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Role of Monocarboxylate Transporters in Drug Delivery to the Brain

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Abstract: Monocarboxylate transporters (MCTs) are known to mediate the transport of short chain monocarboxylates such as lactate, pyruvate and butyrate. Currently, fourteen members of this transporter family have been identified by sequence homology, of which only the first four members (MCT1- MCT4) have been shown to mediate the proton-linked transport of monocarboxylates. Another transporter family involved in the transport of endogenous monocarboxylates is the sodium coupled MCTs (SMCTs). These act as a symporter and are dependent on a sodium gradient for their functional activity. MCT1 is the predominant transporter among the MCT isoforms and is present in almost all tissues including kidney, intestine, liver, heart, skeletal muscle and brain. The various isoforms differ in terms of their substrate specificity and tissue localization. Due to the expression of these transporters in the kidney, intestine, and brain, they may play an important role in influencing drug disposition. Apart from endogenous short chain monocarboxylates, they also mediate the transport of exogenous drugs such as salicylic acid, valproic acid, and simvastatin acid. The influence of MCTs on drug pharmacokinetics has been extensively studied for γ-hydroxybutyrate (GHB) including distribution of this drug of abuse into the brain and the results will be summarized in this review. The physiological role of these transporters in the brain and their specific cellular localization within the brain will also be discussed. This review will also focus on utilization of MCTs as potential targets for drug delivery into the brain including their role in the treatment of malignant brain tumors.

Keywords: Monocarboxylate transporters, γ-hydroxybutyrate, brain, lactate.

INTRODUCTION

Monocarboxylic acids play an important role in energy metabolism in various tissues such as skeletal muscle, heart, brain and red blood cells. Among these monocarboxylates, lactate which is the end product of glycolysis is particularly important. This pathway leads to intracellular accumulation of lactate which must be exported out as high levels of lactate result in inhibition of glycolysis. In addition, some of the tissues such as brain, heart and red skeletal muscle utilize lactate as a fuel for respiration, thus requiring its import into the cell [1, 2]. Monocarboxylate transporters facilitate the transport of lactate and other monocarboxylates and therefore play an important role in cellular metabolism. Proton dependent monocarboxylate transporters (MCTs; SLC16A) are a family of transport proteins that contain 14 members which were identified based on sequence homology [3]. Only 4 members of this transporter family (MCT1-4) have been identified as proton dependent MCTs which catalyze the transport of important monocarboxylates such as lactate, pyruvate, and ketone bodies [4]. Another transporter family that has been demonstrated to be involved in monocarboxylate transport is known as sodium coupled monocarboxylate transporters (SMCTs) which contains only two members, SLC5A8 and SLC5A12 [5-7]. MCTs have a ubiquitous distribution in the body when compared to SMCTs which are more limited in their distribution [7, 8]. Apart from endogenous moncarboxylates, MCTs are also involved in the transport of some exogenous drugs such as salicylate, valproic acid and atorvastatin [8]. Monocarboxylate transporters can significantly influence drug pharmacokinetics due to their presence in the kidney, intestine and brain. MCT1, MCT2 and MCT4 are expressed in the brain and play an important role in transport of endogenous monocarboxylates into and out of brain cells [9]. The present review summarizes the function and distribution of monocarboxylate transporters in the brain. The potential role of these transporters in drug delivery to the central nervous system will also be discussed with specific emphasis on γ-hydroxybutyrate (GHB) which has been shown to be a substrate for both MCTs and SMCTs [10-13].

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MONOCARBOXYLATE TRANSPORTERS

