Origin, utilization, and recycling of nucleosides in the central nervous system

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Ipata PL. Origin, utilization, and recycling of nucleosides in the central nervous system. Adv Physiol Educ 35: 342–346, 2011; doi:10.1152/advan.00068.2011.—The brain relies on the salvage of preformed purine and pyrimidine rings, mainly in the form of nucleosides, to maintain its nucleotide pool in the proper qualitative and quantitative balance. The transport of nucleosides from blood into neurons and glia is considered to be an essential prerequisite to enter their metabolic utilization in the brain. Recent lines of evidence have also suggested that local extracellular nucleoside triphosphate (NTP) degradation may contribute to brain nucleosides. Plasma membrane-located ectonucleotidases, with their active sites oriented toward the extracellular space, catalyze the successive hydrolysis of NTPs to their respective nucleosides. Apart from the well-established modulation of ATP, ADP, adenosine (the purinergic agonists), UTP, and UDP (the pyrimidinergic agonists) availability at their respective receptors, ectonucleotidases may also serve the local reutilization of nucleosides in the brain. After their production in the extracellular space by the ectonucleotidase system, nucleosides are transported into neurons and glia and converted back to NTPs via a set of purine and pyrimidine salvage enzymes. Finally, nucleotides are transported into brain cell vesicles or granules and released back into the extracellular space. The key teaching concepts to be included in a two-to-three-lesson block on the molecular mechanisms of the local nucleotide recycling process, based on a cross talk between the brain extracellular space and cytosol, are discussed in this article.

ectonucleotidases; purine salvage; pyrimidine salvage; nucleotide release; purine and pyrimidine receptors

The brain requires a continuous supply of preformed purine and pyrimidine rings, mainly in the form of nucleosides (NSs), to maintain its nucleotide pool in the proper qualitative and quantitative balance (15). Adenosine (Ado), guanosine (Guo), uridine (Urd), and cytidine (Cyd), the four main natural NSs, can enter the brain through the blood-brain barrier (24). Alternatively, they can be locally produced in central nervous system (CNS) from their respective NS triphosphates (NTPs) by an enzyme cascade system that consists of a series of ectonucleotidases located in the plasma membrane with their active sites oriented toward the extracellular space (31, 32). The products of the ectonucleotidases are then transported into the brain cytosol via the concentrative NS transporter (CNT) system and the equilibrative NS transporter (ENT) system (24). An important source of extracellular NTPs is from synaptic release (1, 32). The present article focuses on these aspects of brain metabolism, with an emphasis on the cross talk between intra- and extracellular metabolic networks. This topic is not generally available in most textbooks. This article aims to provide the key teaching concepts to be included in a two- to three-lesson block based on recent information as well as on traditional knowledge. Students should be aware that the brain is unique among other organs in the extent to which the rates of preformed NS anabolic and catabolic reactions modulate the synthesis of RNA and DNA precursors, purine and pyrimidine signaling molecules, and phosphatides, the main constituents of synaptic membranes.

The Origin of Brain NSs

The brain has a very limited capacity of de novo synthesis of purines and pyrimidines rings and relies on preformed NSs for the synthesis of NTPs. NSs are actively synthesized de novo from simple precursors in the liver and enter the brain through the blood-brain barrier (Fig. 1). The final products of the de novo pathway are nucleotides (21). NSs are not intermediates; thus, cytosolic 5’-nucleotidases play a major role in NS generation in the liver. The liver possesses the entire set of enzymes for NS degradation (21). It is conceivable that the balance between the rates of synthesis and degradation and the export into the bloodstream have a central role in maintaining the homeostasis of circulating NSs to meet the requirement for NTPs in the brain.

Extracellular NS Metabolism in the Brain

The release of nucleotides from brain cells. For most of the last century, research on brain purines and pyrimidines was mainly focused on their intracellular metabolism. New insights into the importance of extracellular nucleotide and NS metabolism followed the recognition that ATP and Ado act as extracellular signals. Extracellular ATP was identified as a cotransmitter in sympathetic and parasympathetic nerves in 1976 (5). Since then, there has been an ever-increasing stream of new ideas and information related to the purinergic receptor proteins (1, 6, 7) and to extracellular ATP metabolism in the peripheral nervous system and CNS (3, 31, 32). After exocytotic neuronal vesicular release, ATP interacts with P2X receptor subtypes and exerts a robust excitatory and neuromodulator action (6, 7). There is also evidence for the vesicular release of ATP from astrocytes (22, 23).

The existence of a plasma membrane pyrimidine-activated receptor was first proposed in 1989 (27), and a direct demonstration of cellular release of UTP was provided by Lazzarowski et al. (18) in 1997. To date, it is well established that UTP, UDP, and Urd diphosphoglucone have important signaling roles in the CNS via interactions with P2Y receptor subtypes (19).

