Area, Age and Gender Dependence of the Nucleoside System in the Brain: a Review of Current Literature

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Abstract: Nucleosides, such as uridine, inosine, guanosine and adenosine, may participate in the regulation of sleep, cognition, memory and nociception, the suppression of seizures, and have also been suggested to play a role in the pathophysiology of some neurodegenerative and neuropsychiatric diseases. Under pathological conditions, levels of nucleosides change extremely in the brain, indicating their participation in the pathophysiology of disorders like Alzheimer's disease, Parkinson's disease and schizophrenia. These findings have resulted in an increasing attention to the roles of nucleosides in the central nervous system. The specific effects of nucleosides depend on the expression of their receptors and transporters in neuronal and glial cells, as well as their extracellular concentrations in the brain. A complex interlinked metabolic network and transporters of nucleosides may balance nucleoside levels in the brain tissue under normal conditions and enable the fine modulation of neuronal and glial processes via nucleoside receptor signaling mechanisms. Brain levels of nucleosides were found to vary when measured in a variety of different brain regions. In addition, nucleoside levels also depend on age and gender. Furthermore, distributions of nucleoside transporters and receptors as well as nucleoside metabolic enzyme activities demonstrate the area, age and gender dependence of the nucleoside system, suggesting different roles of nucleosides in functionally different brain areas. The aim of this review article is to summarize our present knowledge of the area-, age- and gender-dependent distribution of nucleoside levels, nucleoside metabolic enzyme activity, nucleoside receptors and nucleoside transporters in the brain.

Keywords: Nucleosides, area, age, and gender dependence, brain.

1. INTRODUCTION

Nucleosides and their metabolic derivatives have specific roles in the regulation of cellular functions in all tissues and organs of the organism, including the brain [1-10]. Therefore, nucleosides have a role in the pathological mechanisms of several illnesses, and their derivatives are effective in the treatment of a wide variety of diseases, from cancer to psychiatric diseases [1, 5, 11-17]. Nucleosides and endogenous nucleoside metabolites are directly coupled to the synthesis of DNA and RNA, to gene transcription, and to the energy storage and conversion. In addition, some nucleosides have neuromodulatory functions [4, 18]. Adenosine (Ado), guanosine (Guo), inosine (Ino) and uridine (Urd) may participate in physiological and pathophysiological processes in different brain areas such as sleep regulation, immunomodulation, epilepsy, psychiatric disorders, Parkinson's disease and Alzheimer's disease [1, 14, 19-29]. Nucleoside derivatives [5, 30, 31] adenosine uptake inhibitors [32, 33] and adenosine kinase (ADK) inhibitors [34-36] are used in clinical applications to treat brain diseases such as epileptic seizures [5, 32, 33, 36]. Therefore, recently, increasing attention has been paid to nucleoside mechanisms in the central nervous system (CNS).

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Nucleoside concentrations [37-41], nucleoside transporters [42-46] and nucleoside receptors [1, 42, 47-52] are unevenly distributed in the CNS. It has been demonstrated that the activities of nucleoside metabolic enzymes are also regionally different [53-56]. Similarly, gender- and/or age-dependent distribution of nucleosides, nucleoside transporters and receptors, and nucleoside metabolism have been shown [37, 40, 56-63]. All of these results suggest that the regionally different effects of nucleosides in the brain are modulated by age and gender. However, nucleoside receptors, nucleoside transporters, ecto-nucleotide and ecto-nucleoside metabolizing enzymes form a complex molecular interaction network responsible for the effects of extracellular (EC) purines and pyrimidines [64]. Therefore, in this review, first, we briefly summarize what is actually known about nucleoside metabolism, nucleoside transporters and nucleoside receptors. Then, we give a summary of area-, age- and gender-dependent distribution of nucleoside metabolic enzyme activity, nucleoside levels, nucleoside transporters and nucleoside receptors in the brain.

1.1. Metabolism of Nucleosides in the Brain

Nucleosides are composed of purine and pyrimidine bases connected to a pentose (D-ribose in ribonucleosides or 2-deoxy-D-ribose in deoxyribonucleosides) moiety, and after polymerization into long chains, they make up ribonucleic acids (RNA) and deoxyribonucleic acids (DNA). The major purine and pyrimidine bases and their ribonucleosides are

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adenine (Ade)/Ado, guanine (Gn)/Guo, hypoxanthine (Hyp)/Ino and cytosine (Cyt)/cytidine (Cyd), uracil (Ura)/Urd, and thymine (Thy)/thymidine (Thd) [65]. 5-phosphoribosyl-1-pyrophosphate (PRPP), glutamine, glycine, formyl groups, carbon-dioxide and aspartate, and carbamyl-phosphate and aspartate can be the precursors of *de novo* synthesis of purines and pyrimidines in mammals [66-69].

The degradation pathway of adenine nucleotides in the brain is shown in Fig. (**1**). The reaction route can lead from adenosine monophosphate (AMP) to inosine monophosphate (IMP)-Ino-Hyp (IMP pathway) or from AMP to Ado-Ino-Hyp (adenosine pathway). The converting enzymes are as follows: cytoplasmic 5'-nucleotidases (cN), AMP deaminase (AMPDA), adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) [70-72]. Ado can also be synthesized from S-adenosylhomocysteine (SAH) by adenosylhomocysteinase (SAHH, S-adenosylhomocysteine hydrolase) [73]. The catabolism of guanine-ribonucleotides is performed mainly in the guanosine monophosphate (GMP)→Guo→Gn→xanthine (Xn) pathway [74] by cN, PNP and guanine deaminase GDA; Fig. (**1**).

There is no inosine- and guanosine-kinase activity in the brain [71, 74], and the plasma concentrations of deoxynucleosides are very low [75]. In addition, in the brain of adults, *de novo* synthesis of nucleosides is negligible [67]. Therefore, the main precursors of purine-nucleotides in the brain are Ado, Ade, Hyp and Gn [76] converted by way of different metabolic pathways Fig. (**1**). The synthesis of nucleotides from xanthosine (Xao) has been described [77]. The enzyme responsible for uric acid (UA) production is xanthine oxidase XO; Fig. (**1**) but its activity is very low in the CNS [78, 79]. No uricase activity is in human brain, it was lost during evolution, therefore UA is the end product of purine metabolism [80, 81]. The maintenance and regulation of EC Ado concentration is done by ecto-5'-nucleotidase (eN), ecto-adenosine kinase (ecto-ADK) and ecto-adenosine deaminase (ecto-ADA) [82-84].

The intracellular (IC) salvage mechanism preserves the purine nucleosides and bases and maintains the synthesis of ribo- and deoxyribonucleotides. The first step of Hyp and Gn salvage is performed by hypoxanthine phosphoribosyltransferase (HGPRT; hypoxanthine-guanine phosphoribosyltransferase), which converts Hyp and Gn to corresponding purine-monophosphates Fig. (**1**). GMP reductase (GMPR), GMP synthetase (GMPS) and IMP dehydrogenase (IMPDH) enzymes catalyze GMP→IMP and IMP→GMP conversion, respectively. Ado is converted to AMP by ADK Fig. (**1**), and Ado can also be metabolized to IMP through Ino and Hyp in salvage reactions. Ade is used for AMP synthesis, which forms an additional salvage pathway by the adenine phosphoribosyltransferase (APRT) salvage enzyme [85, 86].

