Chemicals (St. Louis) and Beckton Dickinson Microbiology Systems (Sparks, MD), respectively. All other reagents, unless otherwise noted, were purchased from Sigma Chemicals or Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Preparation and Primary Culture of Rat Sertoli Cells. Twenty-day-old Donryu rats were purchased from Saitama Experimental Animal Supply Co. Ltd. (Saitama, Japan). Rats were housed three per cage with free access to commercial chow and tap water, maintained on a 12 h dark/light cycle (8:00 a.m. to 8:00 p.m. light) in an air-controlled room (temperature, 24.5 ± 1 °C; humidity, 55 ± 5%). All animal care and experimentation were conducted according to the guidelines of the Tokyo University of Science. Sertoli cells were isolated from twenty-day-old Donryu rats according to the method described previously[13,14] that was based on the reports by Nagao and Shiratsuchi et al.[31,32] Briefly, testes were decapsulated, and seminiferous tubules were gently isolated and then incubated in 35 mL of 0.25% collagenase in phosphate-buffered saline (PBS) for 20 min at 37 °C with occasional stirring. The seminiferous tubules were washed with serum-free F12-L15 medium and then incubated with occasional gentle pipetting in 35 mL of 0.25% trypsin in PBS for 20 min at 37 °C. F12-L15 medium was composed of a 1:1 mixture of Ham's F12 medium (ICN Biomedicals Inc., Irvine, CA) and L-15 medium (ICN Biomedicals Inc.), containing 15 mM HEPES, 10 units/mL penicillin, 0.1 mg/mL streptomycin, and 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA). Trypsin treatment was terminated by adding 5 mL of FBS and 10 mL of F12-L15 medium containing 10% FBS. The resultant cell suspension was filtered twice through four sheets of gauze to remove cell aggregates and tissue debris, after which the cells were collected by centrifugation (300*g*, 10 min). The cells were suspended in 30 mL of F12-L15 medium containing 10% FBS and washed by centrifugation (150*g*, 10 min). Finally, the cells were suspended in F12-L15 medium containing 10% FBS and passed once through nylon mesh (70 μm, BD Biosciences, Bedford, MA). The isolated testicular cells (1×10^8 cells/well) were seeded on the culture dishes (100 mm diameter, #353003, Beckton Dickinson Biosciences) and were grown in F12-L15 medium, containing 1 μg/mL norepinephrine, in a humidified incubator at 32.5 °C for 3 days and at 37 °C for 3 days. Sertoli cells were isolated after removal of the germ cells floating on the surface of the testicular cells. About 90% of the cells adhering to the culture dish were Sertoli cells as judged from staining by Nile red (Molecular Probes Inc., Eugene, OR), which is a marker for Sertoli cells.[33] By about the sixth day of culture after seeding, the cells reached confluence and were used for the measurements of transport activity and RT-PCR analysis. For the uptake experiment across the basolateral membrane of rat primary cultured rat Sertoli cells, the cells were cultivated on 24-well Matrigel invasion chambers (BD Biosciences) at 32.5 °C for 3 days and at 37 °C for 3 days.[28−30]

Transport Experiments. For the transport experiments using suspensions of the primary-cultured rat Sertoli cells, cells were harvested with a cell scraper and suspended in transport medium containing 137 mM NaCl, 5 mM KCl, 0.39 mM $NaHCO_3$, 0.44 mM KH_2PO_4, 0.95 mM $CaCl_2$, 0.8 mM $MgSO_4$, 25 mM D-glucose, and 10 mM HEPES, adjusted to pH 7.4. The cell suspension was preincubated at 37 °C for 15 min in the transport medium and then centrifuged, and the resultant cell pellets were suspended again in 250 μL of transport medium containing [^{14}C]TEA. After the designated time, the cell suspension was diluted with 1 mL of ice-cold transport medium and centrifuged immediately (7000*g*, 1 min) to terminate the uptake reaction. Then, the cells were suspended 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 $NaHCO_3$ in the standard transport medium with 137 mM *N*-methyl-D-glucamine (NMG) Cl or 137 mM KCl and 0.39 mM $KHCO_3$, respectively, and was used to assess the uptake in the absence of Na^+.

