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REVIEW

Vitamin transport and homeostasis in mammalian brain: focus on Vitamins B and E

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Abstract

With the application of genetic and molecular biology techniques, there has been substantial progress in understanding how vitamins are transferred across the mammalian blood–brain barrier and choroid plexus into brain and CSF and how vitamin homeostasis in brain is achieved. In most cases (with the exception of the sodium-dependent multivitamin transporter for biotin, pantothenic acid, and lipoic acid), the vitamins are transported by separate carriers through the blood–brain barrier or choroid plexus. Then the vitamins are accumulated by brain cells by separate, specialized systems. This review focuses on six vitamins (B_1, B_3, B_6, pantothenic acid, biotin, and E) and the newer genetic information including relevant 'knockdown' or 'knockout' models in mice and humans. The overall objective is to integrate this newer information with previous physiological and biochemical observations to achieve a better understanding of vitamin transport and homeostasis in brain. This is especially important in view of the newly described non-cofactor vitamin roles in brain (e.g. of B_1, B_3, B_6, and E) and the potential roles of vitamins in the therapy of brain disorders.

Keywords: cerebral endothelium, choroidal epithelium, CSF homeostasis, ependyma, niacinamide, pyridoxine, thiamine, vitamer transporters, α-tocopherol.

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For proper functioning, the mammalian brain requires macronutrients (e.g. glucose and amino acids) and micronutrients from blood (Spector and Johanson 2006). In this review, we focus on certain vitamins [B_1, B_3, B_6, pantothenic acid (PA), biotin, and E), a subclass of micronutrients not synthesized (B_3 excepted) in the brain or body. Recently, we reviewed the transport and homeostasis of vitamins B_2 (riboflavin), C, and folate, and also inositol, in the mammalian CNS (Spector and Johanson 2006). The choroid plexus (CP) plays a major role in transferring these micronutrients into the CNS (Spector and Johanson 2006). For an expanded discussion of the definitions and classification of micronutrients, vitamins, vitamin-like substances, and hormones, please see Spector (1989).

A compelling reason for reviewing this topic now is that there is substantial new molecular and genetic information in animals and humans that requires coherent integration into previous work to advance knowledge in this field. Moreover, newly appreciated roles for certain of these vitamins (beyond their enzyme cofactor roles) have been defined (e.g. for B_1,

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Abbreviations used: AVED, ataxia associated with vitamin E deficiency; BBB, blood-brain barrier; BCSFB, blood–CSF barrier; CoA, coenzyme A; CP, choroid plexus; FD, facilitated diffusion; HDL, high-density lipoprotein; KO, knockout; K_T, half-saturation constant for transport (analogous to K_m); PA, pantothenic acid; PAL, pyridoxal; PAM, pyridoxamine; PIN, pyridoxine; PIN-P, PAL-P and PAM-P (phosphorylated forms of PIN, PAL and PAM); PK, pyridoxal kinase; PLTP, phospholipid transfer protein; PS, permeability-surface area product; Pyr Phos, phosphorylated forms of vitamin B_6; Pyr, non-phosphorylated B_6; RFC, reduced folate carrier; RRR-α-tocopherol, preferred form of Vitamin E in mammals; SLC, family of thiamine transporters; SMVT, sodium-dependent multivitamin transporter; SR-B1, scavenger receptor class B; TMP, TDP TTP, thiamine mono-, di-, and triphosphate; TNSALP, tissue non-specific alkaline phosphatase; TRMA, thiamine responsive megaloblastic anemia syndrome; αTTP, α-tocopherol (binding) transport protein.

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B_3, B_6, biotin, and E) in recent years and will be briefly discussed below. Finally, these newly defined roles for vitamins coupled with understanding of their transport and homeostasis in the CNS have important implications for the therapy of certain diseases (e.g. head trauma, stroke, and Alzheimer's disease).

Background

To enter the extracellular space of brain and CSF from blood, a vitamin must cross the cerebral capillaries, the locus of the blood–brain barrier (BBB), or the CP, the main locus of the blood–CSF (BCSFB) barrier (Spector and Johanson 1989). The CP secretes most of the CSF, which serves as a conduit for convecting the transported substances to multiple regions within the CNS (Johanson 2003; Johanson *et al.* 2005). Both the brain capillary endothelial and the CP epithelial cells have tight intercellular junctions known as *zonulae occludentes* (Smith *et al.* 2004). These tight junctions are the anatomical basis of the restrictive passage of water-soluble substances like vitamins through the BBB or BCSFB (Spector and Johanson 2006). There is, however, minimal anatomical impediment to molecules moving across the ependymal and pia-glial interfaces, respectively, that separate the intraventricular and subarachnoid CSF spaces from the extracellular (interstitial) space of brain. Thus, upon gaining access to the CSF, a vitamin such as ascorbate can then penetrate deeply into the brain substance (Spector 1981).

For the vitamins described in this paper, normally less than 10% of their transfer into the CNS can be accounted for by simple diffusion with the rest being mediated by specialized carrier systems at the BBB and/or CP to transport these vitamins from blood into the CSF and extracellular space of brain (Spector and Johanson 1989). Once within the extracellular space, these vitamins must then be transferred into brain cells for transformation into cofactors and compartmentalized for various uses. Like the transport systems at the BBB and CP, the accumulation systems in brain cells are also specialized; simple diffusion cannot provide enough of these nutrients for the cells. Finally, although these vitamins are not generally irreversibly degraded in brain, they are continuously lost from the CNS to blood through the BBB and BCSFB and by bulk flow of CSF into blood (Spector and Johanson 1989). Consequently to balance vitamin clearance out of CSF and brain, there is a steady transport of vitamins into the CNS to provide a stable concentration (homeostasis) in the brain, as exemplified in the concept of turnover discussed under thiamine below (Table 1).

There are several types of transport systems involved in the transfer of vitamins among blood, CSF, and brain. They include facilitated diffusion, active sodium-dependent and independent systems, receptor-type systems, and other mechanisms (Spector 2000). We will indicate, where known, the type of system involved and where it is located e.g. at the BBB, BCSFB barrier (CP), or brain cell membrane. It should be noted that many of the brain parenchymal cell transport systems involve transfer of the transported moiety (vitamer) by facilitated diffusion into (or out of) the cell with subsequent vitamer phosphorylation or other energy requiring processes inside the cell, processes that cause the vitamer to be accumulated inside the cells, and incorporated into cofactors.

General considerations in vitamin homeostasis in brain

Before describing the transport and homeostatic mechanisms for the individual vitamins, several important general considerations that apply to varying degrees to the different vitamins will be described.

Moiety of vitamin (vitamer) transported

In general, the moiety transported in the CNS is the non-phosphorylated or non-cofactor form but thiamine monophosphate (TMP) may be a partial exception discussed

Table 1 Total vitamin concentration and turnover[a]

	Plasma (μmol/L)	Cisternal CSF (μmol/L)	Brain (μmol/kg)	Brain turnover[c] (% per day)	CSF turnover[c] (% per 3 h)
Vitamin					
B_1	0.41* [31][b]	0.36* [26]	~10*,+ [3]	60–100*,+	42*
B_3	0.5*	0.7*	500*,+	8*	50*
B_6	0.30* [55]	0.39 [26]*	8.9 [23]*	17*	24*
PA	~2*	~2*	~100+	18*,+	100*
Biotin	0.006*	0.008*	0.3+	18*	100*
E	20+ (25)	(0.03 lumbar; 0.11 ventricular)	10+ (~15)	~1+	–

[a]Data are in μmol/l or kg from rabbit (*) or rat (+); human data for vitamin E are in parentheses. All data in this and other tables are from work fully documented in the text. [b]Percent non-phosphorylated in brackets. [c]In calculating brain and CSF turnover from vitamin entering from plasma, the assumption is made of steady state and a single uniform intracellular brain or CSF pool (see text).

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below. Moreover, the CP, after accumulating non-phosphorylated thiamine or non-phosphorylated B_6 (Pyr), releases thiamine monophosphate (TMP) or pyridoxal phosphate (Pyr Phos), respectively, into CSF, thus explaining, in part, the presence of TMP and Pyr Phos in CSF (see below).

