

Uridine Function in the Central Nervous System

Arpád Dobolyi^{1,*}, Gábor Juhász², Zsolt Kovács³ and Julianna Kardos⁴

¹*Neuromorphological and Neuroendocrine Research Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University and the Hungarian Academy of Sciences, H-1094 Budapest, Hungary;* ²*Laboratory of Proteomics, Institute of Biology, Eötvös Loránd University, H-1117 Budapest, Hungary;* ³*Department of Zoology, University of West Hungary, Savaria Campus, Szombathely, Hungary;* ⁴*Department of Neurochemistry, Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences, H-1025 Budapest, Hungary*

Abstract: In the adult nervous system, the major source of nucleotide synthesis is the salvage pathway. Uridine is the major form of pyrimidine nucleosides taken up by the brain. Uridine is phosphorylated to nucleotides, which are used for DNA and RNA synthesis as well as for the synthesis of membrane constituents and glycosylation. Uridine nucleotides and UDP-sugars may be released from neuronal and glial cells. Plasmamembrane receptors of 7 transmembrane domains have been identified that recognize UTP, UDP, and UDP-sugar conjugates. These receptors are called P2Y2 and P2Y4, P2Y6, and P2Y14 receptors, respectively. In addition, binding sites for uridine itself have also been suggested. Furthermore, uridine administration had sleep-promoting and anti-epileptic actions, improved memory function and affected neuronal plasticity. Information only starts to be accumulating on potential mechanisms of these uridine actions. Some data are available on the topographical distribution of pyrimidine receptors and binding sites in the brain, however, their exact role in neuronal functions is not established yet. There is also a scarcity of data regarding the brain distribution of other components of the pyrimidine metabolism although site specific functions exerted by their receptors might require different metabolic support. Despite the gaps in our knowledge on the neuronal functions of pyrimidine nucleosides, their therapeutic utilization is appealing. They have been suggested for the treatment of epileptic and neurodegenerative diseases as neuroprotective agents. In addition, the development of traditional drugs acting specifically on pyrimidine receptor subtypes is also promising as a new direction to treat neurological disorders.

Keywords: Epilepsy, neural function, neuronal plasticity, nucleoside transport, nucleotide receptor, pyrimidine salvage, sleep, therapeutic application.

INTRODUCTION

Uridine has crucial role in the pyrimidine metabolism of the brain. It supplies nervous tissue with the pyrimidine ring, and in turn, participates in a number of important metabolic pathways. Uridine and its nucleotide derivatives may also have an additional role in the function of the central nervous system as signaling molecules. There are established signaling molecules in the brain, such as amino acids, and adenosine, which are also available in large pools as metabolites. Adenosine is not a typical neuronal signaling molecule since it is not released from synapses on an action potential trigger. Rather, it is produced with the extracellular degradation of ATP or is released via nucleoside transporters [1, 2], which are not highly specific to any particular nucleoside [3]. In contrast to adenosine, the neuromodulator role of uridine is not established yet with certainty. However, an increasing body of evidence suggests that uridine participates in the control of physiological and pathophysiological brain functions. This review is an attempt to summarize the available data supporting that uridine plays a role in neuronal functions by collecting information from the literature about the potential mechanisms behind its putative neuromodulator

function. Transport of uridine as well as its metabolism in brain cells is summarized and we also refer to excellent recent reviews on this topic [4, 5]. Finally, the potential therapeutic applications of uridine are discussed.

SOURCE OF PYRIMIDINES IN THE CENTRAL NERVOUS SYSTEM

The purine and pyrimidine bases are mostly absorbed from the intestine in the form of nucleosides after degradation from nucleic acids and nucleotides [6]. As opposed to purines that are further degraded in intestinal epithelial cells to uric acid and pentose moiety, a portion of uridine is transported in the plasma [4, 7]. The liver plays a pivotal role in purine and pyrimidine metabolism because virtually all of the nucleosides in the blood stream derive from their secretion from the liver following *de novo* nucleoside synthesis to keep a steady level of nucleosides in the plasma [8, 9]. There are species differences in the plasma levels of pyrimidines. In human and gerbil, uridine is the predominant form due to cytidine deaminase activity in plasma, while in rats the plasma concentration of cytidine is higher [4]. Although a low level of *de novo* pyrimidine synthesis has been demonstrated in the brain [10], most of the pyrimidine content of the brain is supplied by the uptake of pyrimidine nucleosides, particularly uridine from the plasma [11], which is required for the maintenance of electrophysiological activity in the brain [12]. There are 4 genes encoding the equilibra-