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For the experiments by basolateral- and apical-membrane uptakes using Matrigel invasion chambers (BD Biosciences), the medium in each membrane side was replaced with transport medium containing [^{14}C]TEA after preincubation in the transport medium for 15 min. The medium in the opposite side was also replaced with transport medium. At the designated time, the cells on the invasion chamber were rapidly washed twice with ice-cold transport medium, solubilized in 1 M NaOH, and the cell-associated radioactivity was measured by means of a liquid scintillation counter. [^{14}C]TEA uptake was corrected for nonspecific uptake by subtraction of [^{14}C]TEA (20 μM) uptake at 4 °C.

RNA Isolation and RT-PCR. Total RNA was extracted from cultured cells with the Isogen RNA extraction solution (Wako Pure Chemical Industries) according to the manufacturer's protocol. cDNA was prepared from the extracted RNA by means of reverse transcription with Improm-II reverse transcriptase (Promega, Madison, WI) 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 rOCT1 was 5′-CTCTGGCTACAGGAGAACGAC-3′ and 5′-CCTGGTACAGCACAGCACAA-3′, which generated a 403-bp OCT1 PCR product. For rOCT2, 5′-CATCGAGGATGCCGAGAA-3′ and 5′-ACAGACCGTGCAAGCTAC-3′ generated a 246-bp OCT2 PCR product. For rOCT3, 5′-TCAGAGTTGTACCCAACGACATT-3′ and 5′-TCTGCCACACTGATGCAACT-3′ generated a 266-bp rOCT3 PCR product. For OCTN1, 5′-ACCTCAGTGGGTTACTTTGCTC-3′ and 5′-CTCCGCTGTGAAGACGTACA-3′ generated a 297-bp OCTN1 PCR product. For OCTN2, 5′-TTTCGTGGGTGTGCTGATAGTCGC-3′ and 5′-GTGGAAGGCGCAACAATCCCATT-3′ generated a 487-bp OCTN2 PCR product. Reactions were carried out under the following conditions: denaturing at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s. Thirty-five cycles of reaction were performed. PCR products were analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide.

Real-time PCR was performed using Full Velocity SYBR Green QPCR Master Mix (Stratagene, Cedar Creek, TX). The amplification mixture included cDNA as template, 2 × Reaction Buffer (Applied Biosystems), and 0.3 μM primers (OCT3 forward primer, 5′-GCAGCCATATGGCTAGAACTGC-3′; reverse primer, 5′-CGATGCCCTTAGTTTCAGGC-3′; OCTN2, forward primer, 5′-AGAAGCCTCAGTCGCACCA-3′; reverse primer, 5′-TGTCCCCATGCAAGTTAGGAG-3′; forward primer as GAPDH, 5′-GGCTGCTTTTAACTCTGG-3′; reverse primer, 5′-CGGTGCCATGGAATTTGCC-3′), and primers for OCTN1 and OCT1 used quantiTect Primer Assay (Qiagen, Mainz, Germany). Amplification and detection were performed using Mx3000p Real-Time PCR system (Stratagene) with the following profile: 1 cycle at 95 °C for 10 min, 50 cycles each at 95 °C for 15 s and 60 °C for 1 min.

Analytical Method. Cellular protein content was determined according to the method of Lowry et al.[34] with bovine serum albumin as the standard. Cellular uptake was usually expressed as cell-to-medium ratio (μL/mg protein), which was obtained by dividing the uptake amount by the concentration of test compound in the transport medium. The apparent kinetic parameters, K_m (Michaelis constant) and V_{max} (maximal transport rate) of TEA uptake by Sertoli cells, were calculated by nonlinear least-squares regression analysis using MULT1 program[35] according to the following Michaelis−Menten type equations, where v and [s] are the velocity of substrate uptake and substrate concentration, respectively.

$$v = V_{max}[s]/(K_m + [s]) \quad (1)$$

$$v = V_{max1}[s]/(K_{m1} + [s]) + V_{max2}[s]/(K_{m2} + [s]) \quad (2)$$

The data were fitted to eq 2 with two saturable transport components, where the indices 1 and 2 indicate the high- and low-affinity components, respectively.

All data were expressed as means ± SEM, and statistical analysis was performed with Student's t test. The criterion of significance was taken to be $p < 0.05$.