Transport regulation and homeostasis

Notwithstanding the fairly rapid turnover of water-soluble vitamins (Table 1), the brain is generally better protected than any other organ from either deficiency states or excess vitamin intake. The mechanisms involved in keeping brain vitamin levels relatively constant will be described below. In some cases of vitamer transport from blood into CSF and brain (e.g. for B_1, and to a lesser extent, B_6), the K_T, defined as the half-saturation concentration for transport through the BBB and/or BCSFB, is approximately equal to the vitamer concentration normally in plasma (Tables 1 and 2). Thus, entry is controlled in a sense by a saturable 'gate.' In these cases, raising (or lowering) the plasma concentration will decrease (or increase) the relative amount (proportion) entering CSF and brain via the carrier-mediated process. Hence, the carrier-mediated process plays a crucial transport and regulatory role because simple diffusion is relatively unimportant. However, PA, biotin, and niacinamide have K_T values at the BBB much higher than their plasma concentrations (Table 2) and employ different mechanisms for homeostasis, specifically, cellular mechanisms that regulate the total vitamin concentration in brain cells. For these vitamins, the transport systems at the BBB or CP only facilitate vitamer transport into the CNS. For comparative purposes, the permeability-surface area (PS) products at the BBB for mannitol, a substance that traverses the CNS by simple diffusion, and leucine, by facilitated diffusion, are shown in (Table 2).

Table 2 Vitamin transport through the blood–brain barrier

Vitamin	Vitamer transported	PS product (10^{-4}/s)	K_T (μmol/L)	Mechanism
B_1	Thiamine*,+	10^+	0.1–0.3*,+	FD*,+
B_3	Niacinamide*	10^+	$>10^{4+}$	FD*,+
B_6	Pyr* (see text)	$<10^*$	~1–2*	?
PA	PA*,+	2^+	19^+, 30*	AT*,+
Biotin	Biotin	1^+	20*, $\sim100^+$	AT*,+
E	RRR α-tocopherol	–	–	–
Mannitol	Mannitol	0.2^+	–	Diffusion
Leucine	Leucine	158^+	30	FD

*Rabbit; +Rat. PS, permeability-surface area product; FD, facilitated diffusion; AT, Active sodium-dependent and independent systems; PA, pantothenic acid.

Overall, then, there are five general processes that must be understood to explain mechanistically vitamin homeostasis in brain:

(i) Transport from plasma in and out of the extracellular space of brain and CSF;

(ii) Uptake and release of transported vitamers by brain cells;

(iii) Vitamer activation by kinases or other enzymes, i.e. incorporation into cofactors and transfer into appropriate compartments in the brain cells;

(iv) When appropriate, reformation of the transported vitamer for release from the brain cell and then the CNS; and

(v) Finally, mechanisms in kidney and gut including saturable uptake, excretion, and secretion (kidney) mechanisms that tend to keep plasma vitamin levels constant, thus contributing to brain homeostasis.

In rat and rabbit plasma, the total vitamin concentration (the sum of all the forms) of these vitamins varies from 6 nmol/L to 20 μmol/L with similar variability in brain (Table 1). In brain, vitamin homeostasis in some cases can be classified as excellent (e.g. vitamin C, PA, riboflavin, and folate) where it is very difficult to cause a deficiency state in an adult mammal with dietary deficiency (Spector and Johanson 2006). However, the vitamins in Table 1 (except PA) have only 'good' homeostasis wherein it is difficult but not impossible by dietary means to cause brain deficiency states in adults. Moreover, there is a margin of safety. For example, with B_1, symptomatic deficiency occurs when total B_1 levels in brain fall below 20% of normal (McCandless and Schenker 1968).

Specificity of the transport systems

The transport systems for the vitamins in Table 1 are all different with one exception, the PA/biotin/lipoic acid system at the BBB. Moreover, in some cases, there are different systems for the same vitamer with varying specificities at the BBB and CP, as well as in brain cells. For each vitamer traversing CP epithelium or brain capillary endothelium, there is a transfer system at both sides of the cell for entry and exit (e.g. the basolateral and apical choroid epithelial membranes or correspondingly the luminal and abluminal cerebral capillary endothelial cell membranes (Smith *et al.* 2004)).

Types of evidence

To present a comprehensive review of vitamin transport and homeostasis in the CNS, we have employed traditional anatomical, biochemical, and physiological data from animals and humans. Moreover, we have also incorporated recent molecular and genetic data, including animal gene 'knockdown' and 'knockout' (KO) experiments or human equivalents (Spector and Johanson 2006). Our goal is to

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place all these data into coherent, consistent explanatory paradigms. Implicit in our analyses is the assumption that vitamin homeostasis and function in brain are similar in animals and humans, thus enabling cross-genus extrapolations. The available data support this assumption (Spector 2000). Moreover, we have only used data from experiments that meet acceptable standards previously outlined in detail (Spector 2000).

For each vitamin, we provide summary background information, the physiology and biochemistry of transport through the BBB, and where available the kinetic constants. Also discussed are the specificity of the systems, the vitamers transported and the mechanisms of vitamin transport in the CNS. Where available, we will also discuss the ability of CP and brain slices *in vitro* or brain cells *in vivo* to accumulate the particular vitamer(s). *In vitro* transport studies of brain slices and CP often (but not always) furnish relevant data to help understand the *in vivo* findings. Relevant molecular and genetic data are next analyzed, focusing on human data where available. The conclusion includes a brief discussion of the newer roles of vitamins in brain and their potential therapeutic implications.

Throughout this review, we rely on a series of standard physiological techniques. The specific methods and studies will be referenced as we review the specific vitamin studies below (see also Pardridge 1999). To study unidirectional transport through the BBB, there is a series of useful methods including single pass intracarotid injections or more prolonged intracarotid infusions of up to 1 min and intravenous infusions varying from periods of seconds to steady state infusions of many hours (Pardridge 1999). In these experiments, radiolabeled compounds are used and, at the end, the concentrations of radioactive compounds (and their nature) are measured in various brain regions, CSF, and plasma. The prolonged, sensitive intracarotid infusion method was used to generate the PS products and K_T values in Table 2 (Pardridge 1999). Transport from brain to blood can also be measured with this method. The turnover values in Table 1 were generally obtained from intravenous infusion studies, either bolus or steady state (constant blood level). Intravenous infusion studies have an advantage; the animal does not have to be anesthetized during the infusion.

In vitro, both brain slices and the isolated CP can be incubated in artificial CSF at 37°C containing radiolabeled compounds. At various times up to 1 h, the amount and nature of the radioactivity in the tissue and medium can be measured. Release of accumulated radioactivity can also be easily measured. A commonly procured specimen for rodent brain slice studies is the cortical tissue above the lateral ventricle.

Intracerebroventricular injection into temporarily anesthetized rodents is a convenient way to access the CSF. To standardize CSF data interpretations, a passively distributed marker-like radiolabeled mannitol is often co-instilled to judge the adequacy of the injections and the degree of carrier-mediated removal of the test substance. Accordingly, because mannitol removal from the CSF is solely by simple diffusion, it serves as a useful reference marker to compare with the distribution of radiolabeled vitamers cleared mainly by transporters (active or facilitated). After an appropriate time, the rodents are killed and the amount and nature of the radioactivity in various brain regions, CSF, and CP were analyzed. Such experiments must be interpreted with caution because of the complexity of the system: radioactivity can stay in CSF, diffuse into brain through the ependyma or pia and be accumulated inside brain cells, pass through the BBB into blood, pass through the CP into blood, and/or pass into blood via bulk flow of CSF. With careful interpretation, the data are often surprisingly revealing as shown below.

In all these studies, unlabeled compounds can be included to assess saturability, specificity, and kinetic transport constants like K_T (Table 2) and energy or sodium requirements. Coupled with careful analytic and biochemical analyses and, in some cases, after genetic manipulations, mechanistic understanding of vitamin transport, homeostasis, and function is obtainable.