Similarly to the purine (deoxy)nucleotides, pyrimidine (deoxy)nucleotides are synthesized in salvage reactions in the brain [87-98]. Urd and Cyd are salvaged by cytidine deaminase (CDA) and uridine-cytidine kinase (UCK) [94, 97, 99]. It has been shown that the ribose donors Ino and Guo may provide the ribose 1-phosphate (R1P) for uracil-uridine salvage [93,100], therefore, there is a link between purine and pyrimidine salvage in the brain [97,101].

Purine and pyrimidine deoxyribonucleotides are synthesized by the ribonucleotide reductase enzyme (RNR) from purine-ribonucleotides [102]. The deoxyuridine monophosphate (dUMP)→deoxythymidine monophosphate (dTMP) conversion is catalyzed by thymidylate synthetase (TS), which is also found in the brain Fig. (**1**) [90]. Deoxycytidine kinase (dCK) performs the first step in the salvage of deoxyadenosine (dAdo), deoxyguanosine (dGuo) and deoxycytidine (dCyd), while in the case of deoxyuridine (dUrd) and deoxythymidine (dThd), this step is done by thymidine kinase (TK) [91,92,103].

Deficiencies of nucleoside metabolic enzymes cause metabolic diseases that are accompanied by neurological or psychological symptoms such as seizures, self-destructive and aggressive behavior, and mental retardation. Improving the treatment of these diseases is an important requirement [96,104-106]. Inhibitors of nucleoside metabolism can be applied in the treatment of several brain disorders. ADK and ADA inhibitors that increase EC Ado concentration Fig. (**2**) have neuroprotective, anti-nociceptive, anti-seizure, anti-inflammatory, pain killing and anti-Parkinsonian effects and they can also be used for the treatment of schizophrenia [14,34-36,107]. In addition, ADK-deficient, stem cell-derived implants releasing Ado may be one of the most effective tools for the treatment of certain types of epilepsy, so there is intensive research on these implants [108,109]. The XO inhibitor allopurinol can be used in the treatment of pain and certain kinds of seizures. It has neuroprotective effects during ischemia because it increases Ado and Ino levels in the brain [110].

1.2. Nucleoside Transporters in the Brain

Nucleoside concentration is high in brain tissue and the EC space [see below; 40, 41, 63, 81, 111-115]. Nucleosides are water soluble and diffuse readily in the EC space, and their derivatives also have large pools in the CNS [116]. Nucleosides and nucleoside analogues are hydrophilic, and they are transported through the cell membrane by specific transporters [117, 118]. Nucleosides are released by reverse transport through nucleoside transporters (Table **1**) [13, 33].

Both concentrative nucleoside transporters (CNT transporters; unidirectional, sodium-dependent) and equilibrative nucleoside transporters (ENT transporters; bidirectional by facilitated diffusion; Table **1**) are present in the brain [13,119]. ENT transporters can be classified as S-(4-nitrobenzyl)-6-thioinosine (NBTI) sensitive transporters, which are inhibited by NBTI in a nM concentration range ("es": **e**quilibrative, NBTI **s**ensitive type of ENTs), and NBTI insensitive transporters, which are inhibited by NBTI only in a μM concentration range ("ei": **e**quilibrative, NBTI **i**nsensitive type of ENTs). Subtypes of equilibrative nucleoside transporters (ENT1-ENT4) that contain 11 transmembrane (TM) domains were cloned, and they are detectable in the brain [43, 114, 119-121].

Expression of CNT transporters has also been demonstrated in the CNS [45, 121-126]. Six CNT transporter types (N1-N6) can be classified on the basis of transported nucleosides and sodium transport coupling [32, 127, 128]. Nucleosides and nucleoside bases are transported by different transporters [129, 130], but there are data those suggest they can