Results

Expression of Organic Cation Transporters in Primary-Cultured Rat Sertoli Cells and Whole Testis. To clarify which transporter proteins for organic cation are expressed in the Sertoli cells, we examined the expression of previously known organic cation transporters, OCT1, OCT2, OCT3, OCTN1, OCTN2, and MATE1, in rat whole testis and in primary-cultured Sertoli cells by means of RT-PCR analysis. As shown in Figure 1A, OCT1, OCT3, OCTN1, and OCTN2 were expressed in both rat testis and primary-cultured Setoli cells, while OCT2 and MATE1 were not detected in Sertoli cells. The copy number of OCTN2 mRNA was higher than OCT1, OCT3, and OCTN1, while that of OCT1 mRNA was lower than other three genes (Figure 1B).

Time Course of TEA Uptake by Primary-Cultured Rat Sertoli Cells. To examine the transport mechanism of organic cations in primary-cultured rat Sertoli cells, uptake of [^{14}C]-TEA was measured as the model substrate of organic cation transporters. The time course of TEA (concentration 20 μM) uptake by the Sertoli cells is shown in Figure 2. The uptakes of [^{14}C]TEA increased linearly up to 10 min in Sertoli cells. Thus, uptake at 5 min was routinely used for the measurement of the initial uptake rate in the subsequent studies. In addition, uptake of [^{14}C]TEA by Sertoli cells at 4 °C was significantly lower than that at 37 °C (0.47 ± 0.02 and 1.15 ± 0.05 μL/mg, respectively), suggesting an involvement of a carrier-mediated transport mechanism.

Concentration Dependence of TEA Uptake by Primary-Cultured Rat Sertoli Cells. To characterize the observed

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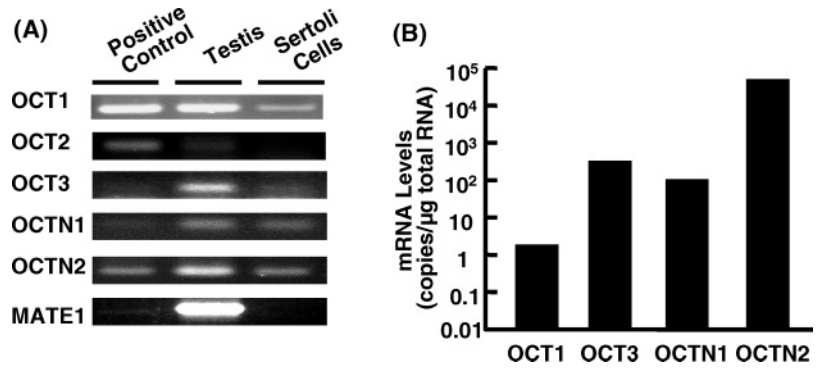

Figure 1. Expression of organic cation transporters in primary-cultured rat Sertoli cells and testis evaluated by RT-PCR analysis. (A) The specific primers described under Experimental Section were used for determining expression of organic cation transporters in rat testis tissues and primary-cultured rat Sertoli cells at 6 days of culture. (B) Expression levels of organic cation transporters were quantitated by real-time PCR assay ($n = 2$). Controls of OCT1, OCTN1, and OCTN2 are liver cDNA, while the controls of OCT2 and MATE1 are kidney cDNA.

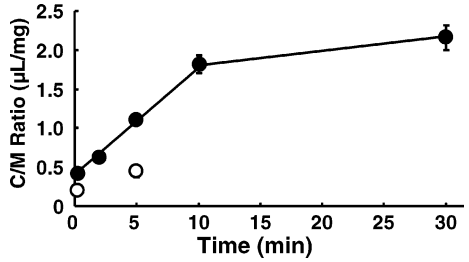

Figure 2. Time course for uptake of [^{14}C]TEA by primary-cultured rat Sertoli cells. Primary-cultured Sertoli cells in suspension were incubated with [^{14}C]TEA (20 μM) for different periods of time at 37 °C (closed circles) or 4 °C (open circles) in the transport medium (pH 7.4). Each point represents the mean ± SEM ($n = 3-4$).

carrier-mediated transport of TEA in Sertoli cells, the concentration dependence of TEA uptake was investigated (Figure 3). Uptake of TEA was saturable and an Eadie−Hofstee plot for the uptake rates after correction for non-saturable uptake (0.56 μL/mg protein/5 min, estimated from the first-order rate constant obtained by nonlinear least-squares regression analysis) indicated the involvement of two saturable transport systems (Figure 3). The kinetic parameters were as follows: high-affinity transport system (K_{m1}= 27.0 ± 13.8 μM, V_{max1} = 8.53 ± 3.3 pmol/mg protein/5 min), and low-affinity transport system (K_{m2} = 7881 ± 414 μM, V_{max2} = 4352 ± 147 pmol/mg protein/5 min).

