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Review

Uridine and cytidine in the brain: Their transport and utilization

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ABSTRACT

The pyrimidines cytidine (as CTP) and uridine (which is converted to UTP and then CTP) contribute to brain phosphatidylcholine and phosphatidylethanolamine synthesis via the Kennedy pathway. Their uptake into brain from the circulation is initiated by nucleoside transporters located at the blood–brain barrier (BBB), and the rate at which uptake occurs is a major factor determining phosphatide synthesis. Two such transporters have been described: a low-affinity equilibrative system and a high-affinity concentrative system. It is unlikely that the low-affinity transporter contributes to brain uridine or cytidine uptake except when plasma concentrations of these compounds are increased several-fold experimentally. CNT2 proteins, the high-affinity transporters for purines like adenosine as well as for uridine, have been found in cells comprising the BBB of rats. However, to date, no comparable high-affinity carrier protein for cytidine, such as CNT1, has been detected at this location. Thus, uridine may be more available to brain than cytidine and may be the major precursor in brain for both the salvage pathway of pyrimidine nucleotides and the Kennedy pathway of phosphatide synthesis. This recognition may bear on the effects of cytidine or uridine sources in neurodegenerative diseases.

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1. Introduction

The circulating pyrimidines uridine and cytidine, besides being incorporated into nucleic acids, can serve as substrates for the salvage pathway of pyrimidine nucleotide synthesis; as precursors of the cytidine triphosphate (CTP) needed in the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) biosynthetic pathway (Kennedy and Weiss, 1956); and as precursors for the UDP (uridine diphosphate) and UTP (uridine triphosphate) that activate brain P2Y receptors (von Kugelgen, 2006) and that promote brain glycogen synthesis via UDP-glucose (Brown, 2004). In humans, the predominant circulating pyrimidine is uridine (Wurtman et al., 2000); in rats, it is cytidine (Traut, 1994); these variations probably reflect the species differences in cytidine deaminase, the enzyme that converts cytidine to uridine in the body. The transports of these pyrimidines into the brain's extracellular fluid, and then into neurons and glia, are essential prerequisites for these nucleosides to be utilized in brain. This review describes the mechanisms currently believed to mediate their passage across the blood–brain barrier (BBB) and then into brain cells. It also considers the biochemical consequences of changing brain uridine and cytidine levels, and related therapeutic implications.

CDP-choline is an endogenous intermediate which is produced in the rate-limiting step of PC biosynthesis via the Kennedy pathway (Kennedy and Weiss, 1956) (Fig. 1). Thus, CDP-choline has been extensively studied for its role in neuronal membrane synthesis. Exogenously administered CDP-choline is metabolized to cytidine and choline in rats (Weiss, 1995), while it is metabolized to uridine and choline in humans (Wurtman et al., 2000). Thus, besides choline, cytidine and uridine have attracted attention as precursors for endogenous CDP-choline production. An efficient mechanism mediating the brain uptake of circulating cytidine has not yet been demonstrated. When double-labeled CDP-choline was administered orally to rats, brain cytidine radioactivity peaking 4–6 h after the labeled CDP-choline was very low compared with that in liver (i.e., 0.2% vs. 60% of administered dose for brain and liver, respectively [Galletti et al., 1991]). Cytidine administered intracerebroventricularly (i.c.v.) does enter the brain (Trovarelli et al., 1982), but uptake via this route bypasses BBB transport.

