Inner Blood–Retinal Barrier Mediates L-Isomer-Predominant Transport of Serine

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ABSTRACT: D-Serine, a coagonist for N-methyl-D-aspartate-type glutamate receptors, which mediate visual signal transmission, is thought to be generated from L-serine via serine racemase in the retina. However, the source of L-serine and D-serine in the retina are yet to be determined. The purpose of the present study was to investigate the characteristics of the blood-to-retina transport of serine at the inner blood–retinal barrier (BRB). *In vivo* study revealed the blood-to-retina transport of [^{3}H]L-serine with an influx clearance of 49.9 μL/(min·g retina), which is greater than that of [^{3}H]D-serine. This was consistent with the L-isomer-predominant uptake of serine by conditionally immortalized rat retinal capillary endothelial cell line (TR-iBRB2 cells), an *in vitro* inner BRB model. [^{3}H]L-Serine and [^{3}H]D-serine uptake by TR-iBRB2 cells took place in an Na^+-dependent and a concentration-dependent manner with Michaelis constant values of 97.5 μM and 9.63 mM, respectively. The uptake process of [^{3}H]L-serine and [^{3}H]D-serine was significantly inhibited by system ASC (alanine–serine–cysteine) substrates. Polymerase chain reaction analysis and immunocytochemistry revealed the expression of ASC transporters ASCT1 and ASCT2 in TR-iBRB2 cells. These results suggest that the system ASC at the inner BRB is a potent pathway for supplying serine in the form of the L-isomer from the circulating blood to the retina. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:3892–3903, 2011

Keywords: L-serine; D-serine; system ASC; blood–retinal barrier; blood–brain barrier; transporters; active transport; distribution; pharmacokinetics; endothelial cell

INTRODUCTION

D-Serine is the physiologically relevant ligand for the glycine modulatory site of N-methyl-D-aspartate (NMDA)-type glutamate receptors,[1] which mediate visual signal transmission in the retina.[2] Indeed, the D-serine concentration determined from microdialysis samples of salamander retina is in the low micromolar range,[3] which is sufficient to activate NMDA receptors.[4] Because a reduction in the cerebral D-serine level causes NMDA receptor hypofunction, which is likely to occur in a neuronal disorder such as schizophrenia, changing levels of D-serine in the retina may also affect the transfer of visual signals via NMDA receptors. It has been proposed that D-serine in the retina is produced by L-serine isomerization via serine racemase[5] because serine racemase is localized in neurons such as ganglion cells, as well as glial cells such as Müller cells and astrocytes.[1,6,7] L-Serine is present in the retina at a concentration of approximately 500 nmol/mg protein, greater than that of D-serine in rats.[1,8] These findings imply that the availability of L-serine is a key determinant of normal NMDA receptor function in the retina. However, the source of L-serine and D-serine in the retina remains largely unexplored.

In the brain, L-serine is derived mostly from *de novo* biosynthesis involving D-3-phosphoglycerate dehydrogenase (Phgdh), which catalyzes the first step of L-serine synthesis via a phosphorylated pathway. Indeed, brain-specific *Phgdh* gene deletion results in marked simultaneous reductions in the L-serine and D-serine contents of the brain.[9] However, Oldendorf and Szabo[10] proposed carrier-mediated transport of L-serine from the circulating blood to the brain across

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the blood–brain barrier (BBB). Intraperitoneal single administration of L-serine (105–131 μmol/mouse) caused significant increases in the L-serine and D-serine contents of the adult mouse cortex with *Phgdh* gene deletion.[9] These findings suggest that the blood-to-brain transport of L-serine across the BBB plays an important role in maintaining the levels of L-serine and D-serine in the brain, at least when the capacity of *de novo* L-serine synthesis in the brain is reduced. Like the BBB, the inner blood–retinal barrier (BRB) is formed by complex tight junctions of capillary endothelial cells, and it plays a crucial role in supplying amino acid from the circulating blood to the neural retina via influx transporters.[11] This prompted us to hypothesize that the L-isomer-predominant transport system at the inner BRB could be involved in the control of serine levels in the retina.