Effect of pH and Extracellular Cations on Uptake of TEA by Primary-Cultured Rat Sertoli Cells. Next, we examined the effect of pH and extracellular cations on the uptake of [^{14}C]TEA (20 μM) in the absence and presence of unlabeled 20 mM TEA. Apparent uptake of [^{14}C]TEA was increased at acidic pH both in the presence and in the absence of unlabeled TEA. However, the saturable uptake rates evaluated by the difference in uptake between the presence and absence of excess unlabeled TEA were almost comparable, showing pH-independent transport activity (data not shown). When extracellular Na^+ in the transport medium was replaced with NMG^+ at equimolar concentration, the uptake was not changed, while replacement with K^+ caused some decrease of the TEA uptake (Figure 4).

Inhibitory Effect of Various Organic Cations on Uptake of TEA by Primary-Cultured Rat Sertoli Cells. To characterize the substrate specificity of the transport system, the inhibitory effects of various organic compounds on [^{14}C]TEA uptake by Sertoli cells were examined (Table 1). Specific uptake rates were evaluated after correction of the uptake obtained at 4 °C. Carnitine (10 mM), a substrate of OCTN, decreased the uptake to 19% of the control. Guanidine and norephinephrine, which are an inhibitor and a substrate of OCT, respectively, decreased uptake by about 30 to 40% of the control uptake at 10 mM. Other cationic compounds, including other inhibitors of OCTs,[36] also exhibited significant inihibitory effects, including amantadine, cimetidine, imipramine, *N*-methylnicotinamide, pramipexol, and serotonin. In addition, the selective OCT inhibitor, corticosterone, showed significant inhibitory effect.

Basolateral and Apical Uptake of TEA by Rat Sertoli Cells Primary-Cultured on Invasion Chamber. Sertoli cells cultivated on Matrigel were used to evaluate the apical and basolateral uptakes separately, since the culture on Matrigel facilitates the formation of polarized cells.[28] Uptake rates of [^{14}C]TEA (20 μM) from apical (face to seminiferous tubular lumen in vivo) and basolateral (face to blood side) sides of Sertoli cells were decreased in the presence of 20 mM unlabeled TEA (Figure 5), while 200 μM unlabeled TEA reduced only basolateral uptake but not apical uptake. We examined the uptake of TEA at both apical and basolateral transport systems in Sertoli cells based on the results of Figure 5. As a result, K_m of the apical membrane transport should be over 200 μM. Since 200 μM unlabeled TEA completely inhibited basolateral uptake of [^{14}C]TEA by Sertoli cells, it was expected that the K_m of the basolateral transport system should be below 200 μM. In addition, to

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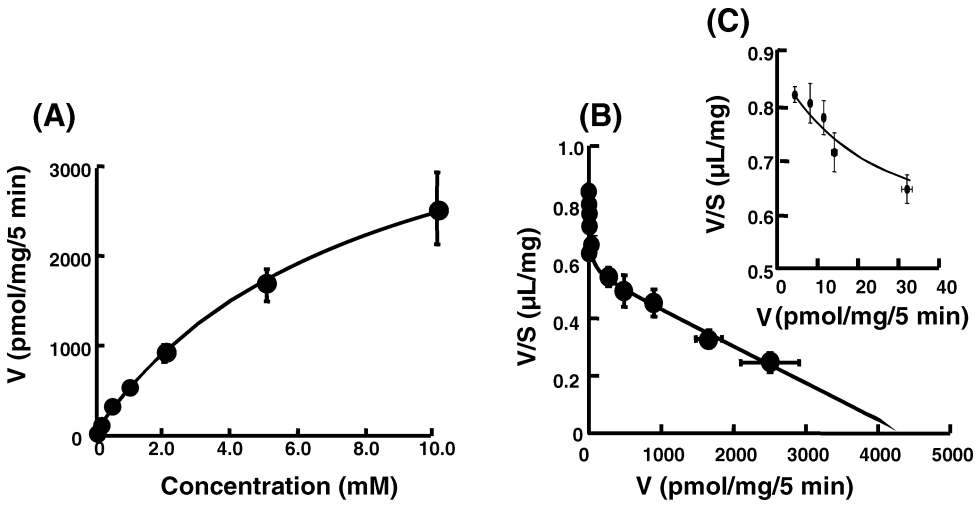