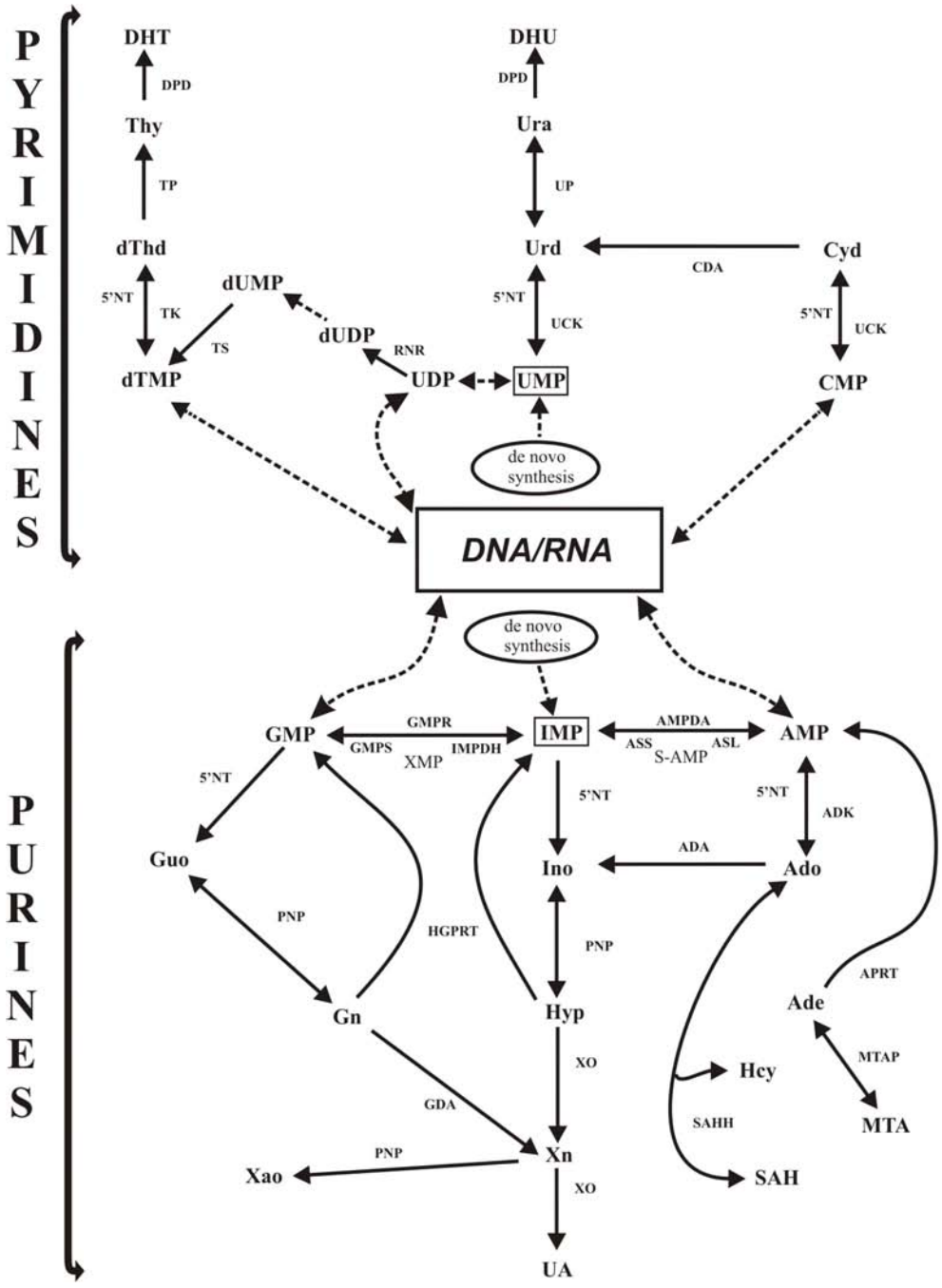

Fig. (1). Metabolism of purine and pyrimidine nucleosides in the brain

Abbreviations: 5'NT: 5'-nucleotidase; ADA: adenosine deaminase; Ade: adenine; ADK: adenosine kinase; Ado: adenosine; AMP: adenosine monophosphate; AMPDA: AMP deaminase; APRT: adenine phosphoribosyltransferase; ASL: adenylosuccinate lyase; ASS: adenylosuccinate synthetase; CDA: cytidine deaminase; CMP: cytidine monophosphate; Cyd: cytidine; DHT: dihydrothymine; DHU: dihydrouracil; DPD: dihydropyrimidine dehydrogenase; dThd: deoxythymidine; dTMP: deoxythymidine monophosphate; dUDP: deoxyuridine diphosphate; dUMP: deoxyuridine monophosphate; GDA: guanine deaminase; GMP: guanosine monophosphate; GMPR: GMP reductase; GMPS: GMP synthetase; Gn: guanine; Guo: guanosine; Hcy: homocysteine; HGPRT: hypoxanthine phosphoribosyltransferase (hypoxanthine-guanine phosphoribosyltransferase); Hyp: hypoxanthine; IMP: inosine monophosphate; IMPDH: IMP dehydrogenase; Ino: inosine; MTA: 5'-deoxy-5'-methylthioadenosine; MTAP: 5'-deoxy-5'-methylthioadenosine phosphorylase; PNP: purine nucleoside phosphorylase; RNR: ribonucleotide reductase; SAH: S-adenosylhomocysteine; SAHH: adenosylhomocysteinase; S-AMP: adenylosuccinate; Thy: thymine; TK: thymidine kinase; TP: thymidine phosphorylase; TS: thymidylate synthetase; UA: uric acid; UCK: uridine-cytidine kinase; UDP: uridine diphosphate; UMP: uridine monophosphate; UP: uridine phosphorylase; Ura: uracil; Urd: uridine; Xao: xanthosine; XMP: xanthosine monophosphate; Xn: xanthine; XO: xanthine oxidase; we didn't show nucleoside mono- and diphosphate kinases or nucleoside di- and triphosphate phosphatases. [18, 37, 71, 72, 83, 84, 90, 91, 93, 94, 96-99, 102]