The presence of proton coupled MCTs was first recognized by lactate and pyruvate transport into human red blood cells with transport being significantly inhibited by α-cyano-4-hydroxycinnamate (CHC) [14-16]. Currently, this family of transporters contains 14 members out of which only 4 members (MCT1-MCT4) have been demonstrated to mediate the proton dependent transport of monocarboxylates such as lactate, pyruvate, and ketone bodies [3, 8]. They provide electroneutral co-transport of monocarboxylates along with protons in a stoichiometric ratio of 1:1. MCT8 is a thyroid hormone transporter and MCT10 is an aromatic amino acid transporter and is also known as T-type amino acid transporter1 (TAT1). The functional characterization of other members of this family has not been done and they are known as orphan transporters. MCTs have 12 transmembrane domains with C- and N-termini within the cytoplasm and an intracellular loop between TMDs 6 and 7 [17]. The conservation of sequence between different isoforms of the mammalian MCTs is the greatest for MCT1-4 whereas sequence is least conserved between other members of the family. The TMDs are highly conserved between the family members with high variations in the C- and N- termini including the intracellular loop [3]. The variations in the sequences of different isoforms may lead to differences in substrate specificity and regulation of MCTs [18]. The regulation of MCTs has been shown to occur both by transcriptional as well as post-transcriptional mechanisms [19, 20]. Although these proteins are not glycosylated, they require association with glycosylated protein, for their functional activity. This ancillary protein is called basigin or CD147 for MCT1 and MCT4 whereas MCT2 differs from its isoforms as it requires embigin instead of basigin for its functional activity [21]. The tissue distribution and substrate specificity of each MCT isoform has been outlined in Table **1**. The key features of each functionally characterized MCT isoform will be further discussed in detail in this section.

Table 1. Tissue distribution and substrate specificity of various MCT and SMCT isoforms.

Protein name	Unigene name	Tissue distribution	Cellular localization in brain	Predominant substrates	Transport mechanism	Reference
MCT1	SLC16A1	Ubiquitous	Brain endothelial cells, astrocytes, some neurons in rats	Lactate, pyruvate, butyrate, acetoacetate, β-hydroxybutyrate, XP13512, GHB	H^+ cotransporter or monocarboxylate exchanger	[8, 9, 87]
MCT2	SLC16A7	Liver, kidney, brain, testis, heart, spleen, pancreas	Neurons	Pyruvate, lactate	H^+ cotransporter	[8, 9, 34]
MCT3	SLC16A8	Retinal pigment epithelium, choroid plexus		Lactate	H^+ cotransporter	[8, 40, 43]
MCT4	SLC16A3	Skeletal muscle, brain, kidney, placenta, leukocytes, heart, lung, chondrocytes	Astrocytes	Lactate, pyruvate, acetoacetate, β-hydroxybutyrate	H^+ cotransporter	[8, 9, 44, 87]
MCT6	SLC16A5	Kidney, muscle, brain, heart, placenta, intestine, prostate, lung, pancreas		Bumetanide, nateglinide	Orphan	[8, 46]
MCT8	SLC16A2	Liver, brain, heart, kidney, placenta, ovary, prosatate, thymus, pancreas		T_3, T_4	Orphan	[8, 48, 49]
MCT10 (TAT1)	SLC16A10	Skeletal muscle, intestine, kidney, heart, liver, placenta		Aromatic amino acids (L-tryptophan, L-tyrosine, L-phenylalanine, L-DOPA	Facilitated diffusion/ exchanger	[8, 50]
SMCT1	SLC5A8	Intestine, kidney, brain, retina	Neurons	Lactate, pyruvate, butyrate, nicotinate, acetoacetate, β-hydroxybutyrate, α-ketoisocaproate, salicylates, benzoate, GHB	Na^+ cotransporter	[5, 54, 88]
SMCT2	SLC5A12	Intestine, kidney, brain, retina	Astrocytes and glia	Lactate, pyruvate	Na^+ cotransporter	[7, 54, 57]

MCT1 (SLC16A1)

MCT1 was first identified as a mutation of the wild type protein which enhanced the uptake of mevalonate into Chinese-hamster ovary cells [22]. This protein has been shown to mediate inhibitor sensitive transport of monocarboxylates. MCT1 has now been cloned from mice, rats and humans and shows 95% sequence homology to Chinese hamster ovary MCT1 [23-26]. The functional

activity of MCT1 is dependent on a proton gradient and it acts as a proton dependent cotransporter/exchanger [27]. Transport was determined to follow an ordered, sequential mechanism through kinetic studies of lactate into red blood cells [16, 28]. A proton first binds to the transporter followed by binding of lactate. The proton and lactate are further translocated across the membrane with their sequential release on the other side. The return of the free transporter binding site across the membrane determines the net flux of lactate and thus forms the rate limiting step of transport. Transport can be stimulated by a pH gradient (low to high). The predominant role of MCT1 is to facilitate the unidirectional proton-linked transport of monocarboxylates across the plasma membrane. This may represent either influx or efflux of substrate depending of the intracellular and extracellular substrate concentrations and the existing pH gradient across the plasma membrane. However, MCT1 can also function as an exchanger, with transport occurring bidirectionally with the exchange of one monocarboxylate for another without the net movement of protons [3].