Vitamin B_1

The concentrations of total thiamine which includes thiamine and thiamine mono- (TMP), di- (TDP), and triphosphate (TTP) in plasma, CSF, and whole brain are shown in Table 1 (McCandless and Schenker 1968; Spector 1976, Spector 1982). The rapid turnover of total thiamine in brain and CSF is also given in Table 1. In this context, turnover is defined as the amount of vitamin per unit time that enters brain or CSF from plasma at steady state divided by the total vitamin content in the brain or CSF. The assumption of a single vitamin pool in brain or CSF is made. Of course, the turnover of the individual components (e.g. TTP) is quite different from the total turnover; moreover, the turnover in various compartments (e.g. mitochondria) is also important. The reader is referred to Rindi *et al.* (1984) for discussion of the turnover of total thiamine and the individual thiamine vitamers in brain. In brain, TDP is an essential cofactor for several enzymes in the Krebs cycle and TTP plays a role in nerve membrane function (Na^+ gating).

In mammals, it is difficult to increase CSF and brain total thiamine levels significantly after large oral doses, thus attesting to the homeostatic mechanisms (Spector 1976, 1982). However, in severe deficiency states, brain levels can fall to below 20% of normal with devastating damage to the CNS unless massive doses of intravenous thiamine are injected. After gastrointestinal absorption, thiamine (and TMP released from liver) circulates in plasma (Spector 1976).

At the BBB, thiamine is transferred across the BBB bidirectionally by a high-affinity, specific, saturable, low-

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Table 3 Thiamine transport in CNS*

1) Transport through BBB
   a) Vitamer transferred = thiamine (see text)
   b) Directionality = bidirectional (facilitated diffusion)
   c) K_T∼0.1–0.3 μmol/L
   d) PS product = 10×10^{-4}/s
2) Transport in CP *in vitro*
   a) Vitamer accumulated = thiamine
   b) Accumulation mechanism = active transport
   c) Vitamer released = thiamine; TMP
3) Transport in brain slices
   a) Vitamer accumulated = thiamine (see text)
   b) Accumulation mechanism = probably facilitated diffusion with subsequent pyrophosphorylation
   c) Vitamers released = thiamine; TMP
4) Specificity for transport at BBB, CP, and brain
   a) See text
5) Molecular biology of transport
   a) SLC 19A1, 2, 3; SLC 29A19

*Data are from rat and rabbit. In this table and in Tables 6–8, the directionality of transport across choroid plexus is not specified because further research is needed for a firm conclusion to be drawn (see text).

capacity facilitated diffusion system (Tables 2 and 3) (Spector 1976; Lockman *et al.* 2003).

Choroid plexus accumulates thiamine by an active transport system and, like liver, releases thiamine and TMP, presumably on the CSF side, thus explaining, in part, the substantial amount of TMP in CSF (Tables 1, 3 and 4) (Spector 1976, 1982). Additional 'polarized transport' experimentation, i.e. using chambers or transwells to orient the apical and basolateral membranes, will be necessary to corroborate our working hypothesis that thiamine and TMP are extruded at the CSF-facing surface of the choroidal epithelium.

Brain slices accumulate thiamine by a saturable process known as facilitated diffusion (Tables 3 and 5) (Sharma and Quastel 1965; Nose *et al.* 1976). At tracer concentrations, the concentration of intracellular thiamine is approximately equal to the medium concentration. However, intracellularly

Table 4 Transport into CSF via rabbit choroid plexus*

Vitamin	Principal vitamer transported	K_T for accumulation (μmol/L)	Vitamer released
B_1	Thiamine*	–	Thiamine; TMP
B_3	Niacinamide	0.2	–
B_6	Pyr	7.0	Pyr Phos; Pyr
PA	PA	∼10 μmol/L	PA

*See text. TMP, thiamine monophosphate; PA, pantothenic acid; Pyr Phos, pyridoxal phosphate; Pyr, non-phosphorylated B_6.

Table 5 Uptake by brain slices

Vitamin	Principal vitamer transported	K_T for accumulation (μmol/L)	Vitamer released
B_1	Thiamine$^{+,*}$	0.1–0.3$^{*,+}$	Thiamine; TMP$^+$
B_3	Niacinamide*	0.8*	Niacinamide*
B_6	Pyr*	0.5*	Pyr*
PA	PA*	∼15 μmol/L*	PA*

* = rabbit; $^+$ = rat. TMP, thiamine monophosphate; PA, pantothenic acid; Pyr, non-phosphorylated B_6.

in brain slices and in brain *in vivo*, thiamine is rapidly phosphorylated to TDP by thiamine pyrophosphokinase and thus it accumulates. Both thiamine and TMP can be released by brain slices (Table 5) (Sharma and Quastel 1965; Nose *et al.* 1976).

Two hours after the intraventricular injection of tracer thiamine and tracer mannitol (as a passive control, distributed in the CNS by simple diffusion) into rabbits, the radioactive thiamine was cleared from CSF into blood and brain much faster than mannitol (Spector 1976). In cisternal CSF, 2 h after the injection, 92% of the thiamine radioactivity was associated with TMP and in brain, 85% was thiamine phosphates. When 1.4 μmol unlabeled thiamine was injected along with the tracer thiamine, only 22% and 12% of the radioactivity in CSF and brain, respectively, were phosphorylated. Moreover, the unlabeled thiamine decreased the amount of thiamine leaving CSF (into blood) and penetrating into brain (Spector 1976).

In summary, thiamine enters the CNS via a facilitated diffusion system at the BBB, and an active transport system in the CP which releases both thiamine and TMP into CSF (Tables 3 and 4). These systems are half saturated at the normal plasma concentration. Brain cells then accumulate thiamine (by facilitated diffusion and pyrophosphorylation) and, as discussed below, possibly TMP from the extracellular space of brain and CSF (Tables 1 and 2). The brain cell thiamine accumulation system is also approximately half saturated at the normal CSF concentration (Table 1). These saturable systems in series (i.e. at the BBB and CP, and at the brain cell membrane) provide an important degree of thiamine homeostasis in brain.

When we first described the transport of thiamine into brain and CSF *in vivo* and *in vitro*, we wondered why there was so much TMP in CSF (Table 1). TMP seemed to come from both brain cells and CP (Table 3). We raised the question whether TMP itself could be transported through cell membranes (Spector 1982). Rindi *et al.* (1984) provided experimental data that TMP itself, in plasma, could traverse the BBB by a saturable mechanism but with a PS product of about 10% that of thiamine (Tables 2 and 3) (Reggiani *et al.* 1984; Patrini *et al.* 1988). Although these experiments

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involved intravenous injection of labeled TMP, they were only 20 s in length (Patrini *et al.* 1988). However, one cannot be certain that the labeled TMP was not dephosphorylated in plasma to labeled thiamine which was the moiety transferred. Both plasma (and CSF) can slowly dephosphorylate TMP. Therefore, to prove conclusively that TMP itself is transported across cell membranes, one requires double-labeled TMP with ^{32}P labeled phosphate (see below under vitamin B_6). Until recently, the above description constituted the knowledge of thiamine and TMP transport and homeostasis in the CNS.

In the last decade, however, the molecular details of thiamine transport have been clarified with the discovery and cloning of SLC 19A1, 2, and 3 and SLC 29A19 (Oishi *et al.* 2002; Lindhurst *et al.* 2006; Subramanian *et al.* 2006). SLC 19A2 is involved in the release of thiamine from the abluminal side of renal and enterocyte cells into blood but this may not be an essential function for brain (Subramanian *et al.* 2006). In humans, the absence of SLC 19A2 protein is associated with the thiamine responsive megaloblastic anemia syndrome (TRMA) (Oishi *et al.* 2002). TRMA in both humans and KO mice (on a low thiamine diet) consists of anemia, diabetes, and sensory neural hearing loss but not the devastating CNS consequences of thiamine deficiency (Oishi *et al.* 2002). Both humans with TRMA and KO mice have a normal plasma thiamine concentration (Oishi *et al.* 2002). Thus, for many transport functions (e.g. into plasma and CNS) SLC 19A2 is not necessary.