Cellular transport of the neutral amino acid L-serine is mediated by several systems, distinguished primarily by their substrate specificity and ionic requirements: Na^+-dependent transporters of system ASC (for alanine, serine, and cysteine-preferring)[12–15] and system A (for alanine-preferring),[16] Na^+- and Cl^--dependent transporter of system B^{0+},[17] and Na^+-independent transporters of system asc[18] and system L.[19] The transport of D-serine is mediated by system ASC,[20] system B^{0+},[17] and system asc.[18] Of these transport systems it has been reported that alanine–serine–cysteine transporter 1 (ASCT1/SLC1A4)[21] and ASCT2/SLC1A5[22,23] (isoforms of system ASC), sodium-coupled neutral amino acid transporter 2 (SNAT2/ATA2/SLC38A2)[24,25] (an isoform of system A), and L-type amino acid transporter 1 (LAT1/SLC7A5)[26,27] (an isoform of system L) are expressed at the inner BRB and/or BBB. Because reverse transcription polymerase chain reaction (RT–PCR) analyses reveal that amino acid transporter B^{0+} (ATB^{0+}) (an isoform of system B^{0+}) mRNA is not expressed in isolated brain capillaries and conditionally immortalized rat retinal/brain capillary endothelial cell lines (TR-iBRB2 and TR-BBB13 cells), an *in vitro* model of the inner BRB/BBB,[23,28] the contribution of ATB^{0+} to L-serine and D-serine uptakes can be negligible at the inner BRB and BBB.

The purpose of the present study was to investigate the characteristics of the blood-to-retina transport of serine at the inner BRB by means of *in vivo* integration plot analysis in rats, and the uptake study using TR-iBRB2 cells.[29] The serine uptake by TR-iBRB2 cells was compared with those in TR-BBB13 cells.[30]

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 250–300 g, were purchased from Japan SLC (Hamamatsu, Japan). The investigations using rats described in this report conformed to the provisions of the Animal Care Committee, University of Toyama, and the Association for Research in Vision and Ophthalmology (ARVO) statement on the Use of Animals in Ophthalmic and Vision Research.

Reagents

[^{3}H]L-Serine (29.5 Ci/mmol) and [^{3}H]L-glutamine (49.8 Ci/mmol) were obtained from Perkin–Elmer Life and Analytical Sciences (Boston, Massachusetts). [^{3}H]D-Serine (20 Ci/mmol) was obtained from Moravek Biochemicals (Brea, California). All other chemicals were commercial products of analytical grade.

In Vivo Blood-to-Retina [^{3}H]L-Serine and [^{3}H]D-Serine Transport Analysis

The apparent blood-to-retina influx permeability clearance ($CL_{retina,influx}$) of L-serine and D-serine was determined by integration plot analysis, as described previously.[31,32] Briefly, the rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg body weight) and then [^{3}H]L-serine or [^{3}H]D-serine (3 μCi/rat) dissolved in 400 μL extracellular fluid (ECF) buffer consisting of 122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM K_2HPO_4, 10 mM D-glucose, and 10 mM Hepes (pH 7.4) was injected into the femoral vein. After collection of blood samples, rats were decapitated and their retinas were removed. The sampling time points were 3, 5, and 10 min for L-serine and 5, 10, and 20 min for D-serine. The retinas were dissolved in 2 N NaOH and subsequently neutralized with 2 N HCl. The radioactivity was measured in a liquid scintillation counter (LSC-5000, Aloka, Tokyo, Japan). As an index of the tissue distribution characteristics of each compound, the apparent tissue-to-plasma concentration ratio ($K_{p,app}$) was used.[32] This ratio [$K_{p,app}(t)$] (mL/g retina) was defined as the amount of [^{3}H] per gram retina divided by that per milliliter plasma, calculated over the time period of the experiment. In brief, the retinal uptake rate of [^{3}H]L-serine and [^{3}H]D-serine can be described by Eq. 1:

$$Kp,\,app(t) = CL_{retina,influx} \times AUC(t)/Cp(t) + Vi \quad (1)$$

where $C_p(t)$ (dpm/mL) and V_i (mL/g retina) represent the plasma concentration at time t and the rapidly equilibrated distribution volume of [^{3}H]L-serine or [^{3}H]D-serine, respectively; $AUC(t)$ [(dpm·min)/mL] is the area under the plasma concentration–time curve of [^{3}H]L-serine or [^{3}H]D-serine from time 0 to t.