Figure 3. Concentration dependence for uptake of TEA by primary-cultured rat Sertoli cells. (A) Cultured cells were incubated at 37 °C for 5 min. The uptake of TEA by Sertoli cells was measured within the concentration range between 5 μM and 10 mM. (B) Eadie−Hofstee plots of the uptake of TEA by primary-cultured Sertoli cells. (C) Eadie−Hofstee plots of the uptake of TEA at low concentration in Figure 3B. These results were obtained by subtraction of [^{14}C]TEA (20 μM) uptake at 4 °C as the nonspecific uptake from the apparent uptake. Each point represents the mean ± SEM (n = 3−4).

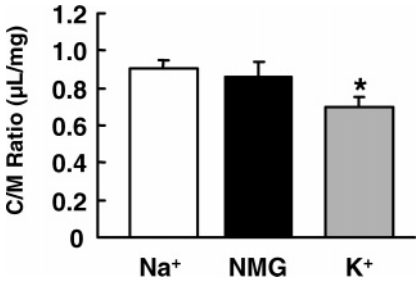

Figure 4. Effect of extracellular cations on uptake of [^{14}C]-TEA by primary-cultured rat Sertoli cells. Uptake of [^{14}C]TEA (20 μM) by primary-cultured rat Sertoli cells was measured for 5 min at pH 7.4. Na^+ (open column) was replaced with *N*-methylglucamine (NMG^+) (closed column) or K^+ (gray column). These results were obtained by subtraction of [^{14}C]-TEA (20 μM) uptake at 4 °C in the presence of Na^+ as the nonspecific uptake. Each point represents the mean ± SEM (n = 3−4), and (*) indicates a significant difference from the control ($p < 0.05$).

Table 1. Inhibitory Effect of Several Compounds for Uptake of [^{14}C]TEA by Rat Primary Cultured Sertoli Cells[a]

inhibitor	concentration mM	[^{14}C]TEA % of control
control		100.0 ± 3.6
carnitine	10	18.7 ± 4.3
guanidine	10	68.9 ± 9.0
norepinephrine	10	61.2 ± 16.4
amantadine	5	2.3 ± 7.0
choline	5	22.3 ± 12.6
cimetidine	5	9.9 ± 5.3
imipramine	5	3.1 ± 4.4
NMN	5	39.7 ± 12.1
pramipexol	5	51.1 ± 1.1
serotonine	5	62.4 ± 5.8
corticosterone	0.5	7.8 ± 8.8

[a] The uptake of [^{14}C]TEA (16.7 μM) by primary cultured rat Sertoli cells in suspension was measured for 5 min at 37 °C in transport buffer (pH 7.4) containing each compound. The data were obtained by the subtraction of the [^{14}C]TEA uptake at 4 °C. Each value represents the mean ± SEM.

estimate the contribution of OCT1 and OCTN2 in basolateral uptake of TEA by Sertoli cells, we demonstrated the inhibitory effect of procainamide and carnitine on TEA uptake by Sertoli cells. As a result, procainamide decreased the uptake by about 30% of the control uptake at 100 μM, while 1 mM carnitine did not affect the uptake of TEA.

Discussion

Normal physiological function of male reproductive tissues is maintained by the selective transfer of physiological and xenobiotic compounds across the BTB, and a part of such transfer is ascribed to membrane transporters. Information on the expression and function of BTB transporters, however, is still limited. In the present study, we examined the transport of organic cations at the BTB using cultured Sertoli cells obtained from 20-day-old rats. This cell culture model is known to form the tight junction between Sertoli cells[30] and to cause the differentiation of spermatogenic cells in vitro.[31] In addition, we previously characterized the transport mechanisms of carnitine, nucleobase, and nucleoside at the BTB using this cultured model.[13,14,37] BTB may have relevance for their pharmacological effect and/or male reproductive toxicity. To characterize the transport of organic cations at the BTB, we evaluated the uptake of TEA, which is a model organic cation, by the primary-cultured rat Sertoli cells. First, we investigated the expression of previously known organic cation transporter molecules (Figure 1). From