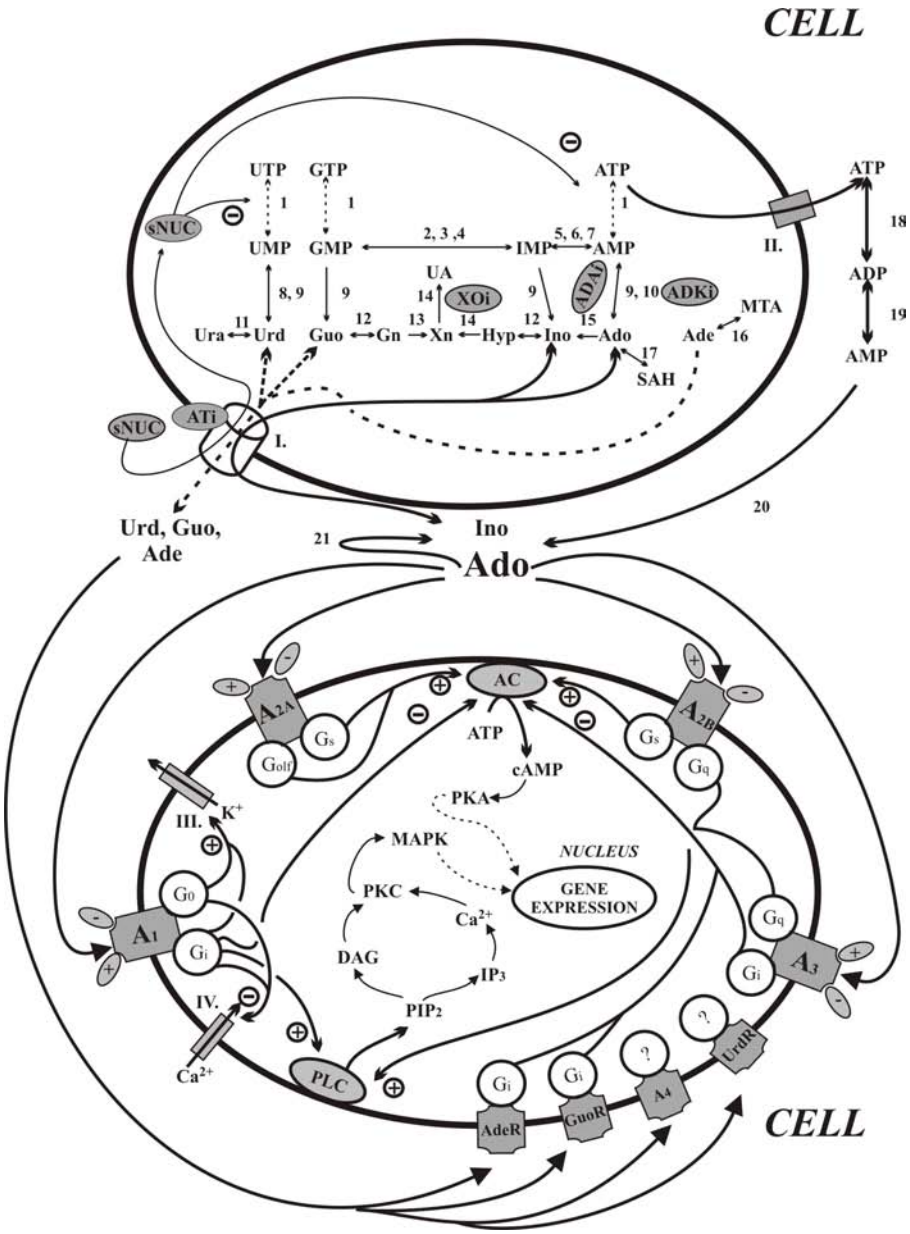

Fig. (2). Pathways of nucleoside production, nucleoside transport and nucleoside receptor coupled signal transduction mechanisms
Abbreviations: *Transporters*: I.: nucleoside transporters; II.: ATP channels and transporters; III.: K^+ channels; IV.: Ca^{2+}-channels [13, 84, 118, 127, 136]; *Metabolic enzymes*: 1.: nucleoside mono- and diphosphate kinases and nucleoside di- and triphosphate phosphatases; 2.: GMPR, GMP reductase; 3.: GMPS, GMP synthetase; 4.: IMPDH, IMP dehydrogenase; 5.: AMPDA, AMP deaminase; 6.: ASL, adenylosuccinate lyase; 7.: ASS, adenylosuccinate synthetase; 8.: UCK.: uridine-cytidine kinase; 9.: 5'NT, 5'-nucleotidase; 10.: ADK, adenosine kinase; 11.: UP, uridine phosphorylase; 12.: PNP, purine nucleoside phosphorylase; 13.: GDA, guanine deaminase; 14.: XO, xanthine oxidase; 15.: ADA, adenosine deaminase; 16.: MTAP, 5'-deoxy-5'-methylthioadenosine phosphorylase; 17.: SAHH, adenosylhomocysteinase; 18.: ecto-ATPase; 19.: ecto-ADPase; 20.: ecto-5'NT, ecto-5'-nucleotidase (eN); 21.: ecto-ADA, ecto-adenosine deaminase [18,37,71,72,83,84,90,91,93,94,96-99,102]; *Nucleotides, nucleosides and their metabolites*: Ade: adenine; Ado: adenosine; ADP: adenosine diphosphate; AMP: adenosine monophosphate; ATP: adenosine triphosphate; GMP: guanosine monophosphate; Gn: guanine; GTP: guanosine triphosphate; Guo: guanosine; Hyp: hypoxanthine; IMP: inosine monophosphate; Ino: inosine; MTA: 5'-deoxy-5'-methylthioadenosine; SAH: S-adenosylhomocysteine; sNUC: synthetic nucleosides/nucleoside analogues; UA: uric acid; UMP: uridine monophosphate; Ura: uracil; Urd: uridine; UTP: uridine triphosphate; Xn: xanthine [13, 31, 37, 71, 72, 94, 96, 98, 128, 134]; *Signal transduction*: AC: adenylate cyclase; cAMP: cyclic adenosine monophosphate; DAG: diacylglycerol; G_i, G_0, G_s, G_q, G_{olf}: several types of G-proteins (f.e. G_i: inhibitory, G_s: stimulatory); IP_3: inositol 1,4,5-triphosphate; MAPK: mitogen-activated protein kinase; PIP2: phosphatidylinositol bisphosphate; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C [1, 4, 5, 35, 143-148]; *Receptors*: A_1, A_{2A}, A_{2B} and A_3, A_4: different subtypes of adenosine receptors [142-144,176]; AdeR: adenine receptor; GuoR: Guo receptor; UrdR: Urd receptor [18, 21, 149-160]; *Inhibitors of nucleoside metabolism and nucleoside transporters*: ADAi: adenosine deaminase inhibitors, ADKi: adenosine kinase inhibitors, ATi: adenosine transporter inhibitors, XOi: xanthine oxidase inhibitors [32-36,107,110]; *Adenosine receptor agonists and antagonists*: „-" in the gray ellipse: antagonists; „+" in the gray ellipse: agonists [4, 5, 143, 144, 176].

Table 1. Nucleoside Transporters in the Brain

Nucleoside Transporters					
Equilibrative Nucleoside Transporters (ENTs)					
Transporter Type (Protein)	**Expression in the CNS**	**Substrate Selectivity**			**Refs.**
		Purines	***Pyrimidines***	***Nucleobases***	[13, 42-44, 99, 136-138]
"es" (ENT1)	Cerebral cortex, hippocampus, thalamus, caudate nucleus, Putamen, amygdala, midbrain, substantia nigra, cerebellum, Medulla oblongata, spinal cord etc.	+	+	-	
"ei" (ENT2)		+	+	+	
"es" (ENT3)	Hippocampus, amygdala, hypothalamus, caudate nucleus, Subthalamic nucleus, substantia nigra etc.	+	+	+	
(ENT4)	Cerebral cortex, hippocampus, caudate nucleus, putamen, Thalamus, cerebellum, medulla oblongata, spinal cord etc.	Ado	-	-	
Concentrative Nucleoside Transporters (CNTs)					
Transporter Type (Protein)	**Expression in the CNS**	**Substrate Selectivity**			**Refs.**
		Purines	***Pyrimidines***	***Nucleobases***	[13, 44, 45, 99]
N1/cif; (CNT2)	Cerebral cortex, thalamus, putamen, midbrain, pons, cerebellum, medulla oblongata etc.	+	Urd (Cyt)	-	
N2/cit; (CNT1)	Cerebral cortex, hippocampus, putamen, midbrain, pons, Cerebellum, medulla oblongata etc.	Ado	+	-	
N3/cib; (CNT3)	Cerebral cortex, corpus callosum, hippocampus, caudate nucleus, putamen, thalamus, amygdala, cerebellum etc.	+	+	-	
N4/cit-like	Unknown	Ado, Guo	+	-	
N5/cs	Unknown	Ado anal.	-	-	
N6/csg	Unknown	Guo	-	-	

Abbreviations: "es": **e**quilibrative, NBTI **s**ensitive type of ENTs; "ei": **e**quilibrative, NBTI **i**nsensitive type of ENTs; Ado: adenosine, Ado anal.: adenosine and its analogues, Guo: guanosine, Urd: uridine; Cyt : cytosine (ENT1-4, CNT1-3, see the text).

be cotransported [118]. Transporters of nucleoside bases are also present in the brain [79, 130-132].

ENT transporters and CNT transporters also participate in the uptake of antiviral and cytostatic nucleoside analogues used in therapy [13, 45]. Like ADK and ADA inhibitors, adenosine transporter inhibitors increase EC concentration of Ado Fig. (**2**). Propentophylline, an Ado transporter inhibitor and A_1 adenosine receptor (A_1 receptor) antagonist, is neuroprotective [32]. It has currently been tested in the treatment of vascular dementia and Alzheimer's disease. It has been claimed that nucleoside transport inhibitors can be used in treatment of ischemia, seizures, sleeplessness and pain [32, 33]. The hypoxanthine derivative drugs AIT-082 and Cladribine are promising for the treatment of Alzheimer's disease and multiple sclerosis, respectively [86,133-135]. Additional information on nucleoside transporters can be found in Table **1** [13, 42-45, 99, 136-138].

1.3. Nucleoside Receptors in the Brain

Nucleoside receptors are not very specific, and they can bind several nucleosides, agonists as well as antagonists with different affinities [5, 27]. Adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) are present both in the peripheral nervous system and in the CNS in neurons, astrocytes and microglial cells [1,4,139,140]. The fifth subtype of adenosine receptors (A_4) is also expressed in the brain Fig. (**2**) [141, 142]. Signaling mechanisms activated by adenosine receptors have been disclosed in detail Fig. (**2**), Table **2** [1, 4, 5, 35, 143-148]. Besides adenosine receptors, there are data suggesting that Urd [21,149-152], Guo [18,153-155] and Ade [156-160] bind to selective receptors (UrdR, GuoR, AdeR, respectively) in the nervous system Fig. (**2**). Therefore, investigations of AdeR and non-adenosine nucleoside receptors and their putative functions in the CNS are interesting fields for further study.