Purine and pyrimidine signaling molecules are present in vesicles, where the concentrations of ATP (~100 mM) and UTP (~8 mM) are much higher than in the surrounding cytoplasm (31). After the release of a single vesicle, the local extracellular concentration of ATP lies in the range of 5–500 μM, well above the limit for P2X receptor activation.

The extracellular breakdown of nucleotides in the brain. The actions of ATP and UTP at their respective receptors are
LIVER CYTOSOL  
BLOOD  
BRAIN CYTOSOL

Fig. 1. The origin of brain nucleosides (NSs). The brain relies on circulating preformed NSs to satisfy its energy demand and to synthesize nucleic acids, lipid and sugar conjugates, and extracellular purine and pyrimidine signaling molecules. The dephosphorylation of NS monophosphates (NMPs), synthesized de novo in the liver, is the main source of brain NSs. Once taken up from the blood into the brain cells, NSs are salvaged to their respective ribonucleoside triphosphates via three successive phosphorylation steps. NSs may generate ribose-1-phosphate (Rib1-P), a precursor of phosphoribosyl pyrophosphate (PRPP), for the salvage synthesis of purine bases. NDP, NS diphosphate; NTP, NS triphosphate.

terminated by rapid degradation, catalyzed by the ectonucleotidases (3, 32) (reactions 5–8 in Fig. 2 and reactions 4–7 in Fig. 3). The ectonucleotidase enzyme chain includes ecto-NTP diphosphohydrolase: ATP (UTP) → ADP (UDP) + P,
or ADP (UDP) → AMP (UMP) + P,
and ADP (UDP) → AMP (UMP) + 2 P,
and ecto-5′-nucleotidase: (AMP) (UMP) → Ado (Urd) + P.
The final product of the ectonucleotidase chain generally is the NS (32). P1 receptor-mediated actions of Ado in inflammation, sleep, memory, cognition, and ischemic injury have been recognized for many years (9, 11, 17). No receptors for Urd have been identified yet, but there is compelling evidence that Urd is transported into neurons and astrocytes, where it plays an essential role as a precursor of brain UTP, Urd diphosphoglucone, CTP, CDP-choline, and membrane phosphatides (8, 10, 19, 25, 30). In addition to ATP and UTP, other nucleotides, such as GTP, GDP, GMP, inosine (Ino) monophosphate (IMP), and possibly CTP, are stored in vesicles (31). Specific binding sites for GTP (12) and Guo (29), two neurotrophic purines (26), have been identified on the plasma membrane of cultured neuronal cell models. Finally, no specific receptors have been identified for Cyt and CTP. Nevertheless, CTP undergoes successive extracellular dephosphorylation steps in the extracellular space and produces Cyt with the same temporal patterns of the sequential production of extracellular NS diphosphates (NDPs), NS monophosphates (NMPs), and NSs observed when ATP, UTP, and GTP were used as substrates of the ectonucleotidase chain (3, 13, 14).

**NS Transport in the Brain**

A major function of ENT and CNT proteins is to control the concentrations of NSs in the brain to maintain the qualitative and quantitative balance of NTP pools for the stability of genetic information and to regulate the trophic functions of NSs.

The ENT proteins mediate the transport of NSs bidirectionally, depending on the concentration gradient across the plasma membrane (15, 24). The $K_m$ values of ENT1 and ENT2, the two major ENT proteins, are between 100 and 800 μM. Most likely, they mediate the inwardly directed transport only when NSs exceed their normal levels, e.g., when their concentrations have been elevated experimentally or when NS derivatives are administered and activated by antiviral or antitumoral drugs inside the cells by the same enzymes of NS metabolism. CNT2, a member of the family of Na$^+$-dependent, high-affinity CNT proteins, mediates the inwardly directed transport of Ado, Ino, Guo, Urd, and Cyt. Most likely, CNT2 modulates the transport of NSs under physiological conditions, because its $K_m$ value for NSs is in the low micromolar range (9–40 μM) and plasma levels of most NSs are subsaturating (~3–5 μM) (28). Moreover, Cyt is transported less efficiently than Urd (8). Hence, in humans and laboratory rats, the major precursor of brain CTP is Urd, not Cyt (Fig. 3).

**Cytosolic NS Utilization in the Brain**

The specificity and regulatory properties of brain NS kinases. Two cytosolic NS kinases, Ado kinase (AdoK; reaction 3 in Fig. 2) and Urd kinase (UrdK; reaction 1 in Fig. 3), have a high specificity for Ado and deoxyadenosine and for Urd and Cyt, respectively. The $K_m$ value for Ado of AdoK is ~0.2 μM; thus, the kinase becomes saturated with Ado at a low micromolar concentration and phosphorylates Ado at its $V_{max}$. Any excess Ado is irreversible deaminated to Ino by Ado deaminase (AdoD; reaction 1 in Fig. 2), whose $K_m$ value for Ado is ~50 μM. It may be speculated that brain Ado is maintained at the lowest micromolar level among the NSs (16) by the extent to which AdoK and AdoD are saturated by Ado, their common substrate.