*Address correspondence to this author at the Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Tüzolto u. 58, Budapest, H-1094, Hungary;
Tel: +36-1-215-6920 / 53634; Fax: +36-1-218-1612;
E-mail: dobolyi@ana.sote.hu

tive (ENT1-4) and 3 genes encoding the Na⁺-dependent concentrative (CNT1-3) nucleoside transporters [3, 5]. Brain endothelial cells contain ENT1, ENT2 and CNT2 while choroid plexus epithelial cells also express CNT3 [13]. In the human choroid plexus, ENT2 and CNT3 are dominant [14]. In brain endothelial cells, CNT2 and ENT2 are present on the cell surface facing the interstitial fluid. In the choroid plexus, CNTs are present on the surface facing the cerebrospinal fluid. The opposite cell surfaces contain only equilibrative transporters [13]. These transporters can all transport uridine (as well as purines) and are responsible for the transport of uridine in and out of the brain [4]. Since the affinity of CNT2 is higher for uridine than cytidine [15, 16], the main form of pyrimidine uptake into the brain is uridine even in species like rat where plasma cytidine level is relatively high [17].

Neurons and glial cells also take up uridine from the extracellular space *via* equilibrative and concentrative nucleoside transporters. Investigation of the topographical and cellular distribution of the nucleoside transporters has started recently and initial results suggest differences in the localization of different types of transporters within the brain [18-21]. Spatial differences in the uptake process may result in differences in the local extracellular concentration of uridine, which has been reported to be lower in the thalamic extracellular space than in the cerebrospinal fluid [22, 23]. Uridine tissue levels also show uneven spatial distributions in the brain [24, 25] and depend on gender and age as well [26]. Uridine transport may also be affected by the activity of a particular brain region as extracellular uridine levels increased following pharmacological depolarization [27, 28] and experimentally induced epilepsy [29, 30].

URIDINE METABOLISM IN THE BRAIN

Uridine kinase catalyzes the first step of UTP salvage synthesis Fig. (1). This enzyme as well as subsequent uridine-phosphorylating enzymes are of low affinity, and therefore unsaturated with their substrates. Consequently, providing the brain with uridine increases its formation and levels of UTP [4]. On the other hand, uridine kinase is inhibited by UTP and CTP, the final products of the pyrimidine salvage pathway. Thus, at relatively low UTP and CTP level, uridine is mainly anabolized to uridine nucleotides. In contrast, at relatively high UTP and CTP levels the inhibition of uridine kinase channels uridine towards phosphorolysis. The ribose-1-phosphate is then transformed into phosphoribosylpyrophosphate (PRPP), which is used for purine salvage synthesis [31].

UTP can be aminated to CTP by CTP synthetase. This metabolic sequence explains how the brain gets CTP – to use in the Kennedy Cycle – even in species like humans, in which cytidine level is low in the circulation. UTP and CTP are both required for the formation of RNA Fig. (1). CTP also provides CDP-choline and CDP-ethanolamine for membrane formation [32, 33]. Uridine nucleotides can also be used for the synthesis of dUTP, which is further processed to dTTP for DNA synthesis. dUTP pyrophosphatase is a key enzyme of dTTP formation and also serves to prevent the incorporation of dUTP into DNA [34]. Another important metabolic function of UTP is to serve as an intermediate for

sugar conjugates such as UDP-glucose and UDP-galactose (UDP-sugars), which are in turn further metabolized for glycogen synthesis [35] or enter the endoplasmic reticulum (ER) for protein and lipid glycosylation [36]. Relatively recently, an additional function of UTP, UDP, and UDP-sugars was revealed. These uridine derivatives are released into the extracellular space where they act on specific plasmamembrane receptors [37-40]. Considering these important roles of uridine in the neuronal metabolism, it is not surprising that deficiencies in uridine metabolism or its pharmacological alterations can lead to neurological symptoms [41].

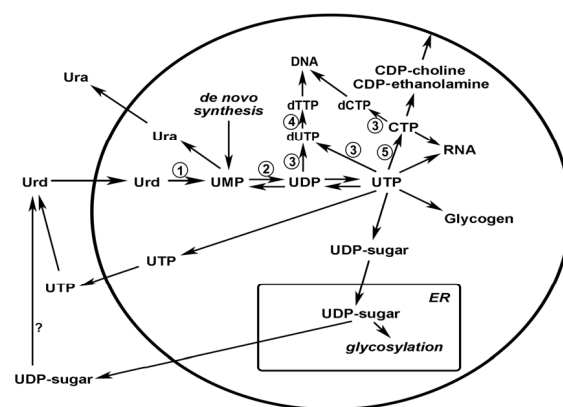


Fig. (1). Schematic diagram of the metabolic pathways of uridine in the brain.