The substrate specificity of MCT1 has been extensively studied in red blood cells by measuring the inhibition of uptake of ^{14}C-lactate [14]. It has been shown that MCT1 is responsible for the transport of a broad range of monocarboxylates including lactate, pyruvate, acetoacetate, β-hydroxybutyrate and GHB [1, 29]. These substrates exist as a monocarboxylate anion under physiological conditions, which is required for a MCT substrate. The K_m value for transport decreases with increasing chain lengths of various monocarboxylates. Monocarboxylates that are substituted in the C-2 and C-3 positions with halides, hydroxyl, and carbonyl groups represent good substrates. The C-2 substitution is preferred over C-3, with the carbonyl group being especially favored. Monocarboxylates with longer branched aliphatic or aromatic side chains have also been found to bind to the transporter, but these are not easily released following translocation and may act as potent inhibitors [3]. Lactate transport has been found to be stereospecific with higher affinity for L-lactate when compared to D-lactate [27].

The inhibitors of MCT1 can be classified into three categories: (1) bulky or aromatic monocarboxylates such as 2-oxo-4-methyl-pentanoate, phenyl-pyruvate and α-cyano-4-hydroxycinnamate (CHC) which act as competitive inhibitors and are blockers of transport function of MCTs [30,31]; (2) amphiphilic compounds with divergent structures which include bioflavanoids such as quercetin and phloretin and anion transport inhibitors such as 5-nitro-2-(3-phenylpropylamino)-benzoate and niflumic acid; and (3) 4,40-substituted stilbene 2,20-disulphonates such as 4,40-diisothiocyanostilbene-2,20-disulphonate (DIDS) and 4,40-dibenzamidostilbene-2,20-disulphonate (DBDS) which act as reversible inhibitors of MCT1 in erythrocytes [32, 33]. It is important to note that CHC is not a specific MCT1 inhibitor and may inhibit one or more isoforms of MCTs. One of the important roles of MCT1 is the unidirectional transport of L-lactate (influx or efflux) which depends on the intracellular and extracellular lactate concentrations as well as the proton gradient across the membrane.

MCT2 (SLC16A7)

A second MCT isoform was cloned from a hamster liver cDNA library and was shown to have higher affinity for monocarboxylates than MCT1 [34-36]. This isoform was named MCT2 and was further characterized following the expression of rat isoform in Xenopus oocytes [37]. MCT2 shares 60% identity with MCT1. MCT2 has similar substrate specificity when compared to MCT1. It has also been shown to be inhibited by similar inhibitors such as CHC, DBDS and DIDS but it has been reported to be insensitive to the organomercurial reagent pCMBS [8, 34]. It has been shown that pCMBS inhibits MCT1 by binding to its associated ancillary protein basigin. This may be the reason for insensitivity to pCMBS as MCT2 has been shown to associate with embigin and not basigin [21, 37, 38]. MCT2 has also been cloned from rat, mouse and human tissues [35, 36]. The sequence of MCT2 is conserved to a lesser extent than MCT1 among these species which results in considerable species differences in the tissue distribution of this isoform [8]. MCT2 expression is limited in major human tissues whereas northern and western blot analysis have shown that this isoform is expressed in liver, kidney, brain and sperm tails in rat, mouse and hamster [8].

MCT3 (SLC16A8)

MCT3 has a very limited distribution and is found only in the basolateral membrane of the retinal pigment epithelium and the choroid plexus in humans, rodents and chickens [39]. The K_m value of chicken MCT3 for lactate has been found to be around 6 mM in a yeast expression system [40]. It has also been found to be resistant against typical MCT inhibitors such as phloretin, CHC and pCMBS. Further information on substrate kinetics of this MCT isoform is not available and further studies are needed. Based on its localization, it is thought to be responsible for the export of lactate produced as a result of glycolysis from the retina [41, 42].