On the other hand, the brain is known to take up circulating uridine. This uptake was initially demonstrated in the early 1970s (Hogans et al., 1971). Subsequently, Cornford and Oldendorf (1975), using a single injection method, showed that adenine, adenosine, guanosine, inosine and uridine all are able to cross the rat BBB, while cytidine, thymidine and their respective bases are not. Further studies on nucleoside transport suggested that nucleoside transporters could be classified based on their sensitivity to the nucleoside analog S-(4-nitrobenzyl)-6-thioinosine (NBTI) into two groups as NBTI-sensitive (*es*) or NBTI-insensitive (*ei*) (Belt, 1983). A few years later, the finding of a totally different type of nucleoside transporter family in mouse intestinal epithelial cells (Vijayalakshmi and Belt, 1988) gave rise to a new classification of nucleoside transporters, i.e., into equilibrative (Na^+-independent, low-affinity) and concentrative (Na^+-dependent, high-affinity) transporter families. The properties and substrate specificity of each family have been reviewed in Table 1. Detailed information can be found elsewhere (Baldwin et al., 2004; Gray et al., 2004; Kong et al., 2004; Podgorska et al., 2005).

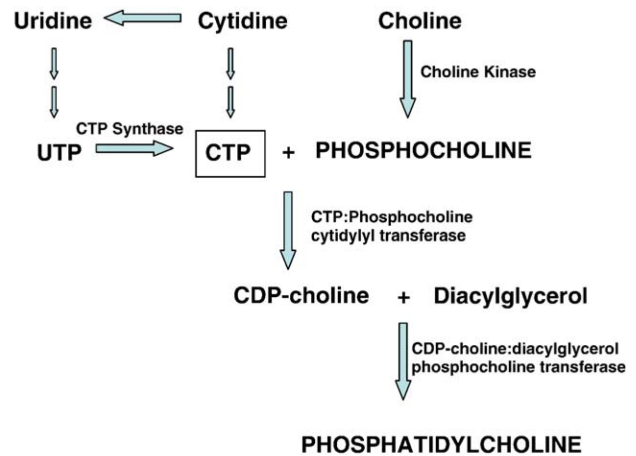

Fig. 1 – Phosphatidylcholine (PC) biosynthesis via the Kennedy pathway. In rats, plasma cytidine is the major circulating pyrimidine, however in gerbils and humans, the primary circulating pyrimidine is uridine. Only small amounts of circulating cytidine are converted to brain CTP, since the rat blood–brain barrier (BBB) lacks a high-affinity transporter for cytidine; uridine, in contrast, readily enters the brain via a high-affinity transporter (CNT2) yielding UTP which is then converted to CTP by CTP synthase. This CTP reacts with phosphocholine to form endogenous CDP-choline, which than combines with diacylglycerol (DAG) to form PC.

Recent studies employing expression cloning and reverse transcriptase-PCR (RT-PCR) methods revealed the expression of a high-affinity nucleoside transporter (CNT2) at the rat BBB for purines like adenosine, and the pyrimidine uridine (Li et al., 2001; Redzic et al., 2005), but not for cytidine. Since BBB transport is the major determinant of brain uptake of most circulating compounds (Pardridge, 2001), this recent finding may open new avenues for exploring the possible effects of cytidine or uridine sources in neurodegenerative disorders like Alzheimer's disease.

2. Transport of cytidine and uridine across the BBB

Circulating cytidine and uridine can both be transported across the BBB by equilibrative transporters. Moreover, a concentrative system, as mentioned above, has also recently been shown to mediate BBB uridine transport. Equilibrative transport proteins (ENT, SLC29 family) exhibit characteristics of low-affinity nucleoside transport with K_m values for their substrates in the high micromolar range (100–800 μM; Pastor-Anglada et al., 1998). Two such transporters, ENT1 and ENT2, have been cloned in rat (Redzic et al., 2005) and mouse (Murakami et al., 2005) BBB. Both ENT1 and ENT2 can

