The blood–brain barrier (BBB) segregates the circulating blood from interstitial fluid in the brain, and restricts drug permeability into the brain. Our latest studies have revealed that the BBB transporters play important physiological roles in maintaining the brain milieu. The BBB supplies creatine to the brain for an energy-storing system, and creatine transporter localized at the brain capillary endothelial cells (BCECs) is involved in BBB creatine transport. The BBB is involved in the brain-to-blood efflux transport of the suppressive neurotransmitter, γ-aminobutyric acid, and GAT2/BGT-1 mediates this transport process. BCECs also express serotonin and norepinephrine transporters. Organic anion transporter 3 (OAT3) and ASCT2 are localized at the abluminal membrane of the BCECs. OAT3 is involved in the brain-to-blood efflux of a dopamine metabolite, a uremic toxin and thiopurine nucleobase analogs. ASCT2 plays a role in l-isomer-selective aspartic acid efflux transport at the BBB. Dehydroepiandrosterone sulfate and small neutral amino acids undergo brain-to-blood efflux transport mediated by organic anion transporting polypeptide 2 and ATA2, respectively. The BBB transporters are regulated by various factors, ATA2 by osmolarity, taurine transporter by TNF-α, and l-cystine/l-glutamic acid exchange transporter by oxidative stress. Clarifying the physiological roles of BBB transport systems should give us important information allowing the development of better CNS drugs and improving our understanding of the relationship between CNS disorders and BBB function.

Key words  blood–brain barrier; transporter; brain capillary endothelial cell; creatine; neurotransmitter; neuro-modulator

The blood–brain barrier (BBB) is formed by complex tight-junctions of the brain capillary endothelial cells (BCECs) and expresses various transport systems. These characteristics of the BBB make it possible to control selective transport across the BBB and to limit the non-selective brain distribution of drugs. Since understanding the mechanism of BBB transport is important for improving the BBB permeability of CNS-acting drugs, the molecular mechanism of the BBB transport has been analyzed mainly with respect to drug transport. At first, blood-to-brain influx transport was regarded as a system for transporting drugs and nutrients to the brain. This resulted in the discovery of transport systems for glucose and amino acids. Subsequently, the importance of efflux transport at the BBB has been recognized after finding that P-glycoprotein (P-gp) is involved in the efflux transport of xenobiotics. Due to these studies, the BBB is now accepted as a complex transport system which is an important determinant of drug distribution to the brain.

In addition to the drug permeability aspects, the physiological function of the BBB is important in the central nervous system (CNS). To date, the BBB transport system has been shown to supply nutrients, such as glucose, lactate, amino acids and nucleotides, to the brain. However, it is conceivable that the BBB have many other physiological functions involving regulation of the CNS milieu. For instance, the brain is a highly energy-consuming tissue and produces various metabolites. In particular, metabolism is essential for the inactivation of neurotransmitters after their secretion from the pre-synapses, as well as re-uptake. The metabolites produced in the brain must be removed in order to maintain proper neural function. Therefore, the BBB can be thought to act as a clearance system to remove various metabolites from the CNS.

Clarifying the physiological role of the BBB is also necessary to understand the role of the BBB in CNS disorders. This is because an alteration in BBB functions may result in a change in CNS conditions leading to CNS disorders. Furthermore, analyzing the BBB functions from a different point of view could result in the discovery of a new transport system that is involved in drug permeability. In this review, I shall introduce the recent advances in research into the molecular basis of BBB transport, focusing on its physiological functions.

The BBB Supplies Creation to the Brain  The brain consumes 18% of total body energy, while its weight is only 2% of the total body weight. The brain cannot store glucose for energy synthesis, and the BBB plays a crucial role in glucose supply to the brain by expressing glucose transporter 1 (GLUT1) (Fig. 1). Besides energy synthesis, an energy-storing system is necessary for maintain energy homeostasis in high energy-consuming tissues. We have shown that the BBB also plays an important role in the energy storing process (Fig. 1).