Abbreviations: CDP - cytidine-5'-diphosphate; CTP - cytidine-5'-triphosphate; dCTP - deoxycytidine-5'-triphosphate; dTTP - deoxythymidine-5'-triphosphate; dUTP - deoxyuridine-5'-triphosphate; ER, endoplasmic reticulum; UDP - uridine-5'-diphosphate; UMP - uridine-5'-monophosphate; Ura - uracil; Urd - uridine; UTP - uridine-5'-triphosphate. Key enzymes are shown by numbers: 1 - Uridine kinase; 2 - UMP kinase; 3 - ribonucleotide reductase; 4 - dUTP pyrophosphatase; 5 - CTP synthetase.

NEURAL ACTIONS OF URIDINE

Several different effects of uridine on the nervous system have been suggested. In this chapter, we summarize the findings that support the neural actions of uridine.

The Effect of Uridine on Sleep

Uridine was identified as an active component of sleep-promoting substance purified from the brainstem of sleep-deprived rats [42, 43]. A 10-h intracerebroventricular infusion of 10 pmol of uridine increased slow wave sleep as well as paradoxical sleep due to increases in the frequencies of sleep episodes but not to their durations [44]. Intraperitoneally injected uridine resulted in a dose-dependent transient excess of slow-wave sleep if administered shortly before onset of the dark period. The sleep latency was remarkably shortened [45]. A brain area in the preoptic region of the hypothalamus, the so-called ventrolateral preoptic nucleus was described as a sleep center [46]. Interestingly, localized electrolytic lesions made bilaterally in the lateral preoptic area in rats eliminated the slow wave sleep-promoting effect of uridine [47] suggesting that the integrity of preoptic sleep

centers is crucial for slow wave sleep-promoting action of uridine.

Uridine in Epilepsy Models

An anti-convulsant effect of uridine has been suggested some decades ago as uridine reduced penicillin- [48, 49], pentylenetetrazole- [50], and electroconvulsion-induced [51] seizures in experimental rodent models of epilepsy. In more recent experiments, uridine was found to be antiepileptogenic in hippocampal kindling models [52, 53]. Uridine decreased kindling rates and afterdischarge durations in rats [53]. On the other hand, uridine did not protect against electroshock-induced convulsions [50]. The inconsistent anticonvulsant effect of uridine in different seizure models from different laboratories may reflect the different amount and dosing applied. For example, three-times-daily intraperitoneal injections of 200 mg / kg uridine reduced kindling rates in rats but once-daily administration was without effect [53].

Thermoregulatory Effects of Uridine

Administration of high-dose uridine resulted in severe hypothermia of 6-10 degrees C in mice and rats [54]. In contrast, low dose of uridine resulted in a slight increase in temperature in rodents [54]. The hypothermia might be related to breakdown products of uridine, since inhibition of uridine breakdown partially prevented hypothermia and since in brain uracil nucleotide levels were only slightly increased after uridine administration, while those of uracil were more markedly increased than in other tissues. [54]. Interestingly, in human and rabbit, high dose of uridine induced fever [55]. The change in body temperature associated with uridine administration was not due to bacterial pyrogens but that one of the degradation products might be involved in thermoregulation [55].

Evidence for Mood Altering Effects of Uridine

Neuroleptic drugs induce disruption of conditioned avoidance responding in rats. While uridine itself does not affect animals' performance in this model, uridine significantly potentiated the disruption of avoidance and avoidance latency induced by the neuroleptic haloperidol, a known dopamine receptor antagonist when coadministered with it [56]. Chronic uridine administration also increased in the stereotypy scores and the catalepsy induced by acute haloperidol injection [57]. Haloperidol-induced [57] and potassium-induced striatal dopamine release was potentiated [58]. The effect of uridine on haloperidol-evoked neural changes may be related to the dopaminergic actions of haloperidol because uridine itself was shown to affect brain dopaminergic systems. In activity tests, uridine-treated rats exhibited a significant increase in the sensitivity to amphetamine [59]. Uridine also potentiated the amphetamine and cocaine-induced rotation in rats with unilateral dopaminergic lesions [59]. Furthermore, chronic uridine treatment reduced the level of dopamine receptors and enhanced their turnover rate in the striatum [60] and might affect the dopamine-dependent prolactin release [61]. In addition to potentiating the action of anti-psychotic drugs, uridine was also suggested to have anxiolytic activity as well [62, 63].