Table 1 – Overview of nucleoside and nucleobase transport

Type	Properties	Protein	Substrate(s)
Equilibrative (SLC29 family)	–Low affinity –Bidirectional –Na - independent facilitated diffusion	ENT1	Purine and pyrimidine nucleosides
		ENT2	Purine and pyrimidine nucleosides and some nucleobases
		ENT3	Purine and pyrimidine nucleosides and nucleobases
		ENT4	Adenosine
Concentrative (SLC28 family)	–High affinity –Unidirectional –Na - dependent active transport	CNT2	Purine nucleosides and uridine
		CNT1	Pyrimidine nucleosides and adenosine
		CNT3	Purine and pyrimidine nucleosides, nucleoside analogues

mediate transport of cytidine or uridine across the BBB if their plasma concentrations (which normally are in low micromolar ranges [Traut, 1994]) are elevated several folds by experimental manipulation. The substrate-selective, Na^+-dependent, unidirectional and high-affinity (with K_m values in the low micromolar range [1–50 μM; Pastor-Anglada et al., 1998]) concentrative transport proteins (CNT, SLC28 family) mediate transport of nucleosides across cellular membranes against a concentration gradient, in concentration ranges resembling their physiological plasma levels. A previous suggestion regarding the existence of an N2 type nucleoside transporter (CNT1) at the BBB of guinea pigs, based on studies using a bilateral in situ brain perfusion technique (Thomas and Segal, 1997), has not yet been confirmed. However, recent studies using expression cloning, RT-PCR and immunoblotting methods have clearly demonstrated that a CNT protein, CNT2, which transports purines like adenosine as well as the pyrimidine uridine, is expressed in rat BBB in vivo (Li et al., 2001; Redzic et al., 2005). Although the principal circulating pyrimidine in rats is cytidine, and its plasma concentrations are two- to three-fold those of uridine (Traut, 1994), available data on BBB nucleoside transporters suggest – at least in rats – that uridine is transported across the BBB more efficiently than is cytidine. This suggestion is supported by an in vivo microdialysis study in which the estimated uridine concentrations in rat brain extracellular fluid were 7-fold those of cytidine (0.76 vs. 0.11 μM) (Dobolyi et al., 1998). It should be noted that Redzic et al. have suggested that CNT2 is likely to be located on the endothelial membrane facing the brain's interstitium, but not blood, and on this basis have suggested that the major function of this protein may be to remove adenosine from the brain interstitial fluid. However, Li et al. (2001) reported that the CNT2 cloned from a rat BBB cDNA library exhibited the properties of a previously defined (Pardridge et al., 1994) sodium-dependent, high-affinity, blood-to-brain transporter for adenosine (and therefore, for uridine).

The possibility that the discrepancies in findings between the above studies reflect methodological differences needs further clarification. However, to date, no direct in vivo evidence has been presented in any species for the presence of any concentrative nucleoside transporter protein at the BBB other than CNT2. Concerning their high-affinity transporters, uridine can be a substrate for all three major CNTs (including CNT2, which is expressed at the BBB), however, cytidine can only be transported by CNT1 and CNT3 proteins which have not so far been demonstrated at the BBB. The affinity constants for cytidine and uridine of their common transporters (i.e., ENT1, ENT2 and CNT1) obtained from various tissues of human and rodents and from various cell lines generally do not differ markedly, or are somewhat lower for uridine (Griffith and Jarvis, 1996; Ward et al., 2000; Ritzel et al., 2001; Smith et al., 2004) which suggests that cytidine transport via any of these transporters would not be favored under physiological conditions. Moreover, hCNT1 may have a higher affinity for uridine, inasmuch as in electrophysiological studies of *Xenopus* oocytes in which this transport protein was expressed, uridine elicited two- to three-fold higher electrical currents than those elicited by cytidine (Lostao et al., 2000; Smith et al., 2004).

Choroid plexus (CP) epithelium can be another portal of entry for compounds into brain. Indeed, previous studies described such transport of nucleosides into brain via the CP epithelium (Spector and Berlinger, 1982; Spector and Huntoon, 1983, 1984; Spector, 1985, 1986); moreover, transport proteins such as ENT1, ENT2 and CNT3 (a concentrative high-affinity transporter with a broad selectivity to both purines and pyrimidines [Gray et al., 2004]) have recently been shown to localize in rat (Anderson et al., 1999a,b; Redzic et al., 2005) and rabbit (Wu et al., 1992, 1994) brain CP epithelial cells. However, the surface area of human BBB is 1000 times that of CP epithelium (i.e., 21.6 m^2 vs. 0.021 m^2 [Pardridge, 2001]). Hence, the BBB is probably the major determinant of brain uptake of pyrimidines, as well as of other compounds.