Creatine plays a key role in this energy-storing process. The phosphate-bound energy of ATP is transferred to creatine and stored as phosphocreatine to regenerate ATP from ADP (Fig. 1). In the human brain, the creatine level is about 180-fold higher than that in plasma. Furthermore, the brain is a key target for creatine deficiency syndrome which involves a reduced level of creatine in the body due to impairment of creatine synthesis. The patients exhibit delayed psychomotor development, hypotonia, seizures, and delayed myelination. Phosphocreatine is also reduced in the brains of mildly demented patients with Alzheimer’s disease. Therefore, the creatine/phosphocreatine shuttle system plays an important role in energy homeostasis in the brain to ensure...
Proper development and function.

Creatine in the body is maintained by biosynthesis mainly in the liver and kidney, and by dietary supplementation. Therefore, muscle creatine is supplied from the circulating blood via creatine transporter (CRT). In contrast, the creatine supply from the circulating blood to the brain has been reported to be limited, since oral administration of 20 g creatine per day for 4 weeks produces only about a 9% increase in total creatine in the human brain. Braissant et al. have also reported that mRNA expression of CRT was not detected in brain capillaries, while that of the enzyme involved in creatine biosynthesis was detected in neural cells.

We have been analyzing neurotransmitter transporters by using conditionally immortalized mouse BCECs (TM-BBB), as an in vitro BBB model. The CRT cDNA fragment was amplified by RT-PCR from TM-BBB cells possibly due to the similarity in the nucleotide sequence between CRT and neurotransmitter transporters. Furthermore, TM-BBB cells exhibited creatine uptake which is significantly inhibited by CRT inhibitors, such as $\beta$-guanidinopropionate and guanidinocacete. Our immunohistochemical analysis revealed that there is intense expression of CRT in mouse BCECs (Fig. 2). Furthermore, 24 h after systemic administration, $[14C]$creatinine uptake which was Na$^+$ dependent with a $K_m$ of 679 $\mu$M corresponds to GAT2 in the mouse, is involved in the efflux transport of GABA at the BBB. TM-BBB cells showed $[3H]$GABA uptake which was Na$^+$-, Cl$^-$-, and concentration-dependent with a $K_m$ of 679 $\mu$M. GAT2/BGT-1 expression in TM-BBB cells and mouse brain capillary-rich fraction has been detected by RT-PCR and Western blot analysis, while mRNA expression of neuronal and glial GABA transporters (GAT1 and 3) was not detected in TM-BBB cells. This result suggests that the GABA transporter expressed at the BBB is not merely as a barrier, it also acts as a regulatory interface for neurotransmitters.

The Brain-to-Blood Efflux Transport of Neurotransmitters

Neurotransmitter inactivation is a critical process for proper neural function. The uptake and metabolism of neurotransmitters by neurons and astrocytes have been well-characterized. The function of the BBB used to be considered to be retention of neurotransmitters in the brain. However, our recent studies have shown that $\gamma$-aminobutyric acid (GABA), a suppressive neurotransmitter, undergoes efflux from the brain across the BBB rather than being retained in the brain. Therefore, the physiological role of the BBB is not merely as a barrier, it also acts as a regulatory interface for neurotransmitters.

Betaine/GABA transporter-1 (BGT-1; SLC6A12), which corresponds to GAT2 in the mouse, is involved in the efflux transport of GABA at the BBB. TM-BBB cells showed $[3H]$GABA uptake which was Na$^+$-, Cl$^-$-, and concentration-dependent with a $K_m$ of 679 $\mu$M. GAT2/BGT-1 expression in TM-BBB cells and mouse brain capillary-rich fraction has been detected by RT-PCR and Western blot analysis, while mRNA expression of neuronal and glial GABA transporters (GAT1 and 3) was not detected in TM-BBB cells. This result suggests that the GABA transporter expressed at the BBB is not merely as a barrier, it also acts as a regulatory interface for neurotransmitters.
different from that expressed at neurons and astrocytes. Indeed, the efflux rate of \(^{3}H\)GABA across the BBB measured by the Brain Efflux Index (BEI) method \(^{19}\) was not inhibited, but increased by nipecotic acid, which is a specific inhibitor of neuronal and glial GABA transporters. \(^{20}\) Immunohistochemical analysis of mouse brain revealed that GAT2/BGT-1 is localized at the BCECs. \(^{18}\) Therefore, GAT2/BGT-1 is suggested to act as a BBB efflux transporter of GABA (Fig. 3).