The Effect of Uridine on Memory

Accumulating evidence suggest that long-term exposure to increased uridine improves certain types of memory function. Rats were assessed for learning and memory skills using 2 versions of the Morris water maze, the hidden platform version that assesses hippocampal-dependent cognitive memory processing, and the visible platform version that assesses striatal-dependent habit memory [64-66]. Chronic, but not acute, dietary supplementation with CDP-choline prevented hippocampal-dependent, memory deficits in aged rats and by younger rats reared under impoverished environmental conditions but did not affect striatal-dependent learning and memory [64, 65]. In rats, dietary CDP-choline is rapidly metabolized into cytidine and choline, the cytidine is then readily converted to uridine, which enters the brain [67]. To confirm that uridine is the compound to affect memory function, a diet supplemented with the uridine source uridine-5'-monophosphate (UMP) was tested similar to the CDP-choline administration. Indeed, UMP was similarly effective to alleviate memory dysfunction tested by Morris water maze as CDP-choline [66]. Increasing uridine levels by dietary UMP administration also improved the performance in gerbils in memory tests using the four-arm radial maze, T-maze, and Y-maze tests [68]. Similar to animal studies, increased uridine formation may have been the mediator of the positive effects of CDP-choline on verbal memory in aging human individuals with relatively inefficient memories [69].

The Effect of Uridine on Neuronal Plasticity

The first clue that uridine may be involved in the regulation of neuronal plasticity came from experiments demonstrating that it enhances neurite outgrowth in PC12 rat pheochromocytoma cells. PC12 cells were differentiated by nerve growth factor and exposed to various concentrations of uridine. After 4 but not 2 days uridine significantly and dose-dependently increased the number of neurites per cell. This increase was accompanied by increases in neurite branching and in levels of the neurite proteins neurofilament M and neurofilament 70 [70]. In subsequent experiments, gerbils received a diet containing UMP as uridine source daily for 4 weeks. This treatment significantly increased the amount of presynaptic protein synapsin-1, postsynaptic protein PSD-95 and neurite neurofibrillar proteins NF-70 and NF-M [71-73]. In contrast, elevated uridine level had no effect on the cytoskeletal protein beta-tubulin [71]. In addition, dietary administration of UMP together with the omega-3 fatty acid docosahexaenoic acid substantially increased the number of dendritic spines in adult gerbil hippocampus. This increase in dendritic spines was accompanied by parallel increases in membrane phosphatides and in pre- and post-synaptic proteins within the adult hippocampus [74]. These actions of elevated uridine levels in the brain on the level of synaptic proteins and the number of dendritic spines were also described during development. When dams consumed UMP for 10 days before parturition and 20 days while nursing, the brains of weanlings exhibited significant increases in membrane phosphatides, various pre- and postsynaptic proteins and in hippocampal dendritic spine densities [75, 76]. These data suggest that chronic uridine treatment promotes synaptogenesis at least in certain parts of the developing as well as the adult rodent brain [72, 77, 78]. To demonstrate the rele-

vance of uridine in the regulation of synaptic function, the effect of oral administration of the uridine source uridine-5'-monophosphate (UMP) was investigated on the release of the neurotransmitters dopamine and acetyl-choline (ACh). Potassium-evoked dopamine release measured by *in vivo* brain microdialysis in the striatum was significantly greater among chronically UMP-treated rats [58]. For ACh, baseline levels in striatum and striatal extracellular fluid were significantly elevated after the rats consumed UMP-containing diet for at least a week [79]. Furthermore, atropine-induced ACh release was also enhanced by chronic elevation of uridine levels [79].

POTENTIAL MECHANISMS OF URIDINE ACTION

Uridine, as a precursor metabolite, can support the synthesis of different molecules, which could potentially account for its neural actions. Thus, it has been hypothesized that elevated uridine level can increase the rate of RNA synthesis under pathological conditions, e.g. following epileptic seizures [48], which might contribute to its anticonvulsant properties. There is more evidence available that uridine exerts some of its actions via elevated synthesis of membrane constituents and of transmitter uridine nucleotides, which could affect neuronal signal transmission. Experimental support of these suggestions is summarized in this chapter. In addition, the existence of a uridine receptor has been proposed, and a potential direct action of uridine will also be discussed.