SLC 19A3, which also transports thiamine, is present in brain and other tissues (Eudy *et al.* 2000; Rajgopal *et al.* 2001). No KO experiments are available and the localization of SLC 19A3 in the CNS is uncertain. However, in both renal cells and enterocytes, SLC 19A3 occurs on the apical border and is probably essential for thiamine reabsorption by kidney and absorption by the gut (Subramanian *et al.* 2006). 'KO' of SLC 19A3, we predict, will result in non-viable animals.

SLC 19A1 is known to be the reduced folate carrier (RFC), a bidirectional carrier (Rajgopal *et al.* 2001; Zhao *et al.* 2002). The RFC is found in substantial amounts on the apical (CSF) side of CP and on neurons. Zhao *et al.* (2002) provided indirect evidence that TMP can be transported weakly by the RFC (K_T = 25 μmol/L). They showed more uptake of single-labeled TMP in non-neuronal tissue culture cells with increased expression of the RFC. They were also unable to inhibit TMP uptake with carrier thiamine in the medium (Zhao *et al.* 2002). Although indirect, these analyses coupled with experiments with TMP *in vivo* (Patrini *et al.* 1988) suggest an alternative low affinity mechanism for thiamine exit from and entry into brain cells, i.e. as TMP via RFC. It is worth noting that TMP transport via RFC may explain why loss of the SLC 19A2 in KO mice and humans is not more devastating. In any event, experiments with double-labeled TMP will be necessary to prove this theory conclusively.

Finally, SLC 25A19 has been shown to transport TDP into mitochondria, thus explaining a previously uncertain mechanism (Lindhurst *et al.* 2006).

In summary, we hypothesize these data support the notion that thiamine is the principal vitamer transported from blood into the extracellular space of brain by cerebral capillaries and into CSF by CP, into the former by a facilitated diffusion system at the BBB, and into the latter by active transport, possibly by SLC 19A3. This latter CP system would be analogous to thiamine transport by renal epithelial cells and enterocytes (Subramanian *et al.* 2006).

Thiamine may be released from CP into CSF possibly via SLC 19A2, by analogy with the renal and intestinal epithelium. Excess intracellular TMP can probably be released from brain cells and by CP into the extracellular space of brain and CSF via RFC, which, as noted above, occurs on the surface of brain cells and at the apical (CSF) side of CP.

Brain cells can accumulate and release both thiamine via the thiamine facilitated diffusion accumulation system and probably TMP by the RFC on neuronal membranes. Of course, the latter mechanistic speculation needs to be unequivocally proven. Moreover, the molecular nature of the facilitated diffusion systems for thiamine at the BBB and brain cell membranes remains unknown.

Vitamin B_3 (niacin)

The concentrations of total niacin in plasma, CSF, and brain are shown in Table 1 (Spector 1979; 87; Spector and Kelley 1979). Approximately, 8% of total niacin in brain turns over per day (Spector 1979). In plasma and CSF, the large majority (if not all) of total niacin is niacinamide (nicotinamide) (Spector 1979). In brain, niacinamide is taken up and converted to niacinamide adenine dinucleotide (NAD) as well as NADH, NADP, and NADPH principally via niacinamide mononucleotide. Nicotinic acid is rapidly converted in the body and brain to niacinamide and NAD (Spector 1979; Spector and Kelley 1979).

Although it is relatively easy to produce symptomatic B_3 deficiency in animals, total niacin and NAD levels are much better maintained in brain than in liver in deficient animals (Spector 1979). On the other hand, at extremely high plasma concentrations, the brain NAD levels, unlike liver, increase only slightly. In humans, inadequate tryptophan (the precursor of niacin) and/or niacin in the diet can lead to the dementia seen with pellagra (Spector 1979).

NAD and its congeners serve as cofactors for many essential enzyme reactions. Recently, it has been established that NAD also donates ADP-ribose in three essential reactions in brain: ADP-ribose transferases, C-ADP ribose synthetases, and sitruins (type III protein lysine deacetylases) (Hisahara *et al.* 2005; Belenky *et al.* 2007). The latter enzymes (sitruins) can deacetylate histones, regulate gene

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Table 6 Niacinamide transport in CNS[a]

1) Transport through the BBB
a) Vitamer transferred = niacinamide (see text)
b) Directionality = bidirectional (facilitated diffusion)
c) K_T > 10 mmol/L
d) PS product 10×10^{-4}/s
2) Transport in CP *in vitro*
a) Vitamer accumulated = niacinamide
b) Accumulation mechanism = active transport with subsequent conversion to NAD
3) Transport in brain slices
a) Vitamer accumulated = niacinamide
b) Accumulation mechanism = facilitated diffusion with subsequent conversion to NAD
c) Vitamer released = niacinamide
4) Specificity at BBB and in brain slices
a) Specific; niacin, quinolinic acid, picolinic acid, *n*-methylniacinamide no affinity

[a]Data are from rat and rabbits.

transcription, and play an essential role in brain (Hisahara *et al.* 2005; Belenky *et al.* 2007).

At the BBB, niacinamide is rapidly transferred across the capillaries bidirectionally by a very low-affinity, high-capacity facilitated diffusion system (Tables 2 and 6) (Spector 1979, 1987). Niacinamide also enters red blood cells by a low-affinity symmetrical facilitated diffusion system (K_T = 6.0 mmol/L) for influx and efflux (Reyes *et al.* 2002). *In vivo* in rabbits, after 3 h intravenous infusions of various concentrations of (^{14}C) niacinamide so as to keep plasma levels constant, there was no saturation of (^{14}C) niacinamide entry into CSF even at a plasma concentration of 1.8 mmol/L (Spector 1979). The ratio of CSF to plasma (^{14}C) niacinamide was 0.7 and 0.9 with tracer and 1.8 mmol/L niacinamide in plasma, respectively. However, about 4 μmol/L unlabeled plasma niacinamide decreased the entry of (^{14}C) niacinamide from plasma into CP and brain by ~50% and 33%, respectively – thereby, showing saturation of entry (Spector 1979). The percent of (^{14}C) NAD in brain also decreased from 56% to 6% as the plasma concentration increased from 0.5 μmol/L (normal) to 1.8 mmol/L (Spector 1979).

Rabbit brain slices accumulate niacinamide by facilitated diffusion (K_T of ~0.8 μmol/L; Table 5) (Spector and Kelley 1979). The accumulated niacinamide is quickly incorporated into NAD. Unchanged niacinamide brain-to-medium ratios did not exceed unity. The brain slices readily released niacinamide (Spector and Kelley 1979). In view of the CSF (and presumably extracellular space of brain) concentration of niacinamide of ~0.7 μmol/L (Table 1), the brain cell accumulation system for niacinamide is normally about one-half saturated (Spector 1979).

Rabbit CP can accumulate niacinamide by high-affinity active transport (Table 4) but it does not readily release the niacinamide (Spector and Kelley 1979). As will be discussed below, the CP uptake system for niacinamide (Tables 4 and 6) seems to be for internal epithelial use and not for substantial net transfer from blood to CSF. However, the existence of a low affinity transport system in CP for niacinamide similar to the facilitated diffusion system in cerebral capillaries cannot be excluded by *in vitro* CP experiments alone.

It was instructive to ascertain how niacinamide, which readily gains access to the CSF via the blood to brain and then through the ependymal route, was transported out of CSF (Spector 1979). Two hours after the intraventricular injection of tracer (^{14}C) niacinamide and (^{3}H) mannitol, only 1% of injected (^{14}C) niacinamide was recovered in CSF and 9% in brain [50% as (^{14}C) NAD; 10% total] when compared with 59% of (^{3}H) mannitol (Spector 1979). When carrier niacinamide was injected intraventricularly with the (^{14}C) niacinamide and (^{3}H) mannitol, a remarkable result was found: only 1% of the injected (^{14}C) was recovered in brain and CSF versus 57% of the (^{3}H) mannitol. Thus, the elevated concentration of carrier niacinamide (41 μmol/L in the withdrawn CSF) saturated (^{14}C) niacinamide entry into and formation of (^{14}C) NAD in brain, allowing very rapid clearance of (^{14}C) niacinamide from CSF and the extracellular space of brain, presumably principally via the facilitated diffusion system in the cerebral capillaries (Spector 1979).