As far as monoamines are concerned, the uptake of norepinephrine and serotonin has been reported using isolated brain capillaries. \(^{21,22}\) TM-BBB cells express norepinephrine transporter (NET) and serotonin transporter (SERT). Immunohistochemical analysis showed that NET is localized at the abluminal membrane of mouse BCECs, and SERT is localized at both the abluminal and luminal membranes (Fig. 3). \(^{23}\) The brain microvasculature is thought to be regulated by monoamines released from adrenergic and serotonergic neurons, since adrenergic and serotonergic receptors are in close apposition to the brain capillaries which express adrenergic and serotonergic receptors. \(^{24-27}\) The abluminally localized NET and SERT would function as an inactivation system for neurotransmitters around the brain capillaries.

Tricyclic antidepressants inhibit the presynaptic terminal reuptake systems to produce their therapeutic effects by increasing monoamine concentrations in the synaptic clefts. The tricyclic antidepressants, amitriptyline and imipramine, increase BBB permeability in addition to their main pharmacological effect. \(^{28}\) Hardebo et al. and Spatz et al. have reported that norepinephrine and serotonin uptake by isolated brain capillaries is inhibited by these tricyclic antidepressants, which inhibit NET and SERT. \(^{21,22}\) Amitriptyline and imipramine may inhibit the activity of NET and SERT expressed at the BBB, resulting in increased levels of monoamines around the brain capillaries and altered BBB permeability.

The function of luminally localized SERT is still unclear. Ganapathy et al. have reported that SERT is localized at the human placental brush-border membrane facing the maternal blood. \(^{29,30}\) They have suggested that the clearance of serotonin from the intervillous space may be necessary to maintain the uteroplacental blood flow, since serotonin is a potent vasoconstrictor. \(^{29}\) Furthermore, in the blood coagulation system, serotonin secreted from platelets enhances blood coagulation. Therefore, maintaining a low plasma serotonin level may prevent inappropriate blood coagulation in the cerebral microvasculature. Based on these pharmacological functions of serotonin, one possible function of the luminally localized SERT is clearance of serotonin from the cerebral intravascular space to maintain cerebral blood flow.

The Stereo-Selective Brain-to-Blood Efflux Transport of Acidic Amino Acids Recently, \(D\)-amino acids have been found to be present in mammals. In the brain, \(D\)-aspartic acid (\(D\)-Asp) functions as a precursor of NMDA and also influences the secretion of several hormones, such as growth and luteinizing hormone, testosterone, melatonin and oxytocin. \(^{31}\) In contrast, \(L\)-Asp is an excitatory amino acid as well as \(L\)-glutamic acid (\(L\)-Glu). \(^{32}\) We have reported that the BBB can carry out stereo-selective efflux transport of Asp, transporting the \(L\)-isomer but not the \(D\)-isomer. \(^{33}\) This \(L\)-isomer-selective Asp efflux transport is consistent with the CNS function of each isomer of Asp, since the accumulation of \(L\)-Asp leads to excitatory neurotoxicity, while \(D\)-Asp needs to be stored as a precursor for NMDA. Therefore, the stereo-selective BBB efflux transport appears to have a role in the stereo-selective regulation of amino acids in the brain as far as expression of the distinct functions of \(L\) and \(D\)-Asp are concerned.

Subtypes of excitatory amino acid transporter (EAATs) have been reported to be expressed in isolated bovine BCECs. \(^{34}\) Nevertheless, the expression of EAATs at the BBB does not explain the stereoselective BBB efflux transport of Asp, since EAATs transport both Asp isomers. \(^{35}\) We analyzed the stereoselective BBB efflux transport of Asp using TM-BBB cells. \(^{36}\) TM-BBB cells exhibit \(L\)-isomer-selective transport activity, like the in vivo BBB. The \(L\)-isomer-selective transport by the cells is \(Na\)^{+}- and pH-dependent, and is inhibited by neutral amino acids, such as \(L\)-alanine (\(L\)-Ala) and \(L\)-serine (\(L\)-Ser). These properties are in good agreement with those of the amino acid transport system ASC. To date, ASC1 and 2 have been identified as members of the ASC system. \(^{37,38}\) TM-BBB cells express both ASC1 and 2 mRNAs, although the mRNA expression of ASC2 in the cells was found to be 6.7-fold greater than that of ASC1. ASC1- and ASC2-expressing oocytes exhibited \(^{3}H\)\(L\)-Asp uptake in an \(Na\)^{+}- and pH-dependent manner, while \(^{3}H\)\(D\)-Asp did not undergo such uptake (Fig. 4). Furthermore, immunohistochemical analysis indicated that ASC2 protein is localized on the abluminal membrane of mouse BCECs. These findings indicate that abluminally localized ASC2 is involved in \(L\)-isomer-selective Asp efflux transport at the BBB (Fig. 3).