MCT4 (SLC16A3)

This isoform was initially named MCT3 based on sequence homology to chicken MCT3 but later was renamed as MCT4 [43]. It is mainly found in glycolytic tissues such as white skeletal muscle fibres, astrocytes, white blood cells, and chondrocytes [3, 8]. It has lower affinity for lactate and pyruvate than MCT1 and is believed to be involved in efflux of lactate from these tissues to prevent intracellular accumulation of lactate which would otherwise inhibit glycolysis [44]. This has been studied by expression of this transport protein in Xenopus oocytes [45]. It has a very high K_m value for pyruvate (150 mM) which helps in preventing its loss from the cell.

MCT 6 (SLC16A5)

MCT6 was first identified by genomic and EST database screening and is predominantly expressed in the kidney and intestine [43]. It is known to transport pharmaceutical drugs such as bumetanide and nateglinide and does not transport short chain monocarboxylates like the other isoforms [46]. This isoform has also been shown to be present in the intestine implicating its role in drug absorption.

MCT 8 AND MCT 10 (SLC16A2 AND SLC16A10)

MCT8 was earlier known as XPCT (X-linked PEST containing transporter) because it contains a PEST domain in its N-terminal [47]. This isoform is also known as the thyroid hormone transporter. Substrate kinetic studies through expression in Xenopus oocytes demonstrated that MCT8 transports both the thyroid hormones (T_3 and T_4) with high affinity with K_m values of 2-5 μM [48]. MCT8 is distributed in many tissues including liver, kidney, skeletal muscle, heart, brain, pituitary, and thyroid [49]. MCT10 is also known as TAT1 and was found to transport aromatic amino acids such as phenylalanine and tryptophan. It has also been expressed in Xenopus oocytes which demonstrated K_m values of around 5 mM for aromatic amino acid substrates such as tryptophan, tyrosine, and phenylalanine [50]. MCT10 is expressed in a variety of tissues including intestine, kidney, liver, skeletal muscle, heart, and placenta [51]. Both MCT8 and MCT10 are known to mediate proton and sodium independent transport of their substrates. Delayed brain myelination which results in variable degrees of mental retardation, hypotonia, spasticity, ataxia and involuntary movements has been attributed to MCT8 deficiency in the brain [52]. Various tyrosine kinase inhibitors have been shown to non-competitively inhibit MCT8 leading to reduced thyroid hormone uptake in brain. Hence tyrosine kinase inhibitors can lead to pharmacokinetic drug interactions leading to increased levothyroxine requirement of thyroidectomized patients [53].

Other isoforms of MCTs, MCT5, MCT7, MCT9, and MCT 11-14 have also been identified but their functional characterization has not been performed.

SMCT

The second transport family that is involved in the transport of monocarboxylates is the sodium coupled monocarboxylate transporters (SMCT), part of the solute carrier gene family SLC5. Only two members of this family have been identified as sodium-dependent monocarboxylate transporters so far, namely SLC5A8 and SLC5A12 [54]. Characterization of SLC5A8 was done by its expression in Xenopus laevis oocytes and it has been shown to transport short chain monocarboxylates [5]. This transporter is dependent on the sodium gradient and typically transports multiple sodium ions along with monocarboxylates in a stoichiometric ratio of 3:1 making it electrogenic. SLC5A8 is expressed in normal colon tissue, and it functions as a tumor suppressor in human colon with silencing of this gene occurring in colon carcinoma. This transporter is involved in the concentrative uptake of butyrate and pyruvate produced as a product of fermentation by colonic bacteria. These are known to act as inhibitors of histone deacetylases, which supports its suppression in tumor cells [55]. SLC5A8 is also expressed in the brush border membrane of renal tubular cells where it has been suggested to mediate the active reabsorption of lactate and pyruvate to minimize their renal elimination and in the brain [56]. SLC5A8 is a higher affinity transporter when compared to MCT1 with K_m values for lactate of 159 μM determined in Xenopus oocytes with heterologous expression of SLC5A8 [5]. The second member of this family, SLC5A12, has been found to be expressed in kidney and intestine with limited distribution in the brain. It is also found to mediate the sodium dependent transport of monocarboxylates but the transport is electroneutral, in contrast to SLC5A8. The affinity of this transporter is lower when compared with SLC5A8, but it exhibits very similar substrate specificity [7, 57].