Several adenosine receptor agonists and antagonists are in clinical and/or preclinical drug development stages. Modulators of A_1 receptors and A_{2A} receptors are candidates for treating Huntington's disease [1,161-163], Alzheimer's disease [1,51,164], dementia and anxiety [1, 165], pain [1,5], psychiatric disorders such as schizophrenia [27,51], epilepsy [1,16,109], ischemic and inflammatory reaction-induced damage in CNS [166,167] and morphine and cocaine withdrawal symptoms [1,168]. For example, A_{2A} receptors have a pivotal role in Parkinson's disease, and an A_{2A} receptor antagonist KW-6002 (istradefylline) is in clinical use for treat-

Table 2. Some of the Physiological Actions and the Mechanisms Involved in the Signal Transduction of Adenosine Receptors in the CNS

Adenosine Receptors in the CNS			
G-Protein and Signal Transduction Pathways	**Expression in the CNS**	**Some of Major Physiological Actions and Therapeutic Potential**	**Refs.**
A_1 Receptor			
G-Protein Coupling: $G_{i,}$ G_0 **Messenger Pathways** (second messengers): cAMP↓ Ca^{2+} channels (N, P, Q type)↓ K^+ channel (f.e. GIRK) ↑ PLC/IP_3/DAG ↑	Cerebral and cerebellar cortex, hippocampus, caudate nucleus, putamen, nucleus accumbens, globus pallidus, thalamus, hypothalamus, amygdala, red nucleus, substantia nigra, medulla spinalis, spinal cord etc.	• Neuronal hyperpolarization; inhibition of neurotransmitter release; regulation of sleep; decreasing of cell metabolism (homeostatic role); modulation of cognition and memory • Activation: antinociceptive; spinal analgesia; neuroprotection; seizure suppression; stroke • Inhibition: dementia; anxiety disorders	[1, 4, 5, 14, 23, 35, 42, 51, 52, 65, 73, 146, 147, 170, 174-186]
A_{2A} Receptor			
G-protein Coupling: G_s, G_{olf} **Messenger Pathways** (second messengers): cAMP ↑ Ca^{2+}- channels ↓ PLC/IP_3/DAG ↑	Cerebral cortex, cerebellum, hippocampus, caudate nucleus, putamen, nucleus accumbens, globus pallidus, thalamus, amygdala, olfactory tubercle, substantia nigra etc.	• Facilitation of transmitter release; sleep regulation; decreasing of motor activity; modulation of cognition and memory • Activation: antiinflammatory; schizophrenia; epilepsy; sleep disorders • Inhibition: neuroprotective; Parkinson's disease; Alzheimer's disease; cocaine abuse; stroke	
A_{2B} Receptor			
G-protein Coupling: G_s, G_q **Messenger Pathways** (second messengers): cAMP ↑ PLC/IP_3/DAG ↑	Hippocampus, striatum, thalamus, hypothalamus	• Neuroprotective effects	
A_3 Receptor			
G-Protein Coupling: G_i, G_q **Messenger Pathways** (second messengers): cAMP ↓ PLC/IP_3/DAG ↑	Cerebral cortex, corpus callosum, hippocampus, caudate nucleus, putamen, thalamus, subthalamic nucleus, amygdala, substantia nigra, cerebellum, medulla oblongata, spinal cord	• Neuroprotective effects • Inhibition: antiinflammatory	

Abbreviations: cAMP: cyclic adenosine monophosphate; DAG: diacylglycerol; $G_{i,}$ $G_{0,}$ $G_{s,}$ $G_{q,}$ G_{olf}: several types of G-proteins (f.e. G_i: inhibitory, G_s: stimulatory); GIRK: G-protein-dependent inwardly rectifying K^+ channels; IP_3: inositol 1,4,5-triphosphate; PLC: phospholipase C.

ing Parkinson's disease [27, 169-173]. The physiological functions of Ado and the therapeutic potential of Ado and its derivatives are summarized in Table **2** [1, 5, 14, 35, 73, 170, 174-186].

Adenosine functions have been investigated extensively Fig. (**2**), Table **2** [1, 4, 5, 147, 178, 180]. However, other nucleosides such as Guo, Ino or Urd have not been the focus of interest, despite accumulating data supporting the participation of Guo, Ino and Urd in physiological and/or pathophysiological processes such as sleep regulation, immunomodulation, ischemia and epilepsy [21, 22, 24, 25, 29, 187-191]. There are some promising new data on the therapeutic application of non-adenosine nucleosides and their derivatives. Ino has immunomodulatory and neuroprotective effects. Therefore, Ino intake can be applied as additional ther-

apy for multiple sclerosis [24, 192]. Guo, Urd and related compounds could be a novel target for drug discovery in epilepsy and neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, Huntington's disease and Lesch-Nyhan disease [18, 29, 193-196]. In addition, other nucleosides, deoxynucleosides and their metabolites may also have physiological functions in the nervous system [157, 197-203], but our knowledge of their role in the brain is very limited. Therefore, in this review, we have focused on four nucleosides, Ado, Ino, Guo and Urd, and their three metabolites, Hyp, Xn and Ura.

2. ANATOMICAL LOCALIZATION OF THE NUCLEOSIDE SYSTEM

2.1. Levels of Nucleosides and Their Metabolites in the Brain

Nucleoside levels and the distribution of structures with low and high nucleoside concentrations correlate with the local activity of the nucleoside metabolic network and with regional differences in nucleoside release/uptake balance among brain regions. The brain concentration of adenosine triphosphate (ATP) is estimated to be 1-5 mM, guanosine triphosphate (GTP) is 0.2-0.6 mM, uridine triphosphate (UTP) is 0.6 mM and that of cytidine triphosphate (CTP) is 0.3 mM. These concentrations are several orders of magnitude higher than the nucleoside concentrations (see below). Levels of deoxynucleosides-triphosphates (dNTPs) may be in the range of 0.2-37 μM [72, 204-206].

Depending on the duration of ischemia, levels of nucleosides and their metabolites can change rapidly. After 5-60 min of ischemia, EC and tissue concentrations of nucleosides and their metabolites such as Ado, Guo, Ino, Hyp and Xn are about 2- to 150-fold higher than the baseline levels, and a rapid degradation of nucleoside triphosphatases, such as ATP, can be observed [111, 112, 204, 207-210]. Therefore, measured tissue levels of nucleosides and their metabolites differ depending on tissue sample collection methods and sample preparation methods [38, 114]. Longer *post mortem* times after manual decapitation of animals and slower sample preparations result in Ado levels in the rat brain that are 6-84 times higher compared with Ado levels in brains of rats sacrificed by high-energy microwave (Table **3**) [38, 39, 211]. Similarly to Ado, increases in Ino concentrations were measured in manually-decapitated guinea pigs [39], and levels of other nucleosides and metabolites also changed. However, similar baseline concentrations of Ado, Ino, Hyp and Xn were measured in animal brains using several different methods that stopped the enzymes, such as freeze-blow techniques, freezing brains *in situ*, freezing of the whole animals or whole head in liquid nitrogen or killing animals with microwave; all of these techniques minimized the *post mortem* breakdown of purines [38, 39, 112, 210, 211]. Thus, killing the animals with high-energy microwave irradiation is one of the most useful methods for estimating the real *in vivo* nucleoside levels of different brain areas in animals. We have published what are probably the most realistic estimates of *in vivo* nucleoside concentrations from *post mortem* tissue samples of the human brain areas using microwave treatment of frozen samples [115]. Levels of Ura (4.0 pmol/mg tissue), Hyp (30.3 pmol/mg tissue), Xn (26.6 pmol/mg tissue), Urd (12.0 pmol/mg tissue), Ino (93.5 pmol/mg tissue), Guo (12.9 pmol/mg tissue) and Ado (9.8 pmol/mg tissue) were measured in human brain cortical samples frozen within 30 sec after neurosurgical removal during temporal lobectomy [115]. The sample preparation method included 10 sec of microwave irradiation of the brain samples before homogenization, which kept the *post mortem* metabolism of purines as low as possible.