EAAT1–3 may play a role in the uptake of \(L\)-Asp, in addition to ASC2 (Fig. 3), because of the report showing that there is expression of EAAs in the abluminal membrane fraction of isolated bovine BCECs. \(^{34}\) ASC2 transports \(L\)-Asp with a low affinity (\(K_m\) = 9.33 mm), and the \(K_m\) of the \(L\)-Asp and \(L\)-Glu transport by EAATs has been reported to be 2–64 \(\mu\)M. \(^{35}\) TM-BBB cells exhibited \(L\)-Asp transport with both low and high affinity, which appears to correspond to the transport by ASC2 and EAATs, respectively. \(^{36}\) However, \(D\)-Asp transport by EAATs at the BBB would be inconsistent.
with our previous in vivo study, demonstrating the absence of brain-to-blood transport of D-Asp.\textsuperscript{33} This discrepancy may be due to the expression and functional level of each transporter at the in vivo BBB and the brain levels of the endogenous substrates. We also cannot rule out the possibility that the D-Asp efflux transport process may not be present on the luminal side. Therefore, further studies are necessary to clarify the contribution of ASCT2 and EAATs and identify the luminal localized transporter involved in the efflux transport of excitatory amino acids.

**The Brain-to-Blood Efflux Transport of Neurotransmitter Metabolites** Homovanillic acid (HVA) is a major metabolite of dopamine. The HVA concentration in blood and urine is widely used as an indicator of dopaminergic neuronal activity in the brain. Abnormalities in cerebral dopamine function have been implicated in many mental and neurological disorders, such as Parkinsonism and various types of seizures.\textsuperscript{39,40} Furthermore, patients with uremic encephalopathy or Reye’s syndrome show markedly elevated HVA concentrations in the CSF.\textsuperscript{41,42} Therefore, the BBB efflux transport system for HVA appears to be an important determinant of dopamine turnover in the brain.

Previous studies have indicated that the HVA concentration in the brain is increased when probenecid or octanoic acid is administered peripherally.\textsuperscript{43,44} Brain microdialysis studies have suggested that the brain-to-blood efflux transport rate of HVA is faster than the blood-to-brain HVA influx rate across the BBB.\textsuperscript{45} These results suggest that a probenecid- and octanoic acid-sensitive transporter(s) mediates the efflux transport of HVA at the BBB. We have investigated the transporter responsible by direct analysis of HVA efflux using BEI methods.\textsuperscript{46} \([\text{H}]\text{HVA}\) was eliminated from the brain across the BBB with a half-life of 41.0 min. The efflux rate of \([\text{H}]\text{HVA}\) was significantly inhibited by a selective inhibitor of organic anion transporter 3 (OAT3).

OAT3 was identified as a brain-expressed member of the OAT family (SLC22A).\textsuperscript{47} Our group and other researchers have reported that OAT3 is involved in the efflux transport of organic anions at the BBB.\textsuperscript{48–50} Our studies using a Xenopus oocyte expression system found that rat and mouse OAT3 can transport HVA, and this transport activity was inhibited by probenecid and octanoic acid (Table 1).\textsuperscript{46,51} Furthermore, immunohistochemical analysis revealed that OAT3 is localized on the abluminal membrane of BCECs in rat and mouse brain. Thus, abluminaly localized OAT3 takes up HVA from the brain interstitial fluid into BCECs and so plays a major role in the brain-to-blood efflux transport system for HVA (Fig. 3).