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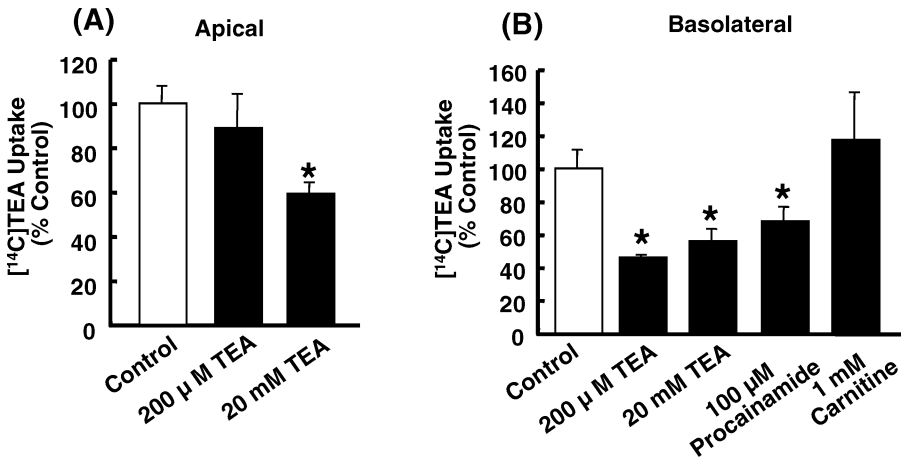

Figure 5. Inhibitory effect of nonlabeled TEA on basolateral and apical uptake of [^{14}C]TEA by rat Sertoli cells primary cultured on Matrigel invasion chamber. Basolateral and apical uptake of [^{14}C]TEA (20 μM) by primary-cultured rat Sertoli cells was measured for 60 min at pH 7.4 in the absence (open column) and presence (closed column) of nonlabeled TEA, 100 μM procainamide, or 1 mM carnitine. Each point represents the mean ± SEM ($n = 3-4$), and (*) indicates a significant difference from the control ($p < 0.05$).

RT-PCR analysis, rOCT1, rOCT3, rOCTN1, and rOCTN2 were detected in the primary cultured Sertoli cells and whole testis tissues of rats. The results are consistent with our previous finding of the expression of rOCTN2 protein and activity in the primary cultured Sertoli cells.[13] Recently, the expression profile of transporters in rat Sertoli cells by the branched DNA signal amplification method was reported by Augustine et al.[25] In that report, they detected rOCT2 as well as several transporters such as rOCT1, rOCT3, rOCTN1, and rOCTN2, while the amount of mRNA of rOCT2 was very low.[25] These results are consistent with the present observation. In addition, we examined the expression of MATE1 in whole testis tissue, which includes spermatogenic cells, Sertoli cells, and Leydig cells, and primary cultured Sertoli cells. As a result, the expression of MATE1 was observed in whole testis, but not in cultured Sertoli cells. It was reported that MATE1 is expressed in Leydig cells.[38] Accordingly, it is possible that other member of MATE1 such as MATE2 and MATE2K might be expressed in Sertoli cells to provide vectorial flux of cationic compounds.

There are no previous studies on the functional activities of organic cation transporter in rat Sertoli cells. In the present study, uptake of [^{14}C]TEA by primary cultured rat Sertoli cells exhibited time dependent increase and the initial uptake at 5 min exhibited significant temperature dependence. The uptake at 4 °C was comparable with the uptake obtained by extrapolating the uptake at 37 °C to time zero. So, the initial uptake at 4 °C was considered to be nonspecific adsorption. Kinetic analysis of TEA uptake by the cells exhibited the presence of two saturable components, with high (26 μM) and low (7881 μM) affinities. The K_m value of the high-affinity component was similar to those reported for rOCT1 (38 μM)[39] and rOCTN2 (63 μM).[40] On the other hand, the K_m or IC_{50} value of the low-affinity component was closer to those of rOCT3 (2.5 mM)[21] and rOCTN1 (0.96 mM).[41] Additionally, the various OCT and OCTN inhibitors significantly reduced the uptake of [^{14}C]TEA (Table 1). Accordingly, apparent uptake of TEA by the primary-cultured rat Sertoli cells is likely mediated by multiple transporters. Uptake of [^{14}C]TEA by the cells revealed Na^+ independence and membrane-potential dependence according to the study of replacement of Na^+ ion with NMG^+ and K^+, respectively. Furthermore, saturable uptake was not essentially pH dependent. The observation suggests that OCT1, OCT3, OCTN1, and/or OCTN2 are possibly involved in the uptake of cationic compounds at the BTB.