In summary, niacinamide, the principal B_3 vitamer in plasma and CSF, rapidly traverses the BBB in both directions by a very low-affinity, high-capacity facilitated diffusion system at the BBB in the cerebral capillaries (Tables 1, 2 and 6). Similarly, niacinamide readily enters and leaves CSF via the facilitated diffusion system at the BBB after transfer through the ependyma and pia. Once within the CSF and the extracellular space of brain, niacinamide is accumulated by brain cells by facilitated diffusion with a K_T~0.8 μmol/L (Tables 5 and 6). Thus, the control of brain tissue levels of total niacin is dependent on entry/exit of niacinamide into brain cells with subsequent incorporation into NAD, binding, compartmentalization, and some use of NAD as an ADP-ribose donor with release of niacinamide (Hisahara *et al.* 2005; Belenky *et al.* 2007). Unlike the case with other vitamins (e.g. folates, ascorbate, and inositol) (Spector and Johanson 2006), the plasma niacinamide levels are a reasonable approximation of what brain cells 'see.' Brain and CSF niacinamide returns to blood by reabsorptive transport across the cerebral capillaries and to a lesser extent by bulk flow of CSF into the venous blood.

At present, there is a tremendous interest in trying to manipulate NAD levels in brain for neuroprotection, where increasing brain NAD appears to be protective (Hoane *et al.* 2006a,b; Belenky *et al.* 2007). Also, manipulation of NAD in animal models of Alzheimer's disease and aging are also active areas of current research (Belenky *et al.* 2007). However, the NAD systems are complex; e.g. niacinamide

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itself inhibits type III protein lysine deacetylases (Belenky *et al.* 2007). The interested reader is referred to current reviews and studies of manipulating brain NAD as a potential therapeutic modality (Hoane *et al.* 2006a,b; Belenky *et al.* 2007).

Vitamin B_6

Vitamin B_6 in the diet consists of pyridoxine (PIN), pyridoxal (PAL), pyridoxamine (PAM), and their respective phosphates (Spector 1978a,b). The three non-phosphorylated forms are designated as Pyr and the three phosphorylated forms Pyr Phos. Pyr can be phosphorylated by pyridoxal kinase (PK) (Spector 1978a,b). PIN-P is converted by PIN-P oxidase in liver, brain, and CP to PAL-P, the active cofactor form (along with PAM-P) (Spector 1978a,b). Pyr is the vitamer transferred through cell membranes as discussed below. Recently, B_6 vitamers have been shown to have potent antioxidant activity thus joining vitamins E and C and glutathione (Bilski *et al.* 2000).

The concentration of Pyr and Pyr Phos in CSF and brain is given in Table 1 (Spector 1978a). Also presented are the turnover rates for total B_6 in brain and CSF. The tissue-phosphorylated forms are mainly PAL-P and to a much lesser extent PAM-P; PIN-P is rapidly converted to PAL-P *in vitro* and *in vivo*. The turnover of total B_6 in brain and CSF is also shown in Table 1 (Spector 1978a).

The entry of Pyr through the BBB and BCSFB is saturable with a $K_T \sim 1$–2 μmol/L (Tables 2 and 6) (Spector 1978a). The exact nature of the transport system at the BBB is uncertain but probably facilitated diffusion.

The CP can accumulate (^{3}H) PIN *in vitro* by facilitated diffusion with intracellular trapping as Pyr Phos via PK (Tables 4 and 7) (Spector 1978b). Remarkably, the CP (like liver) readily releases Pyr Phos (and to a lesser extent Pyr), thus explaining the high percentage of Pyr Phos in CSF (Table 1). Employing (^{3}H and ^{32}P) PIN-P, we have been able to show conclusively that rabbit CP as well as brain slices and red blood cells cannot transport Pyr Phos intracellularly (Spector and Greenwald 1978). The moiety transported is Pyr. Employing (^{3}H) PIN-P causes misleading results because, in artificial CSF, both brain slices and CP can dephosphorylate the Pyr Phos, then accumulate Pyr and rephosphorylate it intracellularly (Spector and Greenwald 1978).

Brain slices accumulate Pyr by facilitated diffusion with a K_T of ~0.5 μmol/L (Tables 5 and 7). The accumulation depends on phosphorylation of Pyr by PK. Brain slices release Pyr but not Pyr Phos (Spector 1978b).

Two hours after the intraventricular injection into rabbits of tracer (^{3}H) PIN, the (^{3}H) PIN was extensively cleared from CSF into blood and brain (Spector 1978a); 74% and 82% of the remaining (^{3}H) PIN in CSF and brain were phosphorylated, respectively. Apparently, as in the case of thiamine, the CP can accumulate PIN from the CSF (as well as the blood), phosphorylate it, and then return Pyr Phos into CSF. When carrier (0.49 μmol/L) PIN was included in the intraventricular injectate, the amount of (^{3}H) PIN entering brain decreased by 65% and the amount of phosphorylated (^{3}H) Pyr of the total in CSF and brain was 8% and 48%, respectively. The mechanism by which CP releases Pyr Phos is unknown.

Table 7 Vitamin B_6 transport in rabbit CNS

1) Transport through the BBB
a) Vitamer transferred = Pyr
b) Directionality = bidirectional (? facilitated diffusion)
c) $K_T \sim 1$–2 μmol/L
2) Transport in CP *in vitro*
a) Vitamer accumulated = Pyr
b) Accumulation mechanism = facilitated diffusion with subsequent phosphorylation
c) Vitamers released; Pyr Phos (major); Pyr (minor)
3) Transport in brain slices
a) Vitamer accumulated = Pyr
b) Accumulation mechanism = facilitated diffusion with subsequent phosphorylation
c) Vitamer released = Pyr
4) Specificity for transport systems at BBB, CP, and brain
a) Pyr Phos; Pyridoxic acid; salicylate < 5% affinity of Pyr
5) Molecular Biology in Brain and CP
a) Accumulation depends on pyridoxal kinase
b) In brain, accumulation probably depends in part on TNSALP* (see text)

Pyr, non-phosphorylated B_6; *TNSALP, tissue non-specific alkaline phosphatase.

Two hours after tracer (^{3}H and ^{32}P) PIN-P was injected intraventriculary into rabbits, the (^{3}H and ^{32}P) was mainly hydrolyzed in the CSF (Spector and Greenwald 1978). The (^{3}H) Pyr was then taken up across the ependyma by brain and phosphorylated to (^{3}H) Pyr P by parenchymal cells. Similarly, in CSF about 70% of the remaining (^{3}H) was (^{3}H) Pyr P, 15% was (^{3}H and ^{32}P) PIN-P, and the remainder (^{3}H) Pyr. Thus, *in vivo* (^{3}H and ^{32}P) PIN-P, like *in vitro*, does not cross the brain cell membranes. As expected, *in vivo* (^{3}H and ^{32}P) PIN-P was cleared from the CNS much more slowly than (^{3}H) PIN. Thus, Pyr Phos in CSF acts as a kind of reservoir for the formation of Pyr for brain. Unlike TMP, however, Pyr Phos itself cannot be accumulated by brain (Spector and Greenwald 1978).

In summary, Pyr is transported by facilitated diffusion across the BBB and into CP by a system that is approximately one-half saturated at a concentration substantially higher (1–2 μmol/L) than the plasma concentration (0.3 μmol/L). The concentration of Pyr to half-saturate the accumulation by brain slices is close to the CSF concentration (0.5 μmol/L). Thus, raising the plasma Pyr concentration decreases the relative amount entering brain cells due to

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proportionately less carrier-mediated flux through the BBB and, more importantly, saturation of PK in brain, thus decreasing the amount phosphorylated and retained (Spector 1978a,b; Spector and Shikuma 1978). At low plasma concentrations, relatively more is accumulated by brain, thus tending to maintain brain B_6 cofactor levels.