Nucleosides and nucleoside metabolites concentrations in the brain can be estimated from cerebrospinal fluid (CSF) samples. In rabbit, rat and human CSF, the concentration of purine and pyrimidine nucleosides and metabolites are in the range of 0.02-24.0 μM [75, 116, 212, 213].

EC levels of nucleosides were measured by *in vivo* microdialysis and cortical cup techniques. The applicability of the cortical cup technique is limited because it allows estimation of nucleoside levels only in superficial areas of the brain such as cerebral and cerebellar cortices. Ado and Ino levels were estimated as 0.03-0.05 μM and 0.075-0.13 μM, respectively, in the cerebral cortex using the cortical cup technique [214]. The microdialysis technique is suitable for measurement of *in vivo* EC level of nucleosides in many different brain areas such as cortex, striatum, thalamus and hippocampus at 1-24 hours after probe insertion. Under basal conditions, EC level of nucleosides and metabolites such as Ado, Ino, Guo, Hyp, Xn, Urd were between about 0.05-12.5 μM in several brain areas as measured by microdialysis [111, 112, 116, 189, 209, 215-220]. EC nucleoside concentrations were also measured using enzyme-based sensors [221]. Basal EC level of Ado, Ino and Hyp were 1.2-2.5 μM [222]. Hagberg *et al*. [112] reported that IC concentrations of Ino and Ado are approximately four- and two-fold higher respectively than their EC concentrations; IC levels of Hyp and Xn were similar to their EC concentrations. However, others have reported that levels of Ado in the EC and IC space are in the same range under physiological conditions [35] due to the activity of ENT transporters in the CNS [43]. *Post mortem* release of Ado, Ino, Guo, Hyp, Urd and Ura was established in the microdialysis studies, supporting the existence of actively-controlled IC pools of nucleosides in living cells [116].

2.2. Distribution of Nucleoside and Nucleoside Metabolite Levels in the Brain

As we have described above, comparison of nucleoside concentrations among studies can be done only when the nucleoside analysis is performed in the same way. In rats, the levels of nucleosides and their metabolites in tissue samples and EC space in different brain areas are uneven (Table **3-6**), and this is also true in the human brain [40,41]. To reduce effects of *post mortem* time on nucleoside levels in human brain bank samples, we developed a back-extrapolation method that allows us to estimate the *in vivo* nucleoside levels in several human brain areas based on *post mortem* degradation curves of these compounds [115]. High Urd (41.2-55.1 pmol/mg) and/or Ino (90.4-161.5 pmol/mg), Guo (15.9-26.1 pmol/mg) and Ado (16.0-23.9 pmol/mg) concentrations were measured in the frontal cortex, the insular cortex, the temporal cortex, the occipital cortex, the caudate nucleus, the substantia innominata, the nucleus basalis, the medial geni-

Table 3. The Effect of Killing and Sample Preparation Methods on Unevenly Distributed Tissue Levels of Adenosine in the Rat or Guinea Pig Brains

Rat Brain Area	Tissue Adenosine Concentration (pmol/mg Protein) [1]	
	Killed by Microwave (10 kW)	Killed by Decapitation
Cerebral cortex	10	840
Striatum	45	2498
Hippocampus	48	2428
Hypothalamus	52	1072
Olfactory bulb	66	1704
Cerebellum	170	1060
Rat/Guinea Pig Brain Area	**Tissue Adenosine Concentration (nmol/mg Protein) [2]**	
	Killed by Microwave (7 kW; Rat/Guinea Pig)	**Killed by Decapitation (Guinea Pig)**
Cerebellum/Vermis	0.063/0.336	1.4
Superior colliculus	0.053/0.102	4.0
Inferior colliculus	-	3.3
Hypothalamus	-	3.6
Cerebral cortex	0.068/0.123	8.6
Hippocampus	0.058/0.149	9.6
Striatum (head)	-	7.7
Thalamus	-	10.3

Based on data published by: [1] [38]; [2] [39]

Table 4. The Area-Dependent Distribution of EC Nucleosides in the Rat Brain (1-2 Hours After Microdialysis Probe Implantation)

Rat Brain Areas	EC Concentrations of Purine Nucleosides and Bases (μM)						
	Ado	Ino	Hyp	Xn	Guo	UA	dAdo
Thalamus [1]	0.95	0.52	4.48	1.17	0.17	1.15	0.17
Hippocampus [2]	0.95	0.42	4.79	0.40	0.26	2.23	1.00
Hippocampus [3]	0.93	1.37	2.43	7.65			
Striatum [4]	1.92	1.50	2.82	3.95			
Striatum [5]	1.00	2.00	4.00	-	0.50		
Rat Brain Areas	**EC Concentrations of Pyrimidine Nucleosides (μM)**						
	Urd	**Ura**	**Cyd**	**Thd**	**dCyd**	**dUrd**	
Thalamus [1]	0.76	1.22	0.11	0.25	1.08	1.06	
Hippocampus [2]	0.71	1.11	-	0.17	0.85	0.90	

Based on data published by: [1] [116]; [2] [189]; [3] [223]; [4] [112]; [5] [216]; Abbreviations: Ado: adenosine; Cyd: cytidine; dAdo: deoxyadenosine; dCyd: deoxycytidine; dUrd: deoxyuridine; Guo: guanosine; Hyp: hypoxanthine; Ino: inosine; Thd: thymidine; UA: uric acid; Ura: uracil; Urd: uridine; Xn: xanthine

culate body, the supraoptic nucleus, the vestibular nuclei, the cochlear nuclei and the cerebellar cortex Fig. (**3**) [41].

The lowest concentrations of Urd (15.7-31.2 pmol/mg) and/or Ino (29.8-60.3 pmol/mg), Guo (4.1-10.8 pmol/mg), and Ado (1.4-6.9 pmol/mg) were found in the entorhinal cortex, the hippocampus, the habenula, the zona incerta, the paraventricular nucleus, the substantia nigra, the inferior colliculus, the locus coeruleus, and the cerebellar nuclei. The

distribution of nucleoside metabolites (such as Hyp, Xn and Ura) was also uneven in the human brain [41].