3. Transport of cytidine and uridine from brain extracellular fluid into cells

Uptake of uridine and cytidine from the extracellular fluid (ECF) into brain cells also is mediated by members of the two nucleoside transport families. Both the low- and high-affinity nucleoside transport proteins has been isolated in whole rat brain homogenates (Lu et al., 2004; Redzic et al., 2005). None of these transporters has been shown to be localized specifically on a particular type of brain cell (e.g., neuron, glia). Available data indicate similar or somewhat higher affinities for uridine than for cytidine among individual nucleoside transporters (no matter whether they are equilibrative or concentrative transport proteins) in tissues other than brain of different species (Griffith and Jarvis, 1996; Ward et al., 2000). Thus, lower ECF concentrations of uridine than of cytidine might be sufficient for the uridine to be taken up into brain cells by equilibrative transporters which mediate facilitated diffusion of nucleosides. This suggestion is supported by a recent study which reported a higher intracellular accumulation of uridine, compared to that of cytidine, via hENT1 and hENT2, the two equilibrative

nucleoside transporters cloned from a human colonic epithelial cell and expressed in nucleoside transporter-deficient PK15 cell line (Ward et al., 2000). However, the main physiological mechanism responsible for pyrimidine nucleoside uptake into cells may be the concentrative system which mediates the active transport of these nucleosides against a concentration gradient since studies have shown that levels of cytidine and uridine in tissues (e.g., liver, brain) are much higher than those in plasmas of several species including mice (Peters et al., 1987), rats (Pillwein et al., 1987) and gerbils (Cansev et al., 2005). If this is true, uridine might again be transported into brain cells more efficiently than cytidine since unlike that of cytidine, uridine's transport can be mediated by more than one specific high-affinity transporter, i.e., both CNT1 and CNT2 (Gray et al., 2004). Furthermore, brain ECF pyrimidine concentrations are mainly determined by BBB transport, and rat brain ECF uridine concentrations are 7-fold those of cytidine (Dobolyi et al., 1998). Taken together, accumulating evidence suggests that uptake of uridine and cytidine into brain cells, once these pyrimidines cross the BBB and are in brain extracellular fluid, favors uridine.

4. Changes in brain levels of cytidine and uridine

In rat brain, de novo pyrimidine synthesis, although at lower rates than those of liver, has been described (Bourget and Tremblay, 1972). It is still unknown to what extent brain pyrimidine levels depend on de novo synthesis, however, it is well documented that the circulating cytidine and uridine are essential for maintaining various brain functions, i.e., electrophysiological activity and restoration of carbohydrate and phospholipid content (Geiger and Yamasaki, 1956; Benzi et al., 1984). Thus, the brain requires that these pyrimidines be taken up from the circulation. Hence, their plasma concentrations, as well as their transport proteins, will be important in modulating brain levels of cytidine and uridine.

Dietary purines and pyrimidines can be absorbed from the gastrointestinal system into the circulation (Sonoda and Tatibana, 1978). The liver has been proposed as the major tissue for regulating plasma pyrimidine levels. Indeed, in rats, it has been suggested that orally administered uridine is eliminated through a single-pass and newly formed uridine is released into the circulation by the liver (Gasser et al., 1981). However, in humans, the liver may not be the major catabolic tissue for pyrimidines; there may be multiple sites of uptake and degradation (Chan et al., 1988). Erythrocytes may also be involved in modulating tissue pyrimidine concentrations since these cells reportedly take up orotic acid, convert it to UDP-glucose, and release uridine and glucose in peripheral tissues and brain (Goetz et al., 1971).