Guo and Ino are not directly phosphorylated to their respective NMPs because of the absence of kinases for the two NSs in mammals. They undergo prior phosphorolysis to guanine and hypoxanthine, respectively, before being anabolized to their respective NMPs via phosphoribosyl pyrophosphate (PRPP), requiring hypoxanthine-guanine phosphoribosyltransferase (PRPT) activity.
ferase (reactions 2, 9, and 12 in Fig. 2). Subsequently, NMPs are further phosphorylated by AMP-specific adenylate kinase (reaction 4 in Fig. 2) and by NMP kinase, specific for UMP, CMP (reaction 2 in Fig. 3), and GMP (reaction 13 in Fig. 2). IMP is not further phosphorylated in the brain cytosol (4). NDP kinase generates UTP, CTP, and GTP from their respective NDPs. ATP, the most abundant NTP in the brain (1/10th that of ATP (28). Brain Urd homeostasis is maintained by the relative rates of its phosphorylation and phosphorolysis, catalyzed by UrdK and Urd phosphorylase, the major entry steps in pyrimidine salvage synthesis and catabolism, respectively (reactions 1 and 9 in Fig. 3). (2, 25). UrdK is competitively inhibited by UTP and CTP, the final products of the pyrimidine salvage pathway. Thus, at relatively low UTP and CTP levels, Urd is mainly anabolized to Urd nucleotides, whereas at relatively high UTP and CTP levels, Urd is mainly catabolized to uracil and ribose-1-phosphate. The sugar phosphate is then transformed into PRPP, which is used for the salvage of adenine, guanine, and hypoxanthine. The UrdK-uridine phosphorylase system might cross regulate the two processes of pyrimidine and purine salvage synthesis in a district where the de novo synthesis is lacking (2).

The transport of NSs, synthesized de novo in the liver, from blood into neurons and glia is an essential prerequisite to enter their metabolic utilization in the brain (Fig. 1). However, the interrelated processes of NTP release into the brain extracellular space, NTP breakdown by the ectonucleotidase enzyme chain, NS transport into brain cells, and cytosolic NS anabolism strongly suggest that, apart from the modulation of ligand availability at nucleotide and NS receptors, the four processes may also serve the recycling of NSs in the brain. The path of NS recycling may be summarized as follows: NS (intracellular) → NTP (intracellular) → NTP (extracellular) → NS (extracellular) → NS (intracellular) (14). As shown in Fig. 2, Ado recycling requires the expenditure of three ATP molecules (reactions 3–5 in Fig. 2), and Urd and Cyt recycling require the expenditure of three ATP molecules each (reactions 1–3 in Fig. 2). Guo recycling requires four ATP molecules (reactions 9 and 12–14 in Fig. 2) because the synthesis of PRPP from ribose-5-phosphate and ATP (reaction 11 in Fig. 2), needed for the salvage synthesis of GMP, generates an AMP molecule rather than an ADP molecule. We recall that no Guo kinase exists in mammals. It may be speculated that under normal aerobic conditions, imported cytosolic NSs are mainly anabolized to NTPs rather than being catabolized or exported from brain cells, whereas NTPs are mainly catabolized in the
extracellular space. In the hypoxic/ischemic brain, the low ATP level would cause NS accumulation, thus favoring the capacity of the brain to achieve reperfusion/recovery of lost nucleotides.

General Conclusions

It is safe to state that a cross talk exists between the extracellular and intracellular metabolism of purine and pyrimidine NSs in the brain. The extracellular space is the major site of brain NS production through successive dephosphorylations of released NTPs, whereas the brain cytosol is the major site of uptaken NS anabolism to NTPs through successive phosphorolytic steps. This NS recycling process has an important role in the CNS, since the brain maintains its nucleotide pools in the proper qualitative and quantitative balance by salvaging preformed purine and pyrimidine rings, mainly in the form of NSs (15). The local recycling of NSs allows their immediate reutilization without NS outflow into the bloodstream, thus avoiding the dilution of NSs, with a considerable spatial-temporal advantage. Finally, students should be aware that dysfunctional NS metabolism has been implicated in the etiopathology of a wide spectrum of neurological, neurodegenerative, and neuropsychiatric disorders (20).

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AUTHOR CONTRIBUTIONS

P.L.I., performed the experiments; P.L.I., analyzed the data; P.L.I., edited and revised the manuscript.

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