The metabolites of monoamine neurotransmitters include many organic anions. We have found that various anionic metabolites of neurotransmitters inhibited HVA transport by OAT3, while the neurotransmitters themselves had no inhibitory effect (Table 1).\textsuperscript{46} Although compounds that have an inhibitory effect on a transporter are not always substrates, the above result raises the possibility that OAT3 mediates the BBB efflux transport of various neurotransmitter metabolites.

Patients with Reye’s syndrome exhibit markedly elevated HVA concentrations in the CSF, although the dopamine concentration in the nucleus caudatus is not abnormal.\textsuperscript{42,52} This observation raises the possibility that accumulation of HVA in the brain may cause encephalopathy. In patients with Reye’s syndrome, the octanoic acid concentration in the blood increases markedly with concomitant increases in the brain.\textsuperscript{53} The plasma level of octanoic acid in severe cases of Reye’s syndrome reaches about 1 mM,\textsuperscript{54} and we have shown that octanoic acid (1 mM) significantly inhibits (by 90%)
rOAT3-mediated HVA transport (Table 1). Therefore, it was proposed that the increased level of octanoic acid in the plasma and brain of patients inhibits the brain-to-blood efflux transport of HVA mediated by OAT3.

The BBB efflux transport mediated by OAT3 is a physiologically multi-functional transport system. We have reported that an endogenous uremic toxin (indoxyl sulfate (IS)) and drugs (6-mercaptopurine (6-MP), 6-thioguanine (6-TG)) are excreted from the rat brain by OAT3-mediated BBB efflux transport (Fig. 3).50,55 During maintenance chemotherapy for acute lymphoblastic leukemia, CNS relapses often occur due to penetration and proliferation of leukemic cells in the brain, because of the limited distribution of thiopurine nucleobase analogs, such as 6-MP and 6-TG.56 Microdialysis studies have shown that the brain-to-blood efflux of 6-MP across the BBB is 20-fold greater than the brain-to-blood influx.57 Thus, the OAT3-mediating BBB efflux transport plays a crucial role in limiting the effects of thiopurine nucleobase analogs in the brain.

The involvement of OAT3 in drug efflux at the BBB shows the importance of analyzing human OAT3 as far as drug development is concerned. Human OAT3 has already been identified in kidney based on the homology with rat OAT1.58 Its amino acid sequence is 75% and 73% identical to those of rat and mouse OAT3, respectively. Since the function and localization of human OAT3 at the BBB have not been analyzed yet, further studies are necessary to clarify the role of human OAT3 at the BBB.

The Brain-to-Blood Efflux Transport of Neuro-Modulators Neuronal function is regulated by various neuro-modulators which must be maintained at low levels for the proper function of neuro-modulators. The BBB efflux transport system is thought to be involved in the clearance of neuro-modulators, like neurotransmitters and their metabolites.

Dehydroepiandrosterone sulfate (DHEAS) is a neurosteroid which can interact with GABA type A receptors and sigma receptors to increase memory and learning ability and to protect neurons against excitatory amino acid-induced neurotoxicity.59 We have reported that DHEAS is eliminated from the brain across the BBB by using the BEI method, and an inhibition study showed that oatp2 was involved in this efflux transport.60 In addition, Gao et al. have reported that oatp2 is localized on both the luminal and abluminal membrane of rat BCECs.61 Thus, oatp2 expressed at the BBB plays a role in the BBB efflux transport of DHEAS (Fig. 3).

Compared with the OAT family, oatp family members accept bulky organic anions.62 Interesting substrates of the oatp family, as far as CNS function is concerned, include opioid peptides and thyroid hormones. These compounds need to cross the BBB to express their CNS functions. Oatp2 is a bidirectional transporter and has been reported to be involved in the entry of a cyclic opioid pentapeptide, [β-phenyl-l-lysine-2,5]-enkephalin (DPPDE).63 In addition to oatp2, recent studies have shown that OATP-A, oatp3 and oatp14 are expressed at the human, mouse and rat BBB, respectively.64—66 Therefore, further identification of the subtypes of the oatp family expressed at the BBB and clarification of their contribution to the BBB transport system will help increase our understanding of BBB function and drug permeability.