Oral administration of cytidine or uridine has been shown to increase their respective concentrations in plasmas of experimental animals or humans (van Groeningen et al., 1991; Wurtman et al., 2000; Cansev et al., 2005; Cansev and Wurtman, 2005). Brain levels of these pyrimidines, particularly uridine, also are altered when plasma concentrations are allowed to vary within a broad range following oral (Cansev et al., 2005) or systemic (Peters et al., 1987) administration. In gerbils, shortly after a single oral dose of a uridine source, i.e., uridine monophosphate (UMP; 1 mmol/kg, yielding approximately 300 mg/kg uridine), a two-fold increase in plasma uridine concentration was observed, accompanied by increased brain uridine levels in the same magnitude (Cansev et al., 2005). In mice, intraperitoneal (i.p.) injection of uridine (3500 mg/kg) caused a three-orders-of-magnitude increase in plasma and a 7-fold increase in brain uridine (Peters et al., 1987). In the same experiment, the same dose of cytidine yielded 3 and 2 orders of magnitude increases in plasma cytidine and uridine levels, respectively. Cytidine injection further increased brain cytidine levels from 42 ± 9 pmol/mg wet weight to 90 ± 32 pmol/mg wet weight (approximately 2.1-fold) and brain uridine levels from 0.1 pmol/mg wet weight to 2.1 pmol/mg wet weight (21-fold). The 10-fold greater magnitude of increase in brain uridine than that in brain cytidine following cytidine administration might be caused both by poor uptake of cytidine into brain and by conversion of some part of administered cytidine to uridine.

A species difference in the activity of cytidine deaminase, the enzyme that converts cytidine to uridine, also influences the principal pyrimidine in the blood. Cytidine is the principal circulating pyrimidine in rats (Traut, 1994), but uridine is the principal pyrimidine in gerbils (Cansev et al., 2005), humans (Wurtman et al., 2000), rabbits (Spector, 1986) and guinea pigs (Olivares and Verdys, 1988). Oral administration of cytidine (in the form of CDP-choline) increases plasma cytidine levels in rats (Lopez-Coviella et al., 1995) but principally elevates uridine in humans (Wurtman et al., 2000) and gerbils (Cansev and Wurtman, 2005). Cytidine

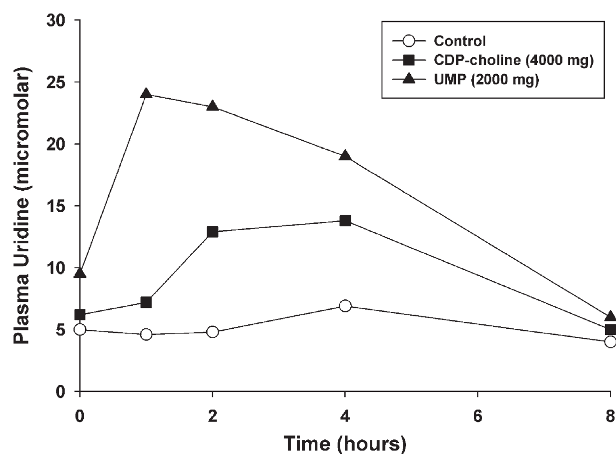

Fig. 2 – Effects of oral UMP or CDP-choline on human plasma uridine levels. Subjects received oral UMP (2000 mg) or CDP-choline (4000 mg), containing 3.3 mmol uridine or 3.2 mmol cytidine, respectively. Plasma uridine peaked at 24 μM after UMP and at 14 μM after CDP-choline; increases in Areas Under the Curves (AUCs) compared to that of control were 345% and 201%, respectively. The elevation in plasma uridine caused by UMP administration was significantly greater than that caused by CDP-choline (Wurtman and Watkins, unpublished observation).

injected i.p. to mice in a high dose has also been shown to be converted in part to uridine, both in blood and in brain (Peters et al., 1987).