To estimate whether organic cation transport systems are functional at the basolateral or apical membranes, we cultivated rat Sertoli cells on a Matrigel-coated invasion chamber according to the previous report by Nakagawa et al.[30] For the confirmation of the formation of polarity of the cultured cells, we evaluated the permeability of inulin and transepithelial electrical resistance of the cells, which can be used for the evaluation of a paracellular permeability.[14]

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(39) Urakami, Y.; Okuda, M.; Masuda, S.; Saito, H.; Inui, K. Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. *J. Pharmacol. Exp. Ther*. **1998**, *287*, 800−805.

(40) Wu, X.; Huang, W.; Prasad, P. D.; Seth, P.; Rajan, D. P.; Leibach, F. H.; Chen, J.; Conway, S. J.; Ganapathy, V. Function characteristics and tissue distribution pattern organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. *J. Pharmacol. Exp. Ther*. **1999**, *290*, 1482−1492.

(41) Wu, X.; George, R. L.; Huang, W.; Wang, H.; Conway, S. J.; Leibach, F. H.; Ganapathy, V. Structure and function characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta. *Biochim. Biophys. Acta* **2000**, *1466*, 315−327.

We observed the reduction of the permeability of inulin and the increase in transepithelial electrical resistance by cultivation of the cells on the Matrigel-coated invasion chamber. In addition, since Nakagawa et al. reported that Sertoli cells formed tight junctions in this culture,[30] our cells cultured on Matrigel also formed the tight junction, suggesting a polarization of the cells. Uptake of [^{14}C]TEA (20 μM) from the basolateral side was reduced by 200 μM and 20 mM concentration of unlabeled TEA, while the uptake from the apical side was reduced only by 20 mM unlabeled TEA. Accordingly, it is considered that the low- and high-affinity organic cation transporters are expressed at the apical and basolateral membranes, respectively. Considering the K_m values of TEA uptake by rOCTs and rOCTNs, it was suggested that the high-affinity transporters rOCT1 and/or rOCTN2 are functional at the basolateral membrane of rat Sertoli cell. On the other hand, the low-affinity transporters rOCT3 and/or rOCTN1 may be functional at the apical membrane. Previously, we reported that rOCTN2 mediates the transport of carnitine from blood to seminiferous tubule at the basolateral membrane of rat Sertoli cell.[13] However, in this study, procainamide decreased the uptake by about 30% of the control uptake at 100 μM, while 1 mM carnitine did not affect the uptake of TEA. Urakami et al. reported that the apparent K_i value of procainamide for TEA uptake of rat OCT1 was 47 μM.[42] In addition, Wu et al. reported that procainamide decreased rat OCTN2-mediated TEA uptake to about 25% of the control uptake at 2.5 mM.[40] Accordingly, it is possible that the transport of TEA is mainly accounted for by OCT1 at the basolateral side in Sertoli cells.

On the other hand, carnitine inhibited the TEA uptake by the cells in suspension, which represents both the basolateral and apical membrane uptakes. It was possible that the inhibitory effect of carnitine on TEA uptake observed in the present study might be accounted for by OCTN1 rather than OCTN2. The testis-to-plasma concentration ratio of TEA was comparable between wild-type and juvenile visceral steatosis mice, which show the phenotype of systemic carnitine deficiency,[43] suggesting that the contribution of OCTN2 to the blood to testis flux of TEA may not be extensive. Therefore, it is considered that the present result shows good coincidence with that observed in TEA disposition in jvs mice. In this study, we especially focused on the basolateral uptake of cationic compounds. Further studies will be required to identify the mechanisms of apical membrane transport from seminiferous tubules into Sertoli cells for organic cations to fully understand the transport of cationic compounds at the BTB.

In conclusion, it was demonstrated for the first time that multiple organic cation transporters are expressed at the BTB and differential transport proteins may be expressed at the apical and basolateral membranes of the Sertoli cells.

Abbreviations Used

BTB, blood testis barrier; FBS, fetal bovine serum; OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; PBS, phosphate-buffered saline; RT-PCR, reverse transcription polymerase chain reaction

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