System A is a transport system for small neutral amino acids that accepts l-Ala, l-Proline (l-Pro), glycine (Gly), and α-methylaminoisobutyric acid (MeAIB) as substrates. Gly modulates the function of NMDA receptors, and l-Pro interacts with NMDA and AMPA receptors. Furthermore, small neutral amino acids function as osmolytes, and induction of system A activity by hypertonicity has been reported in a variety of different cells.57 It has been suggested that system A is present on the abluminal side of the BBB following an uptake study using isolated rat brain capillaries and isolated abluminal membrane vesicles from bovine BCECs.58,69 We have used the BEI method to show that brain-to-blood efflux transport of [3H]-l-Pro and [3H]-Gly takes place across the in vivo BBB.70

[3H]-l-Pro uptake by conditionally immortalized rat BCECs (TR-BBB)71 is an Na+-dependent process with high-affinity and low-affinity saturable components (Km = 425 μM and 10.8 mM).70 The mode of inhibition of [3H]-l-Pro uptake by amino acids is consistent with the involvement of system A. Three Na+-dependent small neutral amino acid transporters have been identified as system A isoforms, namely, ATA1 (GlnT; SAT1; SLC38A1), ATA2 (SAT2; SA1; SLC38A2), and ATA3 (SLC38A4).72—76 All three isoforms of system A have been found to be expressed in the rat brain capillary-rich fraction and TR-BBB cells, and ATA2 mRNA was present in 93-fold and 2140-fold greater levels than ATA1 and ATA3 mRNAs in TR-BBB cells, respectively. This result suggests that ATA2 is the responsible transporter for system A efflux transport at the BBB (Fig. 3).

Under hypertonic conditions (450 mM Osm/kg), ATA2 mRNA in TR-BBB cells is induced by up to 373% concomitantly with activation of [3H]MeAIB uptake.70 This suggests that system A at the BBB is regulated by the osmolarity around the BBB. Osmo-regulation in the brain is important for maintaining a constant milieu in the CNS, since hyperosmolarity causes brain edema. Therefore, osmo-regulation of ATA2 may contribute to the regulation of osmolarity in the brain and the cell volume in BCECs in order to maintain a stable environment in the brain and to permit the BBB to function under pathological conditions.

Regulation of BBB Functions by CNS Conditions To control the CNS milieu as a “CNS supporting and protecting system”, the function of the BBB should be regulated by the factors reflecting the conditions of the CNS and the circulating blood. The expression of transporter of energy sources, GLUT1 and MCT1, at the BBB changes during development,77 and is thought to be regulated by dietary conditions. As noted in the former section, ATA2 in BCECs is regulated by osmolarity.70 Studies to identify transporters and tight-junction proteins at the BBB are now in progress. After the molecular identification, the regulation of these molecules will play a key part in clarifying the physiological and pathological role of the BBB in the CNS.

Taurine (2-aminoethanesulfonic acid) is one of the abundant free sulfur-containing β-amino acids in the CNS and is thought to play a role as an osmoregulator and neuro-modulator.78,79 Taurine also plays an important role in the development of CNS,80 and its brain levels in children is about 4-fold greater than that in adults. The level of an enzyme required for taurine biosynthesis is low in humans, primates and cats.80 Therefore, dietary taurine is important for main-
tain taurine levels in the body. A study using primary cultured bovine BCECs has shown that there is a taurine transport system in both the luminal and abluminal membranes of the BCECs.81) Therefore, this taurine transport system at the BBB may supply taurine to the brain, and be involved in the maintenance of taurine levels in the brain.

We have analyzed the regulation of taurine transport at the BBB focusing on the functions of osmoregulation and neuroprotection.82) Taurine transporter (TAUT) was detected in TR-BBB cells, suggesting the involvement of TAUT in taurine transport at the BBB. Hypertonic conditions induced the uptake activity of taurine and mRNA expression of TAUT in TR-BBB cells. Therefore, regulation of TAUT would play a role in CNS osmoregulation in conjunction with ATA2.