Although cytidine is readily converted to uridine in humans, administration of a uridine source is more effective than cytidine in increasing plasma uridine, and thus probably brain uridine, levels. Wurtman and Watkins (unpublished observation) compared the effects of orally administering cytidine, as CDP-choline, or of uridine, as UMP, on plasma uridine levels in humans (Fig. 2). When subjects received 3.3 mmol of uridine, plasma uridine rose from 9.5 μM to 24 μM, the Area Under the Curve (AUC) increasing by 345%. When they received 3.2 mmol cytidine as CDP-choline (4000 mg) plasma uridine rose from 6 μM to 14 μM, and the AUC increasing by 201%. Thus, the elevation in human plasma uridine caused by UMP administration was significantly greater than that caused by a nearly equimolar dose of CDP-choline.

5. Consequences of changing brain cytidine and uridine levels

Cytidine and uridine exert important effects on a variety of brain functions by, for example, being converted to their respective nucleotides. One of these functions is neuronal membrane phospholipid synthesis. The biosynthesis of PC, the most abundant phosphatide in the brain, via the Kennedy pathway (Fig. 1) requires phosphocholine and cytidine triphosphate (CTP), a cytidine nucleotide, which is involved in the rate-limiting step (Kennedy and Weiss, 1956). The enzyme that converts CTP to endogenous CDP-choline (CTP: phosphocholine cytidylyltransferase) is unsaturated at physiological brain CTP levels (Mages et al., 1988). Thus, one might expect that increased brain CTP levels, which could be achieved by administration of either exogenous cytidine or uridine, could elevate membrane phosphatide synthesis. Data supporting this suggestion indeed came from previous studies which showed elevated brain cytidine and uridine levels following cytidine (Peters et al., 1987; Cansev and Wurtman, 2005) and elevated brain uridine levels following uridine (Cansev et al., 2005) administration. An increase in neuronal cytidine and uridine levels augmented CTP levels both in vitro (Savci and Wurtman, 1995; Richardson et al., 2003; Pooler et al., 2005) and in vivo (Cansev et al., 2005; Cansev and Wurtman, 2005). Moreover, the involvement of elevated CTP levels for the synthesis of major brain phospholipids, particularly PC, has been well documented (Lopez G.-Coviella and Wurtman, 1992; Lopez-Coviella et al., 1995; Savci and Wurtman, 1995). Finally, addition of uridine to cell cultures, or its chronic dietary administration to rats, can modulate such membrane-dependent processes as neurotransmitter release (as evidenced by potentiation of potassium-induced striatal dopamine release [Wang et al., 2005]), and neurite outgrowth (as evidenced by elevated neurofilament proteins which are enriched in neurites [Pooler et al., 2005; Wang et al., 2005]). Together, these observations suggest that both cytidine and uridine are capable of increasing neuronal membrane synthesis via increasing CTP levels.

Pyrimidines can also have effects on the brain by activating the P2Y receptors. Among eight different mammalian P2Y receptor subtypes ($P2Y_{1,2,4,6,11,12,13,14}$) cloned, $P2Y_2$, $P2Y_4$ and $P2Y_6$ accept pyrimidine nucleotides as ligands (von Kugelgen, 2006). While rat $P2Y_4$ can be activated by ATP, UTP, CTP, GTP and ITP in an equipotent manner (Wildman et al., 2003), human $P2Y_4$ (Chang et al., 1995) and $P2Y_6$ (Communi et al., 1996) receptors are activated by pyrimidine (mainly uridine) nucleotides only. Human (Lazarowski et al., 1995) and mouse (Lustig et al., 1993) $P2Y_2$ receptors respond equally well to UTP and ATP. UDP-glucose also has been shown to activate mouse brain $P2Y_{14}$ receptors (Freeman et al., 2001). A possible neurotransmitter role for UDP, UTP and UDP-glucose via pyrimidine sensitive P2Y receptors has been proposed based on studies that showed the release from intracellular stores of these pyrimidines in concentrations capable of stimulating their respective receptors (Lazarowski et al., 1997, 2003). In nerve growth factor-differentiated PC12 cells, uridine or UTP has been shown to mediate neurite outgrowth (Pooler et al., 2005). This effect was accompanied by an increase in phosphatidylinositol (PI) turnover; abolished by apyrase, an agent that degrades nucleotides such as UTP; and blocked by P2Y receptor antagonists, suggesting that uridine, in addition to increasing CTP synthesis, promotes neurite outgrowth by UTP-mediated stimulation of a P2Y receptor-coupled signaling pathway (Pooler et al., 2005). These observations are in agreement with previously reported neurothropic effects of P2Y receptors (Rathbone et al., 1999). Boehm and colleagues reported that UDP and UTP modulate noradrenaline release from cultured rat superior cervical ganglia (Boehm et al., 1995), an effect later shown to be mediated by $P2Y_6$ receptors via activation of protein kinase C (Vartian et al., 2001). Recently, UTP (Molliver et al., 2002) as well as UDP, CDP and CTP (Park et al., 2004) have been shown to be involved in modulation of pain transmission via $P2Y_2$ receptors. Furthermore, UTP has been proposed as an endogenous nociceptive messenger (Stucky et al., 2004).