Neuronal Membrane Formation

The rates of the formation of UTP from uridine and CDP-choline from UTP in the brain seem to depend on the availability of uridine rather than on the activity of the participating enzymes. A similar feature of brain metabolism, the dependence of synthesis on local concentrations of substrates, which are nutrients that cross the blood-brain barrier, has been recognized for other important reactions, including the neuronal production of serotonin, dopamine, or acetylcholine from tryptophan, tyrosine, or choline, respectively [80]. Stimulation of UTP and CDP-choline synthesis by uridine was first demonstrated in PC12 cells. Adding uridine to the incubation medium caused significant elevations in UTP and CDP-choline levels [81]. In a subsequent study, in which adult gerbils received UMP by gavage, plasma and brain uridine levels were markedly elevated but cytidine levels were only slightly increased [82]. At the same time, brain UTP, CTP, and CDP-choline were all elevated 15 min after UMP administration [82]. Since uridine did not affect the synthesis of diacylglycerol or the activity of the phosphotransferase, which catalyzes the synthesis of phosphatidylcholine from diacylglycerol and CDP-choline, it is unlikely that uridine treatment inhibits the conversion of endogenous CDP-choline to phosphatidylcholine [81]. As CDP-choline is an immediate and rate-limiting precursor of phosphatidylcholine synthesis, these results suggest that uridine may also enhance phosphatidylcholine synthesis and membrane formation [81, 82]. This mechanism is likely to play a role in the effects of uridine on memory and neuronal plasticity [73, 77].

Uridine Action Via Released Uridine Nucleotides

The release of uridine nucleotides from brain cells has been proposed [83] after the discovery of receptors recognizing uridine nucleotides based on analogy with the release of adenine nucleotides [84, 85]. Cellular release of uridine nucleotides was first reported from bovine vascular endothelial cells loaded with radiolabeled uridine [86]. Following the development of a sensitive enzymatic assay [87, 88] quantification of physiologically relevant concentrations of UTP became possible. Caspase-dependent release was found in thymocytes [89]. A calcium-dependent release of UTP has been demonstrated from lung cells [90, 91]. The release of UTP from astrocytoma cells in response to mechanical stimulation was also demonstrated [87, 92]. It was verified that the increases in UTP levels were not the result of cell lysis [87]. In addition to UTP, UDP-sugars were also released from a variety of cell types including glioma cells [93-95]. Although these findings suggest that uridine nucleotides are present in synaptic vesicles, direct evidence of such storage has not been reported. However, a strong argument for the release of uridine nucleotides is the existence of uridine nucleotide receptors on plasmamembranes [39]. These receptors belong to the P2Y family of 7 transmembrane domain G-protein coupled receptors as reviewed extensively [2, 36, 40, 96]. The members of the P2Y receptors family that are activated by pyrimidines are shown in Table 1. Other members of the P2Y receptor family are G-protein coupled receptors that are activated by purine and not pyrimidine nucleotides [2, 96].

Information on the localization of pyrimidine receptors mostly derives from RT-PCR [98, 100], western blotting [101], and cell culture studies [102], which did not allow the determination of the precise distribution of pyrimidine receptors. Therefore, these receptors are to be investigated by *in situ* hybridization, immunocytochemical, electronmicroscopical as well as functional approaches in the future. Initial functional studies suggest the involvement of pyrimidine receptors in the proliferation, differentiation, survival of cells, as well as the removal of damaged cells. UTP induces proliferation and neuronal differentiation of olfactory epithelium [103] and augments the proliferation of adult neural progenitor cells via P2Y2 receptors [104]. UTP was also neuroprotective against cerebral ischemia reperfusion injury [105]. Microglia expressing P2Y6 receptors show phagocytosis by the stimulation of UDP [106] while UTP was shown to promote the removal of apoptotic cells by phagocytes via P2Y2 receptors [89].

The experimental data are scarce as to whether the effects of uridine are exerted via pyrimidine receptors. While uridine is known to increase the level of intracellular UTP [81, 82], there is no information whether it also increases the level of the releasable pool of pyrimidine nucleotides. Nevertheless, available evidence suggests that uridine nucleotides and pyrimidine receptors contribute to the neurite outgrowth enhancing effect of uridine in PC12 cells. The increase in neurite outgrowth produced by uridine administration was mimicked by exposing the cells to UTP, and could be blocked by various drugs known to antagonize P2Y receptors, such as suramin, reactive blue 2, and pyridoxal-phosphate-6-azophenyl-2',4' disulfonic acid [70]. Moreover,

Table 1. Pyrimidine Receptors and Their Localization in the Central Nervous System