The B_6 homeostatic system, however, is imperfect and severe deficiency in children and adults can cause brain dysfunction especially seizures (Clayton 2006). Moreover, products of several inborn errors of metabolism and certain drugs can complex PAL-P and lead to B_6 brain deficiency and seizures (Clayton 2006). Other drugs such as theophylline can inhibit PK and cause seizures. Some people have inborn errors in key PAL-P requiring enzymes, defects that in some cases can be overcome by high doses of B_6 (Clayton 2006). Finally, there are individuals born with abnormal PIN-P oxidase who require PAL (not PIN, thus bypassing PIN-P oxidase) for survival (Clayton 2006). Moreover, there are humans born with a condition termed hypophosphatasia (Clayton 2006). These people have low levels of tissue non-specific alkaline phosphatase (TNS-ALP), an ectoenzyme only active in the extracellular space (Whyte *et al.* 1988). In the more severe cases, they have 50–100 times higher levels of plasma PAL-P than normal and low levels of plasma PAL (Whyte *et al.* 1988; Clayton 2006). The increased levels of plasma PAL-P (released by liver) are due to the inability to dephosphorylate PAL-P in plasma (by TNSALP) for release of PAL for tissue uptake. Tissue levels of PAL-P and PAL tend to be normal except in severe cases (Whyte *et al.* 1988). In severe cases, B_6 responsive seizures occur soon after birth. With TNSALP KO mice, with no residual TNSALP, the condition is fatal with seizures at birth, preventable with, as expected, PAL to correct the low brain levels of PAL-P (Waymire *et al.* 1995; Clayton 2006).

Therefore, it appears that in the CNS, the conversion of Pyr to Pyr Phos by CP, the subsequent release by CP of Pyr Phos into CSF (analogous to the liver releasing Pyr Phos into plasma) followed by dephosphorylation of Pyr Phos (presumably by TNSALP in the CNS) is probably a part of the biology for normally providing Pyr to brain, an integral component of the homeostatic mechanism (Spector and Greenwald 1978; Waymire *et al.* 1995; Clayton 2006). Without question in humans and mice, the inability to dephosphorylate extracellular PAL-P to PAL by TNSALP leads to perinatal seizures and death (Clayton 2006). If recognized early, providing PAL can overcome the seizures and prevent death by furnishing PAL for transport through the BBB and uptake by brain cells. In withdrawn rabbit cisternal CSF or artificial CSF kept at 37°C for 1 h, only 12% and 2%, respectively, of the (^{3}H and ^{32}P) PIN-P were hydrolyzed (Spector and Greenwald 1978). These findings are consistent with an important role of TNSALP for Pyr transport and homeostasis in the CNS.

Pantothenic acid

Pantothenic acid, after entry into cells and phosphorylation by PA kinase, the rate-limiting step, is then converted via a series of intermediates to coenzyme A (CoA) in all tissues (Spector and Boose 1984; Spector 1986a,b). The kidney and gastrointestinal tract, working together, keep the plasma levels of PA relatively constant (Spector 1986a).

The concentration of total PA in plasma, CSF, and brain, and the turnover of total PA in CSF and brain are shown in Table 1 (Spector 1986a). Most if not all the total PA in CSF and plasma is PA itself (Spector 1986a). It is very difficult in deficiency states to deplete the brain of PA for reasons discussed below (Spector 1986a).

Pantothenic acid is transferred across the BBB of rats and rabbits by a saturable system in the cerebral capillaries with a K_T of 19 μmol/L in rats and ~30 μmol/L in rabbits (Tables 2 and 8) (Spector *et al.* 1986). Biotin, probenecid, and medium chain fatty acids (all <100 μmol/L) inhibit PA transport through the BBB, whereas penicillin G, hydroxybutyrate, L-leucine, pyruvate, and vitamins B_1, B_3, and B_6 (PIN) – all 1 mmol/L – do not (Spector *et al.* 1986). *In vitro*, bovine endothelial cells contain a sodium-dependent, active transport system termed the sodium-dependent multivitamin transporter (SMVT); see below. The SMVT is almost certainly the mechanism by which PA enters cerebral capillaries. How PA leaves the capillaries and is transferred into the extracellular space of brain are unclear.

In vivo in rabbits infused with tracer PA for 3 h so as to maintain constant plasma levels, PA readily entered brain and CSF by a saturable system (K_T~30 μmol/L), but none of the tracer PA in plasma or the CNS was metabolized (Spector 1986a). Brain and CSF PA divided by plasma PA

Table 8 Pantothenic acid transport in the Rabbit CNS

1) Transport through the BBB*
a) Vitamer transferred = PA
b) Directionality = into brain
c) PS product = 2×10^{-4}/s; active transport
d) K_T = 19 μmol/L
2) Transport in CP *in vitro*
a) Vitamer accumulated = PA
b) Accumulation mechanism = active transport (K_T~10 μmol/L)
c) Vitamer released = PA
3) Transport in brain slices
a) Vitamer accumulated = PA
b) Accumulation mechanism = saturable with subsequent phosphorylation (K_T~15 μmol/L)
c) Vitamer released = PA
4) Specificity of transport (see text)
5) Molecular biology of transport at BBB and CP
a) SMVT (see text)

*BBB data are from rats.

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levels just exceeded one in 3 h (Tables 1, 2 and 8) (Spector 1986a).

In vitro rabbit CP accumulated labeled PA from medium by an active, sodium-dependent transport system with a half-saturation concentration of ~10 μmol/L (Table 4) (Spector and Boose 1984; Spector 1986b). Unchanged PA was readily released by a cold-sensitive release mechanism of unknown type. The CP uptake system was weakly inhibited by probenecid and *n*-caproic acid but not nicotinic acid or cysteine (1 mmol/L).

In vitro rabbit brain slices weakly accumulated (^{14}C) PA with ~1/3 phosphorylated in 30 min with 0.5 μmol/L PA in the medium (Tables 5 and 8) (Spector and Boose 1984; Spector 1986b). The uptake system was not sodium-sensitive (unlike the SMVT system at the BBB and CP) but PA uptake was inhibited by probenecid, ouabain, and medium-chain fatty acids. The IC_{50} for probenecid and decanoic acid were 0.1 and 0.05 mmol/L, respectively. Pyruvate, acetate, and hydroxybutyrate (all 1 mmol/L) had no effect. (^{3}H) phospho-PA was also not accumulated by CP or brain slices *in vitro*. In efflux (release) experiments, PA was readily released by brain slices (Spector and Boose 1984; Spector 1986b).

Most interesting was the partitioning of accumulated (^{3}H) PA inside brain slices (Spector 1986b). After homogenization, 64% of (^{3}H) was associated with the pellet – approximately half of which was (^{3}H) PA – not (^{3}H) CoA or (^{3}H) phosphorylated intermediates. This suggests the PA itself is concentrated in brain cellular organelles, e.g. in mitochondria. It is worth noting that the concentration of free PA in brain is 20 μmol/L or ~20% of the total PA in brain (Table 1) (Spector 1986a).

Two hours after the intraventricular injection of tracer PA and mannitol into rabbits, PA was more quickly cleared than mannitol from the CSF by a probenecid-sensitive mechanism (Spector 1986a). Some PA penetrated brain but after 2 h (^{14}C) PA in brain or CSF was not metabolized. When carrier PA was injected intraventricularly along with the tracer PA, the entry of tracer PA into brain was diminished and the efflux of PA from CSF was slowed. It was unexpected that tracer PA in brain was not phosphorylated after 3 h intravenous injections or 2 h intraventricular injections in view of the easily detectable phosphorylation of PA in brain slices. To detect phosphorylation *in vivo*, we injected 37 μCi (^{3}H) PA into rabbit left lateral ventricle (Spector 1986a). The rabbits were allowed to wake and then killed 18 h later. Only 0.3% of the (^{3}H) PA injected was recovered in CSF (all (^{3}H) PA) but in the left forebrain 4.1% was recovered, with 43% as (^{3}H) CoA (Spector 1986a). We think that part of the reason for the *in vitro–in vivo* discrepancy is that in brain slices, a significant portion of the endogenous PA leaks out of the slices into the medium, thus desaturating PA kinase and therefore enhancing (^{3}H) PA phosphorylation *in vitro*, whereas evidently this does not happen *in vivo*.