FUNCTION OF MONOCARBOXYLATE TRANSPORTERS IN THE BRAIN

Transport of lactate across the plasma membrane is important under hypoxic conditions when glycolysis becomes the predominant pathway and also for tissues that rely on glycolysis to meet their normal energy demands [3]. Under hypoxic conditions, glycolysis results in the formation of lactate which needs to be exported out of the cell for continued glycolysis to occur [58, 59]. The transporters have lower affinity for pyruvate thus ensuring that it is not lost from the cell and further converted to lactate which results in regeneration of NAD^+ and continued glycolysis. In the brain, glucose serves as the major energy source under normal conditions, but during prolonged starvation and diabetic ketoacidosis as observed in diabetes, other monocarboxylates such as lactate and ketone bodies (β-hydroxybutyrate and acetoacetate) become an important energy substrate and their transport into the brain is required [60-62]. The endothelial cells of the blood vessels in the brain have been reported to express MCT1 which probably mediates the transport of lactate and ketone bodies across the blood brain barrier (BBB) [63, 64]. The capacity of the brain to use ketone bodies such as β-hydroxybutyrate was found to increase in starvation and diabetes by 50-60% in rats [62]. This study also showed that BBB permeability to ketone bodies increased by both starvation and diabetes.

Under certain conditions such as hypoxia or ischemia, glycolysis is the only pathway for the production of ATP resulting in increased brain concentrations of lactate [3]. There are different isoforms of MCTs that are expressed in different subcellular regions of the brain with MCT1 and MCT4 being predominantly found in the astrocytes and MCT2 being the major isoform in the neurons [65]. This ensures export of lactate from astrocytes formed as a product of rapid glycolysis which is then taken up by the neurons to be used as a respiratory fuel for further oxidation [9]. Glucose is considered to be the predominant energy fuel for neurons. However, several studies have shown that neurons can efficiently utilize monocarboxylates, especially lactate as oxidative energy substrates in addition to glucose [66]. In contrast, astroglial cells are a major source of lactate and they predominantly metabolize glucose into lactate in the brain followed by lactate efflux [67]. In some cases, it has been shown that astrocytes can use lactate as an energy substrate, but to a very limited extent when compared to neurons [67]. The export of lactate along with a proton also helps in maintaining the intracellular pH by preventing cellular acidification. This has been demonstrated by disrupting the expression of MCT1 or MCT4 in astrocytes in the hippocampus of rats which resulted in loss of memory of learned tasks [68]. This loss in memory could be reversed by injecting L-lactate locally whereas the injection of glucose was not able to reverse this. Similar loss in memory in rats was obtained by disrupting MCT2 in neurons but this could not be reversed by injection of either L-lactate or glucose demonstrating that MCT2 is required for the uptake of these respiratory fuels into the neurons for proper functioning of the brain [68]. This is commonly known as the astrocyte-neuron lactate shuttle hypothesis. Exposure to glutamate has been shown to stimulate glucose utilization and the release of lactate by astrocytes [69]. This provides a coupling mechanism between neuronal activity and glucose utilization. It has also been demonstrated that certain neurotransmitters such as noradrenaline, vasoactive intestinal peptide and adenosine that activate glycogenolysis also increase lactate release [70].

MCTs are also involved in the uptake of ketone bodies in the neurons in conditions with low glucose utilization [8]. Neurons have the ability to oxidize lactate under both physiological and hypoxic conditions similar to heart and red skeletal muscle and they contain the same isoform of lactate dehydrogenase (LDH) as present in heart (LDH-1 subunit) [71]. The LDH-5 subunit (muscle type) is present in glycolytic tissues, favoring the formation of lactate from pyruvate whereas the LDH-l subunit (heart type) preferentially drives the reaction toward the production of pyruvate. It has been shown that LDH-1 subunits are present in neurons. However, LDH-5 subunit is predominantly present in the astrocytes [72]. This selective distribution of lactate dehydrogenase isoenzymes in astrocytes and neurons is consistent with the proposed astrocyte-neuron lactate shuttle.