	Endogenous Agonists	Receptor Localization
P2Y2	UTP=ATP	Neurons and glial cells in several brain regions [96]
P2Y4	UTP>ATP	Neurons and glial cells in several brain regions [96]
P2Y6	UDP	Neurons and glial cells in several brain regions [96]
P2Y14	UDP-sugars	Mostly astrocytes, microglia [97]
GPR17	UDP, UDP-sugars, cysteinyl-leukotrienes	Neurons in several brain regions but not in astrocytes [98,99]

There are five currently known receptors that recognize uridine derivatives. These are listed in the first column. The recently orphanized G-protein-coupled receptor 17 (GPR17) has not yet been renamed. The second column indicates the endogenous agonists and also their relative affinities. The third column of the table describes the localization of the receptors and the corresponding references.

degradation of nucleotides by apyrase blocked the stimulatory effect of uridine on neuritogenesis [70].

Evidence for a Uridine Receptor in the Brain

Released uridine nucleotides can be degraded to uridine in the extracellular space by nucleotide-converting ectoenzymes [1, 37, 107, 108]. Indeed, depolarization leads to elevated extracellular uridine levels. Labeled uridine taken up by synaptosomes in a dipyridamole-sensitive process was shown to be released by 4-aminopyridine-induced depolarization [109]. *In vivo* depolarization of neuronal cells by ouabain, high-potassium ion concentration, and glutamate receptor agonists lead to increased concentrations of uridine in the extracellular space measured by brain microdialysis [27, 28]. Elevated level of uridine was also found during seizures in the extracellular space of the hippocampus in an aminopyridine-induced rat model of epilepsy and the levels of uridine correlated with seizure activity [29]. Furthermore, local administration of uridine inhibited hippocampal unit activity in anaesthetized rats without any change in the local extracellular adenosine levels [28]. In analogy with the adenosine system where ATP as well as its extracellular degradation product adenosine have their own receptors [96], the existence of uridine receptors has also been suggested. Although uridine receptors have not been cloned yet, evidence keeps accumulating on the direct binding and action of uridine in the central nervous system.

Evidence that uridine itself may have an active role in the brain extracellular space was first provided by the demonstration that uridine and GABA interacted competitively with GABA binding sites in rat cerebellar buffer-washed membranes. Both high and low affinities of GABA for its receptors were affected by 1 mM uridine administration, whereas the apparent number of binding sites remained unchanged [110]. Subsequently, it was also shown that the GABA binding to membrane preparations from frontal cortex, hippocampus, and thalamus were all competitively inhibited by the *in vitro* addition of uridine [111]. Since intraperitoneal injection of uridine produced a dose-related decrease in the cerebellar content of cyclic GMP and antagonized its increase elicited by bicuculline as well as reduced bicuculline-induced seizures, the anti-convulsant actions of

uridine were suggested to be the result of the modulation of GABA-mediated inhibitory neurotransmission [111].

Another line of research based on the sleep-promoting activity of uridine also lead to the suggestion that uridine exerts some of its actions through its own receptors. Intracerebroventricular injection of uridine derivatives including N³-benzyluridine [112, 113], N³-benzyl-6-azauridine [114, 115] and N³-phenacyluridine Fig. (2). [116] resulted in hypnotic activity and prolonged the duration of pentobarbital-induced sleep. In contrast, a number of structurally related compounds were without effects [114, 115]. Furthermore, an uridine analogue, N³-alpha-hydroxy-beta-phenethyluridine Fig. (2) was identified whose N³-(S)-(+)- but not N³-(R)-(-)-alpha-hydroxy-beta-phenethyluridine isomer had potent hypnotic activity and inhibited N³-phenacyluridine binding [117]. In additional experiments, specific uridine binding was demonstrated in synaptic membranes from bovine thalamus and N³-Phenacyl derivatives of uridine inhibited specific uridine binding [118]. In contrast, N³-phenacyl-2',3'-O-isopropylideneuridine, whose sugar moiety is different from uridine, and N³-benzyluracil and N³-phenacyluracil that have no ribose moiety, did not have hypnotic activity and exhibited no binding affinity [118]. The affinities of N³-phenacyluridine to benzodiazepine, GABA, 5-HT, and adenosine receptors were quite low [119]. Therefore, the binding site of uridine and N³-phenacyluridine was proposed to represent a uridine receptor, which may play a role in the induction of sleep mediated by uridine [119].