In summary, PA is transported into brain and CSF, in large part, by a system in brain capillaries and CP, respectively, that is almost certainly the SMVT discussed below under biotin. It remains to be determined how the PA egresses the cerebral capillaries into brain interstitial space and the CP epithelium into CSF. Probenecid, medium chain fatty acids, and biotin all have affinity for the SMVT system in brain capillaries and CP in the ~20–50 μmol/L range. After passage through the BBB or BCSFB, PA can be accumulated from the extracellular space by brain cells via a saturable, energy-requiring, but sodium-insensitive system. *In vitro*, in brain slices, this system is easily detectable as is the metabolism of (^{3}H) PA to (^{3}H) CoA. *In vivo*, the incorporation of (^{3}H) PA into (^{3}H) CoA is very slow. The brain turnover calculations in Table 1 are based on there being a single uniform pool, but in the case of PA, like thiamine, that assumption is invalid. It is quite clear that the turnover of PA is much faster than that of CoA in the various brain compartments. To understand the turnover of CoA requires much more quantitative work, but it is likely that the slow turnover of CoA helps explain the resistance of brain to PA deficiency. The ready access of plasma PA (2 μmol/L) through the SMVT system (K_T~19–30 μmol/L) to the CSF and brain is clear but not an important part of the homeostatic system for PA/CoA in brain except with very high concentrations of plasma PA.

Biotin

Biotin is an essential cofactor for four carboxylase enzymes (Spector and Mock 1987, 1988). In recent years, biotin has also been implicated in cell signaling and in histone biotinylation via biotindase (Hymes and Wolf 1996). This latter activity helps regulate chromatin structure and hence gene expression. Whether histone biotinylation occurs in human brain is uncertain because of the very low levels of biotinidase (Hymes and Wolf 1996).

The concentrations of total biotin in rabbit plasma and CSF and rat brain are shown in Table 1 (Spector and Mock 1988). In plasma and CSF, the main vitamer is biotin. In humans, the concentration of plasma biotin is ~1 nmol/L. The turnover of biotin in CSF and brain is also shown (Table 1) (Spector and Mock 1988).

Biotin penetrates the BBB and BCSFB (Tables 1 and 2) with a PS product of 10^{-4}/s and a K_T of ~20–100 μmol/L in mice, rabbits, and rats (Table 2) (Spector and Mock 1987; Park and Sinko 2005). The entry of biotin into rat brain is strongly inhibited by PA, probenecid and medium chain fatty acids, e.g. nonanoic acid, but not biocytin, niacinamide, thiamine, or PIN (Spector and Mock 1987). In conscious adult rabbits after a 3 h intravenous infusion of tracer (^{3}H) biotin so as to keep the plasma levels constant, there was rapid entry of (^{3}H) biotin into CSF and brain (Spector and Mock 1988). The concentration of (^{3}H) biotin in brain

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equaled that of plasma and was four and two times higher in CP and CSF, respectively. Raising the biotin level in plasma from a normal of ~6 nmol/L to 20 μmol/L decreased the tissue to plasma levels of ^{3}H biotin in brain by 50% and those in CP and CSF by about 75%. In these experiments, no metabolism of (^{3}H) biotin was observed. Thus in the range of 6 nmol/L to 1 μmol/L plasma biotin, increasing the plasma biotin concentration would decrease the entry of biotin into brain relatively little, as the K_T is ~20 μmol/L (Spector and Mock 1988).

In calf brain and bovine endothelial cells grown in culture, two groups of investigators have shown that biotin is accumulated by a sodium-sensitive accumulation system with a K_T~50 μmol/L. PA, lipoic acid, nonanoic acid, and probenecid but not biocytin inhibit (^{3}H) biotin transport (Baur and Baumgartner 2000; Park and Sinko 2005). They also found that SMVT was present and expressed in these cultured endothelial cells (Park and Sinko 2005).

In vitro in adult rabbit brain slices or isolated CP, we were unable to determine the nature of biotin penetration into these tissues (Spector and Mock 1988). For example, the uptake of 1 nmol/L (^{3}H) biotin by CP in 5, 15, and 30 min showed tissue-to-medium ratios of 1.1 or less and was not saturable with 1 mmol/L biotin. It is worth noting that two groups of investigators have reported a very high affinity system (K_T~3 nmol/L) for biotin transport in white cells and keratinocytes (Zempleni and Mock 1999; Grafe *et al.* 2003). However, in enterocytes and retinoblastoma cells only the SMVT system was found (Balamurugan *et al.* 2003; Kansara *et al.* 2006). We also did not detect accelerated transfer of biotin (i.e. an increase in the PS product through the rat BBB (Table 2) with 3 nmol/L biotin in the injectate (Spector and Mock 1987)). However, it is possible that a very high affinity system exists in brain cell membranes, an eventuality that needs to be tested.

Two hours after the intraventricular injection of tracer (^{3}H) biotin (10 μCi; final tracer concentration in withdrawn CSF 9 nmol/L) and (^{14}C) mannitol, the (^{3}H) was removed from the CSF and brain slightly more rapidly than mannitol (Spector and Mock 1988). Like niacinamide discussed above, the addition of carrier biotin intraventricularly accelerated the clearance of (^{3}H) biotin from CSF and brain by an uncertain mechanism. No metabolism of (^{3}H) biotin in brain or CSF was observed. However, after intraventricular injection of 20 μCi (^{3}H) biotin and waiting 18 instead of 2 h, 30–46% of the (^{3}H) in forebrain, cerebellum, and brainstem was covalently bound to proteins, thus demonstrating the adequacy of the techniques and the slow incorporation of (^{3}H) biotin into carboxylases, histones, or other proteins (Spector and Mock 1988).

In summary, there is strong evidence that biotin, PA, and probably lipoic acid and possibly medium chain fatty acids (e.g. nonanoic acid) are transported through the cerebral capillaries into brain by an active, Na^+-dependent transport system facing the capillary lumen. This is almost certainly the SMVT system (Park and Sinko 2005). How biotin exits the capillary on the brain side and rapidly enters CSF from plasma need to be clarified. Moreover, the mechanism by which biotin enters brain cells awaits elucidation. However, what is clear is that (^{3}H) biotin can enter brain cells from CSF and presumably plasma and be covalently attached to proteins over time (Spector and Mock 1988). The concentration of free biotin, pool sizes, and location of bound biotin intracellularly in brain are unknown and confound efforts to understand biotin transport/metabolism and turnover in brain.

What is firm, however, is that (because of the ready transfer of biotin and PA through the BBB into brain and into CSF with a K_T~20 μmol/L; Table 2) the concentration of biotin in the extracellular space of brain and CSF reflects the plasma level and is not involved in regulating biotin (or PA) homeostasis in brain. Rather, biotin uptake by brain cells, covalent incorporation into proteins, compartmentalization, and presumably release of biotin from proteins are the factors involved in homeostasis. Thus, PA, biotin, and B_3 differ dramatically from B_1 and to a lesser extent B_6 in the role played by the BBB (and BCSFB) in their respective brain homeostastic mechanisms.

Vitamin E

In nature, there are four potential Vitamin E (or tocopherol) forms, depending on the degree of methylation (Zingg and Azzi 2004). Each of these four, in turn, has eight potential stereoisomers as there are three asymmetric carbons in tocopherol. The mammalian body, for reasons described below, prefers RRR-α-tocopherol, a highly lipid-soluble compound. Approximately, 90% of brain (and other tissue) tocopherol is RRR-α-tocopherol and this isomer is the focus of this review (~10% is gamma-tocopherol) (Martin *et al.* 1999).