There are only sporadic data on brain tissue levels of nucleosides and their metabolites in different experimental animals. Tissue concentrations of Ado in rat and guinea pig brain areas (cerebral cortex, hippocampus, striatum, hypothalamus, cerebellum, olfactory bulb, superior colliculus) suggests regional differences in nucleosides in animals also (Table **3**, Table **5**, Table **6**) [37-39].

The EC level of nucleosides is influenced by the delay in sample collection after probe implantation in microdialysis studies. Thus, when comparing EC levels of nucleosides measured by microdialysis in different brain areas and species, particular attention has to be paid to sample collection time. EC levels of other nucleosides and metabolites than Ado, Ino, Hyp and Xn were investigated only by some authors using standard microdialysis methods [116,189] in different brain areas. Reportedly, EC levels of nucleosides were not different in the rat thalamus and hippocampus except for Guo, dAdo and Thd (Table **4**) [116, 189]. Similar EC Ado concentrations, but three-fold higher Ino concentrations, were detected in the rat hippocampus by Chen *et al.* [223] than by others [189]. Hagberg *et al.* [112] measured two- to three-fold higher Ado and Ino levels in the rat striatum compared to rat thalamus and hippocampus (Table **4**) [116, 189]. Others measured similar Ado, but higher Guo and Ino levels, in the rat striatum than in the hippocampus and the thalamus [216].

The distribution of EC nucleoside levels in other brain areas has not yet been investigated systematically. All of these results suggest that EC level of nucleosides in different brain areas may be uneven.

Comparing different species, tissue levels of Ado in the brains of guinea pig, rat and human were different [39, 115]. Ado levels in guinea pig brain areas were about two- to five-fold higher than that in the rat brain (Table **5**). Concentrations of Ado, Ino and Urd were 5.1-, 1.6-, 2.1-times higher in the cortex of rats than in human, respectively, while levels of Guo were 1.9-times higher in the human cortex (Table **5**). Extracellular levels of Ado were higher in the rat brain areas (striatum and hippocampus) than those in the gerbil brain areas (Table **5**) [112, 189, 208], but Ado levels of rat and piglet thalamus were similar [116, 224]. All of these results demonstrated that nucleoside concentrations in different brain areas have area and species differences, which confirmed the hypothesis of discrete regional and species-dependent differences in nucleoside neuromodulation in the brain.

However, measuring more nucleosides other than Ado in most of the brain areas of different species, and using of the same methods during comparisons are all necessary to reveal real species-dependent concentrations and distributions of nucleosides in the CNS.

2.3. Distribution of Nucleoside Metabolic Enzymes in the CNS

The distribution of enzyme levels determines the velocity and efficiency of the catalyzed biochemical reactions. Although there could be other factors controlling enzymatic reactions, including separation of enzymes and substrates by lipid membranes, the spatial distribution of nucleoside metabolic enzymes reflects the intensity of the nucleoside metabolic network in a certain brain area. The distribution of 5'-nucleotidases (5'NTs), ADA and GDA activity has been described in detail. In addition, the regional distributions of ADK and PNP in the brain have also been measured [53-56].

The distribution of 5'NT activity in the brain shows species differences among rat, mouse and guinea pig (striatum, cerebral cortex and cerebellar cortex). Uneven distribution of 5'NT activity was revealed in rat and human brain homogenates. Enzyme activity was highest in the medulla oblongata, the thalamus and the hippocampus (2272-2779 nmol/h/mg protein) in rat brain. The dorsal part of the spinal cord shows the lowest enzyme activity (768 nmol/h/mg protein) [225]. In human brain, the highest activity was found in the medial temporal lobe (1123 nmol/h/mg protein). High 5'NT activity was measured in the superior colliculus, the lateral temporal lobe, the amygdala, the thalamus and the caudate nucleus (701-895 nmol/h/mg protein). In the human, enzyme activity was the lowest in the cerebellar cortex and in the white matter (centrum semiovale, 210-273 nmol/h/mg protein) [225].

The activity of ADA in the brain is also significantly different in different brain regions and among species. In rat, ADA activity was the highest in the hypothalamus (391 nmol/30 min/mg protein) and in the olfactory bulb (279 nmol/30 min/mg protein), while it was low in the hippocampus and in the striatum (32 and 37 nmol/30 min/mg protein) [226, 227]. In the guinea pig and rabbit, the highest ADA activity was measured in the cerebellum (273 nmol/30 min/mg protein) and in the superior colliculus (1252 nmol/30 min/mg protein) respectively [227]. Van der Weyden and Kelley [228] found three times higher ADA activity in the human cerebrum (27 nmol/min/mg protein) than in the cerebellum and spinal cord (9 nmol/min/mg protein). Norstrand *et al.* [229] reported 8-11 times higher ADA activities in the white matter of human temporal and frontal lobes (420-579 nmol of ammonia/min/g of wet tissue) than in the human spinal cord (cervical part: 50 nmol of ammonia/min/g of wet tissue). ADA activity in temporal and frontal white matter is 1.5-2 times higher than in the cortical gray matter. In the parietal and occipital lobes, the differences are small. Hippocampus and thalamus showed higher activity (207-270 nmol of ammonia/min/g of wet tissue) than the hypothalamus (157 nmol of ammonia/min/g of wet tissue), while medulla and spinal cord show the lowest ADA activity (16-67 nmol of ammonia/min/g of wet tissue). In contrast, others have published that the highest ADA activity is in the hypothalamus (350 nmol/min/g wet tissue), and there is lower activity in the frontal and temporal cortex (170-220 nmol/min/g wet tissue) [54]. It has been shown that the NBTI binding site-rich brain areas significantly correlated with the density of ADA immunoreactive neurons and fibers, which indicates increased sensitivity to Ado-induced effects [226,230]. The rather *ad hoc* and contradictory data, as well as missing information about ADA activity in specific brain nuclei do not allow us to localize any specific, ADA-dependent regulatory mechanisms or neuronal circuits in the human brain, yet.

The lowest GDA activity of all mammalian brain regions was measured in the cerebellum [53, 231, 232]. The highest

Table 5. Area- and Species-Dependent Distribution of Nucleosides in Brain Tissue (EC Levels were Measured 1-2 Hours After Probe Implantation)

Brain Areas	Species	Tissue Nucleoside and Base Concentrations						
		Ado	Ino	Hyp	Xn	Guo	Urd	Ura
Cerebellum[1] (nmol/mg protein)	Guinea pig	0.336						
	Rat	0.063						
Superior colliculus[1] (nmol/mg protein)	Guinea pig	0.102						
	Rat	0.053						
Cerebral cortex[1] (nmol/mg protein)	Guinea pig	0.123						
	Rat	0.068						
Hippocampus[1] (nmol/mg protein)	Guinea pig	0.149						
	Rat	0.058						
Cerebral cortex[2] (pmol/mg tissue)	Human	9.8	93.5	30.3	26.6	12.9	12.0	4.0
	Rat	50.0	148.7	199.7	50.5	6.7	25.1	25.9
Brain Areas	**Species**	**EC Nucleoside and Base Concentrations (μM)**						
		Ado	**Ino**	**Hyp**	**Xn**	**Guo**	**Urd**	**Ura**
Striatum	Rat [3]	1.92	1.50	2.82	3.95			
	Gerbil [4]	0.52						
Hippocampus	Rat [5]	0.95	0.42	4.79	0.40	0.26	0.71	1.11
	Rat [6]	0.80	0.66	1.16	3.60			
	Gerbil [4]	0.51						
Thalamus	Rat [7]	0.95	0.52	4.48	1.17	0.17	0.76	1.22
	Piglet [8]	1.03						