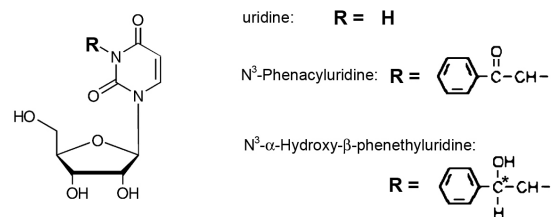


Fig. (2). Chemical structure of uridine and uridine analogues with hypnotic activity. The asymmetric center of N³-α-Hydroxy-β-phenylethyluridine is indicated by *.

In another approach, uridine binding sites were identified in rat synaptosomal membranes [109]. Pyrimidine and purine analogues displayed different rank order of potency in displacement of specifically bound uridine (uridine > 5-F-uridine > 5-Br-uridine similar to adenosine >> 5-ethyl-uridine similar to suramin > theophylline) and in the inhibition of uridine uptake (adenosine > uridine > 5-Br-uridine similar to 5-F-uridine similar to 5-ethyl-uridine) into purified cerebrocortical synaptosomes [120]. Furthermore, dipyridamole did not affect uridine binding and the effective ligand concentration for the inhibition of uridine uptake was about two orders of magnitude higher than that for the displacement of specifically bound uridine supporting that the binding site is not a nucleoside transporter. Actions of uridine, UDP, UTP, ATP, and adenosine were studied by fluorescent labeling of ion fluxes into cortical synaptosomes. Uridine evoked the largest transmembrane Ca^{2+} ion influx, whereas adenosine evoked K^+ ion influx [109, 120]. Also, uridine was shown to increase free intracellular Ca^{2+} ion levels in hippocampal slices [120]. Uridine analogues were found to be ineffective in displacing radioligands that were bound to various glutamate and adenosine-recognition and modulatory-binding sites, which is important because [S]-willardiine, a specific agonist of the AMPA receptor [121] is an uridine derivative [122]. Moreover, the GTP binding site on AMPA/kainate receptor may show affinity for uridine [123, 124]. In additional characterization of the uridine binding site, it was shown that [^{35}S]GTP γ S binding to membranes isolated from the rat cerebral cortex was enhanced by uridine arguing for the involvement of a G-protein-coupled receptor [120]. Altogether, these findings provide evidence for a rather specific, G-protein-coupled binding site of excitatory action for uridine in the brain. However, the direct evidence for the existence of a uridine receptor is still missing. The protein(s) responsible for binding uridine and making alterations in brain functions are not identified yet. The existence of a putative uridine receptor has probability but the final validation of the receptor requires molecular biological and additional pharmacological studies. Thus, identification and characterization of uridine receptor would be the next essential step for understanding the role of uridine in the nervous system.

THERAPEUTIC UTILIZATION OF CENTRAL URIDINE ACTIONS

Uridine, as a natural endogenous molecule, is attractive for therapeutic use because of its low toxicity. Uridine administration was tested in human in order to alleviate the side-effects of anti-cancer drugs [125]. It was established that the major limiting factors in increasing the dose of uridine in human are fever and diarrhea [126, 127]. Since orally administered formulas can increase uridine levels in the plasma as well as in the brain [82], it is relatively straightforward to address the central actions of uridine for therapeutic purposes. Based on the actions of uridine, its potential antiepileptic effects as well as its potential effects on memory in Alzheimer disease have been tested.

Four unrelated patients with a syndrome that included developmental delay, seizures, ataxia, severe language deficit, and an unusual behavioral phenotype were investigated. Examination of the cultured fibroblasts of these patients re-

vealed that the activity of cytosolic 5'-nucleotidase was markedly elevated, which resulted in a decreased incorporation of uridine into nucleotides with normal utilization of purine bases [128]. In response to oral uridine administration, the patients experienced fewer seizures, decreased ataxia, improved speech and behavior, and improved cognitive performance in a double-blind placebo trial. On replacement of the supplements with placebo, the patients rapidly regressed to their pretreatment states. These observations suggest that increased nucleotide catabolism causes the symptoms of these patients, which can be reversed by administration of uridine [128].