The utilization of lactate and ketone bodies as energy substrates has been found to be higher in neonates when compared to adults and this is consistent with higher expression of MCT1 in neonates [59, 73, 74]. MCT1 expression in the membrane of capillary endothelium was found to be 25 times higher in 17-day suckling rat pups than adults using electron microscopic immunogold methods. This transporter was found to be equally distributed in both luminal and abluminal membranes [75]. These results were further confirmed by a report of high mRNA and protein expression of MCT1 in the BBB during suckling and reduction in expression with maturation [76]. This also explains the switch in fuel utilization from a combination of glucose, lactate and ketone bodies in the neonatal brain to complete dependence on glucose in adults. It has been shown in rodents that increased susceptibility of the neurons to acute severe hypoxia, which mimics the disorder of sleep apnea, is mediated by decreased expression of MCT2 in the neurons [77]. MCT1 and MCT4 have also been associated with the transport of short chain fatty acids such as acetate and formate which are then metabolized in the astrocytes [78].

LOCALIZATION OF MCTs IN THE BRAIN

MCTs are widely expressed in rat, mouse and human brain, both at the cellular and sub-cellular levels. MCT1 has a ubiquitous distribution in the body and is found in the liver, kidney, heart, muscle and brain [3]. Of all the identified isoforms of MCTs, it has been demonstrated that MCT1, MCT2 and MCT4 are expressed in

the brain as depicted in (Fig. **1**) [9]. The different subcellular regions of the brain express different MCT isoforms. The mRNA of MCT1 has been found in the cortex, hippocampus and cerebellum of adult rat brain [59, 76]. Earlier studies have shown that MCT1 is significantly expressed in cerebral blood vessels with specific localization on the endothelial cells on both luminal and abluminal membranes and ependymocytes lining the four brain ventricles in rats [73]. MCT1 was also found in the glial end feet surrounding capillaries [73, 75] and in brain parenchymal cells [73]. Confocal microscopy studies have also identified the expression of MCT1 in astrocytic processes both *in vitro* and *in vivo* [64, 79, 80]. Low expression of MCT1 has also been identified in specific subpopulations of neurons in adult rat brain such as those in the cerebral cortex, hippocampus, and hypothalamus [75]. However, MCT1 expression was not observed in the adult mouse brain neuron [64]. Recently, the absolute protein quantities of MCT1 have been determined in freshly isolated human brain microvessels from patients with epilepsy or glioma using quantitative RT-PCR and LC/MS/MS. The results of this study demonstrated the expression of MCT1 in these samples [81].

Regional distribution of MCT2 in the mouse brain includes cortex, hippocampus and cerebellum [59, 65, 80]. MCT2 is the major neuronal isoform as demonstrated by immunohistochemistry results with major localization in the postsynaptic densities of the neurons [80, 82, 83]. There was no co-localization of MCT2 immunoreactivity with presynaptic elements in the neuron. MCT2 has also been found in immunoreactivity in the postsynaptic membrane of parallel fibre-Purkinje cell synapses in the rat cerebellum and in the postsynaptic δ2-glutamate receptors as demonstrated by electron microscopy [63, 84]. In addition, its presence has also been demonstrated at both mRNA and protein levels in cultured neurons [80]. The expression of MCT2 was also observed in some populations of astrocytes in the white matter and glia but such presence was only detected in rat brain and cultured rat brain astrocytes [79, 85]. The mouse brain or the cultured mouse brain astrocytes failed to show such expression suggesting that there could be species differences in the distribution of MCT2 in the brain [64, 80, 83]. MCT2 has also been found in the Purkinje fibers of the cerebellum as demonstrated by immunohistochemistry [84]. In brain endothelial cells, the presence of MCT2 was only observed in a few studies and thus this still needs to be further examined [82, 86]. Although MCT2 expression has been demonstrated in rodent brain, very little MCT2 expression was observed in human brain as shown by Northern blotting results [43]. It is important to understand that there are some discrepancies in results obtained in different studies. This could be due to the differences in specificity of the antibodies used to identify the MCT isoforms which has been discussed in Bergersen *et al.* [84]. Species differences in MCT expression could also contribute to some of these differences. These discrepancies remain to be further evaluated in future studies.