Taurine exerts a neuroprotective effect against excitotoxic agents and oxidative stress, and its level in brain interstitial fluid (ISF) is elevated in ischemia.83—85) We hypothesized that taurine transport at the BBB is involved in the changes in taurine ISF levels under pathological conditions. We have tested TNF-α, LPS and diethyl maleate (DEM), and found that, of these compounds, TNF-α induced the uptake of taurine and mRNA expression of TAUT in TR-BBB cells (Table 2).82) TNF-α is induced in the brain after cerebral ischemia and traumatic brain injury.86) Therefore, taurine transport at the BBB can be up-regulated by TNF-α in response to CNS cell damage. We have also reported that taurine uptake and TAUT expression in TR-BBB cells is suppressed by excess taurine (Table 2).82) It has been reported that the taurine transport system is present in both the luminal and abluminal membranes of the BCEC, and the luminal uptake rate is about 2-fold greater than that of the abluminal uptake.87) Therefore, in order to understand the role of TAUT regulation in BCECs, it is important to see how changes in luminal and/or abluminal taurine transport are affected by regulation of TAUT in BCECs.

We have also shown that blood-to-brain system x_c transport is induced by 12-h DEM infusion,87) and this induction is due to the up-regulation of xCT,88) which consists of system x_c forming a complex with 4F2hc.89) System x_c is an l-cystine and l-Glu exchange transport system. Supplying l-cysteine, which is reduced from l-cystine, to cells is one of the rate-limiting steps of glutathione biosynthesis.90) Glutathione provides protection against free radicals, peroxides and other toxic compounds in the CNS,90) and DEM is used as a reagent to deplete intracellular glutathione to induce oxidative stress. Therefore, the up-regulation of xCT at the BBB would lead to an increase in brain glutathione levels to protect the CNS function. The results regarding the regulation of TAUT and xCT suggest that one of the physiological roles of BBB regulation is protecting the CNS against damage and degeneration.

### CONCLUSION

Understanding the molecular basis of the BBB is important for the development of new CNS drugs. However, BBB research into drug transport can only clarify limited aspects of BBB function. As shown in this review, recent BBB research from the viewpoint of physiological function throws new light on the novel functions of the BBB, including new transporting and regulation systems. These studies show that the BBB is one of the important brain systems for maintaining the CNS milieu. Furthermore, BBB function is closely related to CNS disorders. This means that studies from the viewpoint of neuroscience are necessary for future BBB research. In addition, the new analytical technologies, especially genetic technologies, should be developed for BBB research, e.g. BBB-selective gene transfer and silencing, proteomics of BBB proteins and imaging (histological and functional) of BBB function. This multidisciplinary approach to BBB research will markedly increase our knowledge of BBB function, which will help improve drug delivery to the brain. Furthermore, the new knowledge about the physiological and pathological functions of the BBB may open up new pathways for drug development and offer improved therapeutic strategies for CNS disorders.

### Acknowledgements

I am most grateful to Drs T. Terasaki, K. Hosoya, H. Takanae, and S. Hori for their advice, encouragement and criticism. I wish to thank Drs H. Asaba, K. Tetsuka, M. Tomi and S. Mori, and all graduate and undergraduate students who are involved in this study for their creative work and discussions, and Ms. N. Funayama for her secretarial assistance. This study was supported, in part, by Grants-in-Aid for Scientific Research (B) and for Young Scientists (B) from Japan Society for the Promotion of Science (JSPS). It was also supported in part by the Uehara Memorial Foundation, the Nissan Science Foundation, the Tokyo Biochemical Research Foundation and the Industrial Technology Research Grant Programs in ’00 and ’03 from the New Energy and the Industrial Technology Development Organization (NEDO) of Japan.

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### Table 2. The Uptake of [3H]Taurine by TR-BBB Cells under Different Conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uptake of [3H]taurine (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100±2</td>
</tr>
<tr>
<td>TNF-α</td>
<td>121±4*</td>
</tr>
<tr>
<td>LPS</td>
<td>103±6</td>
</tr>
<tr>
<td>DEM</td>
<td>94.1±6.7</td>
</tr>
<tr>
<td>Taurine</td>
<td>10.4±0.5**</td>
</tr>
</tbody>
</table>

Cells were treated with TNF-α (20 ng/ml), LPS (10 ng/ml), DEM (100 μg) or taurine (50 μM) for 24 h, respectively. After treatment, taurine uptake was measured after a 5 min incubation with [3H]taurine. Each value represents the mean±S.E.M. (n=4). * p<0.01, ** p<0.0001 significantly different from the control. Cited from Kang et al., *J. Neurochem.*, 83, 1188—1195 (2003).