In another human study, the effect of CDP-choline (citicoline) was tested on the verbal memory of older volunteers. Dietary CDP-choline is known to increase the levels of uridine in the brain [67]. In a randomized, double-blind, placebo-controlled, parallel group design study, the subjects took either placebo or citicoline (1000 mg/d) for 3 months. Citicoline therapy improved delayed recall on logical memory only for the subjects with relatively inefficient memories [69]. These subjects with relatively inefficient memories were recruited for a second study that used a crossover design where subjects took both placebo and citicoline (2000 mg/d), each for 2 months. In this study, citicoline was clearly associated with improved immediate and delayed logical memory [69]. Consequently, citicoline may prove effective in treating age-related cognitive decline that may be the precursor of dementia. Therefore, CDP-choline and other nutritional components that increase brain uridine levels may be important in the treatment of Alzheimer's disease [129]. Alzheimer's disease is a progressive memory impairment characterized by neurodegeneration and the dense deposition of misfolded proteins in the brain. There is no cure for Alzheimer's disease and current treatments usually only provide a temporary reduction of symptoms. An Alzheimer's diseased brain contains fewer synapses and reduced levels of synaptic proteins and membrane phosphatides [130]. The ability of nutritional compositions to stimulate synapse formation and effectively reduce Alzheimer's disease neuropathology in these preclinical models provides a solid basis to predict potential to modify the disease process, especially during the early phases of Alzheimer's disease [73, 129]. Whether these potential therapeutic effects of a nutrient approach observed in animal models can also be replicated in a clinical study needs further investigation [72, 131].

Uridine might also be effective in the treatment of other neurodegenerative disorders than Alzheimer's disease. In a recent study, co-administration of UMP and docosahexaenoic acid known to increase synapse formation alleviated the behavioral consequences of 6-hydroxydopamine injection in a rat model of Parkinson's disease [132]. In addition, the levels of striatal dopamine, synapsin-1, and the activity of tyrosine hydroxylase were increased by the treatment at the lesioned side [132]. The finding that uridine and docosahexaenoic acid partially restored dopaminergic neurotransmission in this model of Parkinson's disease suggest that they may also be effective in the human disorder. The potentiation of haloperidol effects by uridine suggests that uridine coadministration might enhance the antipsychotic action of traditional neuroleptics. This would allow for a reduction in the therapeutic dose of the antipsychotic, thereby making it

possible to relieve some of the side effects of neuroleptic therapy [56, 57].

Uridine might also be useful as a nutrition supplement during development. Uridine (as uridine monophosphate) is found in mother's milk and has been proposed to play a role in regulatory mechanism through which plasma composition influences brain development [76]. UMP and docosahexaenoic acid administered orally to rat dams during gestation and nursing increased synaptic elements in brains of weanling pups suggesting that the administration of these phosphate precursors to lactating mothers or infants could be useful for treating developmental disorders characterized by deficient synapses [75].

CONCLUSION

Emerging evidence suggests that uridine is a neuroactive molecule, which is involved in the regulation of certain neural functions apart from its role in pyrimidine metabolism. Uridine has sleep-promoting and anti-epileptic effects, might affect mood, improves memory function and influences neuronal plasticity. Evidence for the existence of uridine-sensitive neurons is also convincing. These actions are likely to be exerted via its actions on membrane formation, by the known uridine nucleotide receptors, or even on its own putative receptor predicted in plasmamembranes or intracellular binding sites in the central nervous system. Since uridine, as a dietary component, is not toxic and has access to the brain from the plasma through transporters, it is an appealing lead molecule for the development of drugs with central site of action. Based on its actions, the therapeutic application of uridine and its derivatives are being explored.

ACKNOWLEDGEMENTS

Support was provided by the Bolyai János Fellowship Grant of the Hungarian Academy of Sciences, and the Hungarian Scientific Research Fund Research Grants NFM-OTKA NNF2 85612, and NKTH-OTKA K67646 for AD, and the Scientific Foundation of NYME SEK (2009-2010) Hungary for ZsK and NKTH TECH_09_A1 for AD and JK, and TAMOP 4.2.1./B-09/1KMR-2010-0003 to GJ.

ABBREVIATIONS

Ach	=	Acetyl-choline
AMPA	=	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ATP	=	Adenosine-5'-triphosphate
CDP	=	Cytidine-5'-diphosphate
CNT	=	Concentrative nucleoside transporter
CTP	=	Cytidine-5'-triphosphate
dCTP	=	Deoxycytidine-5'-triphosphate
dTTP	=	Deoxythymidine-5'-triphosphate
dUTP	=	Deoxyuridine-5'-triphosphate
ER	=	Endoplasmic reticulum
GPR17	=	G-protein-coupled receptor 17

PRPP	=	Phosphoribosylpyrophosphate
UDP	=	Uridine-5'-diphosphate
UK	=	Uridine kinase
UMP	=	Uridine-5'-monophosphate
Ura	=	Uracil
Urd	=	Uridine
UTP	=	Uridine-5'-triphosphate

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