Another metabolic pathway in which uridine is involved in brain is glycogen synthesis. Glycogen has been shown to be synthesized within the brain (Brown, 2004), as evidenced by the expression of glycogen synthase enzyme both in astrocytes (Pellegri et al., 1996) and in neurons (Inoue et al., 1988). Glycogen is synthesized from glucose-1-phosphate and UTP by the action of UDP-glucose pyrophosphorylase to form UDP-glucose, the source of all glucosyl residues added to the glycogen molecule. Elongation of the glycogen chain is governed by a UDP-UTP cycle. Catalyzed by glycogen synthase, glycogen chain elongation causes the release of one molecule of UDP at each addition of a glucosyl residue, which is then converted by nucleoside diphosphate kinase to UTP, recombining with a glucose molecule as long as the chain elongation proceeds.

Besides the involvements of cytidine and/or uridine in brain lipid (e.g., phospholipid) and carbohydrate (e.g., glycogen) metabolism, and the possible neurotransmitter role of uridine nucleotides, it has been proposed that uridine interacts with GABAergic (Guarneri et al., 1985) and dopaminergic transmission (Myers et al., 1995). Further studies are required to confirm and characterize these interactions.

6. Implications for therapeutic uses of cytidine or uridine sources

Neuroprotective effects of exogenously administered CDP-choline (citicoline), a source of plasma uridine in humans (Wurtman et al., 2000), are being evaluated in treatment of neurological disorders such as acute or chronic ischemic stroke, traumatic brain injury and cognitive impairment in both experimental and clinical studies. Beneficial effects have been observed with CDP-choline administration in experimental models of cerebral ischemia (Aronowski et al., 1996; Schabitz et al., 1999; Baskaya et al., 2000; Grieb et al., 2001), embolic ischemic stroke (Shuaib et al., 2000), intracerebral hemorrhage (Clark et al., 1998), brain hypoxia (Alberghina et al., 1981) and cognitive impairment (Alvarez et al., 1997). Grieb et al. (2001) attributed CDP-choline's beneficial effects in cerebral ischemia to its choline moiety, since neither cytidine monophosphate, nor cytidine was effective when given alone. It should be noted that choline alone, or mixtures of choline plus cytidine or choline plus uridine, were not tested in that study. Moreover, no data were provided on plasma cytidine, uridine or choline levels after any treatment. In human studies, CDP-choline treatment improved memory performance (Alvarez et al., 1997) and verbal memory (Spiers et al., 1996) in elderly subjects, and mental performance in Alzheimer's disease patients (Cacabelos et al., 1996). Moreover, CDP-choline yielded positive outcomes in some (Tazaki et al., 1988; Clark et al., 1997, 1999) but not all (Clark et al., 2001) clinical studies of stroke. A meta-analysis further suggested that administration of oral CDP-choline in the first 24 h of ischemic insult in patients with moderate to severe stroke increased the probability of complete recovery at 3 months (Davalos et al., 2002). Several mechanisms, including increased phospholipid synthesis (Lopez-Coviella et al., 1995; Alberghina et al., 1981; Dorman et al., 1983); glucose metabolism (Kakihana et al., 1988); and cholinergic function (Dixon et al., 1997) and decreased activation of PLA_2 (Adibhatla and Hatcher, 2003) and apoptotic pathways (Krupiski et al., 2002) have been proposed for the neuroprotective effects of CDP-choline. Since all these studies considered cytidine and choline as the major metabolites of CDP-choline, they focused mainly on the resynthesis via the Kennedy pathway of CDP-choline in the brain by these two metabolites (Fig. 1).