MCT4 expression has been demonstrated in the astrocytes of adult rat and mouse brain in the cerebral cortex, striatum, hippocampus, paraventricular nucleus in the hypothalamus and capsula internalis [87]. MCT4 has been found to be exclusively expressed in the astrocytes [63, 84]. This is consistent with the high rate of glycolysis in astrocytes, thus requiring continuous efflux of lactate.

Studies have shown that a developmental switch exists in the expression of different MCT isoforms in various regions of the rat brain [76]. The mRNA and protein expression of MCT1 in the BBB has been found to be maximum during suckling followed by a decline with maturation in rats [75]. However, MCT2 found predominantly in the neurons shows constant expression during maturation, thus demonstrating that they play an important role in energy metabolism in the adult brain. In contrast, Pellerin *et al* have observed a decline in expression of both MCT1 and 2 during maturation by Northern blot analysis [87].

SMCT1 has recently been shown to be expressed exclusively in the neurons of mouse brain through immunofluorescence studies and it was reported to co-localize with MCT2 [88]. Studies in mixed cultures of rat brain neurons and astrocytes have also demonstrated its localization in the neurons. This suggests that SMCT1

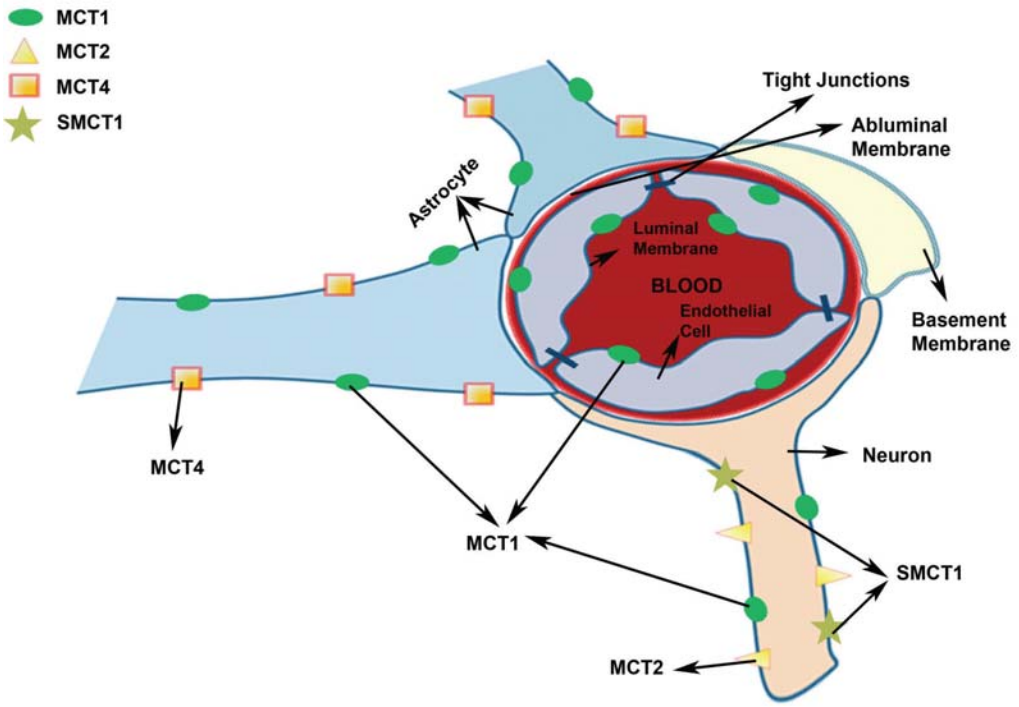

Fig. (1). Cellular localization of different MCT isoforms in brain (adapted from Simpson *et al.* 2007) [125].

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