α-Tocopherol *in vitro* has both pro-oxidant and anti-oxidant properties. *In vivo*, in the presence of vitamin C, glutathione, and other reducing agents, it probably acts as an antioxidant. However, α-tocopherol is postulated to have many non-oxidant properties (e.g. inhibition of protein kinase C and phospholiphase A2 and modulation of gene expression) (Zingg and Azzi 2004). The relative importance in brain of the antioxidant versus the non-oxidant properties e.g. in α-tocopherol deficiency states, is unknown (Zingg and Azzi 2004).

In animals and humans, there is α-tocopherol homeostasis, especially in the brain. The concentration of α-tocopherol in plasma, CSF, and brain is shown in Table 1 (Vatassery 1992). In rats, on very high vitamin E diet (vs. controls) for 4 months, it was only possible to increase the vitamin E concentration in brain by about 40% (Vatassery 1992). In liver, the concentration increased to 460% (Vatassery 1992). A revealing experiment in humans with Ommaya shunts

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showed that giving massive oral doses (400, 800, 1600, 3200, and 4000 IU daily, stepped up each month) only raised the ventricular CSF α-tocopherol concentration from 0.114 to 0.164 μmol/L ($p > 0.05$) after the final 1 month of 4000 IU, a dose 100 times the recommended daily intake. The plasma level rose from 19 μmol/L to 111 μmol/L during the titration, a ∼sixfold rise (Pappert *et al.* 1996). On the other hand, in deficiency states, the brain is the last organ to be depleted. Thus, there are powerful homeostatic mechanisms for α-tocopherol protecting the brain (Vatassery 1992). However, in animals and humans, vitamin E deficiency can occur (on a prolonged, very low α-tocopherol diet) with ataxia, areflexia, dysarthria, sensory loss, and pyramidal signs.

The detailed pharmacokinetics of vitamin E transport into CSF and brain are not known. However, the turnover of α-tocopherol in brain has been measured and is quite slow (Table 1) (Vatassery 1992).

In view of the highly lipid-soluble nature of α-tocopherol and its great affinity for plasma lipoproteins (e.g. high-density lipoprotein; HDL), one would anticipate unusual mechanisms for α-tocopherol transport and homeostasis in brain. Highly protein-bound substances like vitamin E do not readily pass the BBB (Adams and Wang 1994). Recently, three such mechanisms have been described. First, Goti *et al.* (2001) showed that the Scavenger Receptor Class B type 1 (SR-B1), which facilitates the uptake of HDL, promotes the uptake of HDL-associated α-tocopherol in porcine brain capillary endothelial cells. Goti *et al.* (2001) suggested that this receptor is responsible for α-tocopherol transport across the BBB. Subsequently, others have shown that SR-B1 KO mice have high plasma levels of α-tocopherol but low levels in brain, consistent with the postulated role of SR-B1 at the BBB (Mardones *et al.* 2002). However, the brain levels were not low enough to cause deficiency symptoms (Mardones *et al.* 2002).

Second, phospholipid transfer protein (PLTP), which promotes the exchange of α-tocopherol between lipoproteins and cells, is present in brain and may be involved in α-tocopherol transport (Gander *et al.* 2002). In PLTP KO mice, the brain concentration of α-tocopherol is 30% less than controls (Desrumaux *et al.* 2005).

Third, it is now clear that α-tocopherol (binding) transport protein (αTTP) plays a crucial role in α-tocopherol transport and homeostasis in brain (Copp *et al.* 1999; Yokota *et al.* 2001; Kaempf-Rotzoll *et al.* 2003; Qian *et al.* 2006). This protein has high affinity only for RRR-α-tocopherol, and thus is largely responsible for the selectivity of the body and brain for RRR-α-tocopherol (Copp *et al.* 1999; Yokota *et al.* 2001; Kaempf-Rotzoll *et al.* 2003; Qian *et al.* 2006). This protein is present in rodent and human brain although the levels in normal human brain are low (Kaempf-Rotzoll *et al.* 2003). A human KO of αTTP exists and causes ataxia associated with vitamin E deficiency (AVED) (Kaempf-Rotzoll *et al.* 2003). AVED patients have very low plasma vitamin E concentrations and develop CNS symptoms of vitamin E deficiency beginning after age 4 (Kaempf-Rotzoll *et al.* 2003). In αTTP KO mice, at about 1 year of age, the mice develop AVED-like neurological symptoms, thus confirming the importance of αTTP in mice and men (Yokota *et al.* 2001). In the KO mouse, the plasma and brain concentrations were ∼10% of normal (Yokota *et al.* 2001). Moreover, although supplementation with oral vitamin E raised the mouse plasma concentration close to normal, the brain levels only doubled, thus attesting to the importance of αTTP in brain (Yokota *et al.* 2001). However, this small increase in the α-tocopherol concentration in brain eliminated the neurological signs (Yokota *et al.* 2001). In humans with AVED, high dose vitamin E supplementation is helpful (Yokota *et al.* 2001).

In summary, the highly lipid-soluble α-tocopherol concentration in brain is normally regulated. SR-B1 at the BBB, PLTP, and αTTP undoubtedly play a role in the transport and regulation of the α-tocopherol concentration in the CNS. It is worth noting that PLTP is highly expressed in CP (Desrumaux *et al.* 2005). This location of PLPT in CP raises the possibility that PLTP is involved in the transfer of α-tocopherol from plasma into CSF, a phenomenon that remains to be established.

Conclusions

The mammalian CNS contains multiple separate transport systems for vitamins. Initially, to penetrate the CNS, the transported vitamer must traverse the BBB and/or BCSFB (CP). Separate systems exist for each vitamin at these barriers (except for the SMVT at the BBB which transports PA, biotin, and lipoic acid). In some cases, the CP plays the predominant role (e.g. ascorbate and folate) (Spector and Johanson 2006); in other cases, the BBB (e.g. B_1, B_2, B_3, and B_6) plays the main role. However, it worth noting that the CP is also involved in B_1 and B_6 transport (like the liver) in releasing phosphorylated vitamers into CSF. With the exception of the SMVT system, each of these systems is fairly specific for their vitamer.

Once within the CSF and extracellular space of brain, brain cells have specific uptake mechanisms, generally facilitated diffusion with subsequent intracellular trapping (e.g. by phosphorylation), compartmentalization, and ultimately the release of the transported vitamer. In some cases, e.g. PA, biotin, and B_3, the regulation of total vitamin levels in brain takes place at the level of the brain cell with the BBB and/or BCSFB (CP) playing a facilitative role.

Coupled with transport systems in the intestine, liver, and kidney for each vitamin, organs that tend to keep the plasma levels fairly stable, the systems in the CNS at the BBB, CP, and brain cell itself provide even more exquisite homeostasis by the mechanisms discussed above. We are now beginning to understand some of these systems on a molecular level.

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These transport/homeostatic systems have implications for therapy. For example, raising NAD concentrations in brain may be helpful in minimizing brain damage after trauma and stroke (Belenky *et al.* 2007). In animal models, giving large doses of niacinamide (which fairly readily traverses the BBB as noted above) presumably increases the NAD concentration in the injured brain area, a helpful intervention in animal models (Belenky *et al.* 2007). On the other hand, the utility of large doses of vitamin E in human brain diseases has been disappointing. In fact, there is actual increased morbidity/mortality in meta-analyses of the large randomized vitamin E trials (Spector and Vesell 2002, 2006). We also know that giving humans massive does of vitamin E (e.g. 4000 IU daily) does not increase CSF levels appreciably due to the homeostatic mechanisms described above (Pappert *et al.* 1996). Almost certainly, the levels in human brain are not increased more than a trivial amount. Thus, unless small increases in brain vitamin E help, the utility of vitamin E is *a priori* dubious as confirmed with a careful analysis of the controlled trials.

With the increased knowledge of the molecular mechanisms of vitamin transport and homeostasis, we are confident this information is not only of great scientific interest but also has important therapeutic implications. Much more work, however, needs to be performed to obtain a truly in depth knowledge of these mechanisms.

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