On the other hand, uridine did not initially attract attention as a precursor for PC synthesis by Kennedy pathway. However, recent studies exploring the effect of uridine on the levels of phospholipid intermediates have shown that uridine, as well as cytidine, can be a substrate for neuronal membrane synthesis (Richardson et al., 2003; Cansev et al., 2005; Pooler et al., 2005). A dietary regimen combining two phospholipid precursors, uridine and choline, improved selective attention and spatial learning in spontaneously hypertensive rats, a rodent model used for testing cognitive impairment (De Bruin et al., 2003). In a recent study, adding uridine (as UMP) to a choline-containing diet for 4 weeks increased levels of major brain phospholipids of gerbils by 13–47% (Wurtman et al., in press). Combining a polyunsaturated fatty acid, DHA, with this mixture further increased neuronal membrane phospholipids, to 39–74% of controls. Moreover, levels of synaptic proteins such as Synapsin-1 and PSD-95 were elevated in animals receiving both the uridine plus choline diet and that diet supplemented with gavaged DHA, suggesting that the treatment increased synaptic membranes. This effect could be beneficial in treating neurodegenerative disorders. It is well documented that brains of patients with early Alzheimer's disease contain fewer and smaller synapses and reduced levels of synaptic proteins (Terry et al., 1991; Selkoe, 2002; Coleman et al., 2004). Moreover, in brains of a mouse model of Alzheimer's disease neuronal dysfunction and behavioral deficits occur months before abnormal proteins and plaques are deposited (Jacobsen et al., 2006). Thus, uridine might have therapeutic utility as a precursor for neuronal membranes, e.g., when deficiencies in synaptic membranes underlie the symptoms of the disease.

7. Conclusions

It can be concluded that uridine, by crossing the rat BBB via the CNT2 transporter, is taken up by the rat brain more efficiently than is cytidine under physiological conditions. Although low-affinity ENT transporters have been shown to be localized at the rat or mouse BBB, no direct evidence has been presented to date regarding the presence of a high-affinity transporter for cytidine in this location. Whether or not human BBB exhibits similar characteristics to those of rat BBB is unknown, however, uridine's higher physiological plasma levels (compared with those of cytidine's) and the similar or higher affinities of transport proteins for uridine suggest that uridine is also the principal salvaged pyrimidine and the major pyrimidine precursor used for neuronal membrane synthesis by humans.

It is not possible to relate data on the effects of CDP-choline in traumatic brain injury or stroke to BBB pyrimidine uptake because in these disorders affected brain regions generally lack an intact BBB. However, the relative brain uptakes of cytidine and uridine may be factors affecting their use in cognitive disorders. Although species differences as well as the lack of adequate information about specific BBB pyrimidine transporters limit this analysis, uridine sources may be better candidates than cytidine sources for treating human cognitive disorders, since uridine is the principal circulating pyrimidine and probably is transported into the brain more efficiently than is cytidine. The reported beneficial effects of CDP-choline in cognitively impaired humans (Spiers et al., 1996; Alvarez et al., 1997) and in Alzheimer's disease (Cacabelos et al., 1996) may well be mediated by uridine – besides choline – since CDP-choline is principally a source of plasma uridine, but not of cytidine, in humans (Wurtman et al., 2000).

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