Based on data published by [1] [39]; [2] [115]; [3] [112]; [4] [208]; [5] [189]; [6] [219]; [7] [116]; [8] [224]. Abbreviations: Ado: adenosine; Guo: guanosine; Hyp: hypoxanthine; Ino: inosine; Ura: uracil; Urd: uridine; Xn: xanthine

Table 6. Age- and Gender Dependent Distribution of Nucleosides in the Brain

Brain Areas	Age (Gender)	Nucleoside and Base Concentrations						
		Ado	Ino	Hyp	Xn	Guo	Urd	Ura
RAT								
Cerebellum [1] (pmol/mg tissue)	7 weeks	50						
	36 weeks	144						
Hypothalamus [1] (pmol/mg tissue)	7 weeks	63						
	36 weeks	128						
Cortex [1] (pmol/mg tissue)	7 weeks	130						
	36 weeks	309						
Striatum [1] (pmol/mg tissue)	7 weeks	107						
	36 weeks	305						

(Table 6) contd….

Brain Areas	Age (Gender)	Nucleoside and Base Concentrations						
		Ado	Ino	Hyp	Xn	Guo	Urd	Ura
Hippocampus [2] (pmol/ml)	4 months	0.44						
	12 months	1.16						
	24 months	1.23						
Hippocampus [3] (pmol/mg protein)	6 weeks	51						
	24 months	55						
HUMAN								
Frontal cortex [4] (pmol/mg tissue)	38.6 ys. (male)	6.7	78.0	69.2	40.5	14.0	37.1	6.6
	80.8 ys. (male)	9.9*	108.4**	65.1	38.8	13.2	45.4	8.7*
	36.2 ys. (female)	2.8^{++}	96.0^{+}	65.2	34.5	21.5^{+}	48.7^{+}	7.2
	81.2 ys. (female)	$5.2^{**/++}$	118.8*	$55.7^{*/+}$	49.3*	21.6^{+}	43.9	7.0
White matter [4] (pmol/mg tissue)	40.4 ys. (male)	13.7	49.1	55.5	34.3	10.9	19.9	8.5
	78.4 ys. (male)	10.8	62.5**	68.2	39.4	10.3	31.3*	12.0
	40.8 ys. (female)	14.1	82.9^{++}	63.6	35.4	13.7^{+}	39.4^{++}	11.9
	81.2 ys. (female)	14.7	90.8^{+}	63.6	37.6	9.5	33.0	9.6

Based on data published by [1] [37]; [2] [264]; [3] [265]; [4] [63]. Abbreviations: Ado: adenosine; Guo: guanosine; Hyp: hypoxanthine; Ino: inosine; Ura: uracil; Urd: uridine; Xn: xanthine; ys.: years; Statistical significance between 38.6 ys. male cortex v. 80.8 ys. male cortex, 36.2 ys. female cortex v. 81.2 ys. female cortex, 40.4 ys. male white matter v. 78.4 ys. male white matter and 40.8 ys. female white matter v. 81.2 ys. female white matter were signed by * ($p<0.05$) or ** ($p<0.005$) while between 38.6 ys. male cortex v. 36.2 ys. female cortex, 80.8 ys. male cortex v. 81.2 ys. female cortex, 40.4 ys. male white matter v. 40.8 ys. female white matter and 78.4 ys. male white matter v. 81.2 ys. female white matter were labeled by $^{+}$ ($p<0.05$) or $^{++}$ ($p<0.005$).

GDA activities in mouse brain were measured in the cerebral cortex, the olfactory tubercle, the basal ganglia and the amygdala (4.02-5.25 nmol/min/kg dry tissue). GDA activity just reached the detectable levels (<0.15-0.17 nmol/min/kg dry tissue) in the cerebellum. GDA activity in the white matter was only 40-50% of that in the cortical gray matter [232]. In cat brain, the highest GDA activity was found in the parietal cortex (55 nmol/min/mg protein), while the olfactory tubercle shows lower enzyme activity (34 nmol/min/mg protein). The absence of GDA activity in cat vermis and lateral cerebellum resulted in lower concentrations of Xn in vermis than in the cerebral cortex [53]. In human cerebellum, the GDA concentration was also very low (<0.005-0.023 μmol/min/mg protein), but the highest level was in the thalamus (19.2 μmol/min/mg protein), which indicates interspecies differences in GDA distribution. Moderate GDA activity was measured in the basal ganglia and in the hippocampus of the human brain (6.6-7.8 μmol/min/mg protein) [53].

Activity of ADK in the human brain was high in the hypothalamus, the pons and the hind brain (17.3-19.4 nmol/min/g wet tissue), while it was the lowest in the frontal cortex (9.8 nmol/min/g wet tissue) [54].

We have only sporadic data on the distribution of PNP in the brain. Activities in the occipital cortex, the thalamus and the pons were the highest (247-261 μmol/min/g wet tissue), and PNP levels were lowest in the cerebellar cortex and temporal white matter (156 and 162 μmol/min/g wet tissue) in the human brain [233]. Moderate PNP activity was detected, for example, in the putamen.

Compared with other brain areas, the highest level of SAH was found in the striatum in the rat [234] suggesting that the distribution of SAHH activity also may be uneven in the brain.

It has been shown that regionally different activities of nucleoside metabolic enzymes result in uneven distributions of nucleosides and their metabolites in the human brain [41]. Low activity of ADA and high activity of 5'NT can cause high Ado and Ino levels, as is observed in the caudate nucleus and cochlear nuclei. Moderate PNP, low or moderate GDA, and moderate or high 5'NT activities result in high Guo concentrations, as in the basal ganglia. Differences in nucleoside levels in several species have been shown, and these differences are the result of differences in nucleoside metabolic enzyme activities. Therefore, one of the possible explanations of uneven distribution of nucleosides and their metabolites in the brain is the regionally-different glia/neuron ratio [235-240] because neuronal and glial nucleoside metabolism is markedly different [241,242]. However, we did not find strong correlations between neuron/glia ratio and nucleoside levels in the human brain areas [41].

In conclusion, the regional distribution of nucleosides in the brain may reflect the different production of nucleoside metabolism in these brain areas due to differences in the neuron/glia ratio, the metabolism of nucleosides in the glial cells

and neurons, and differences in the activity of the nucleoside metabolic enzymes [41, 54, 225, 233, 239, 241, 243-245]. However, more detailed studies are required for the better knowledge and understanding the functional significance of nucleoside metabolic enzyme activity and nucleoside levels in different brain areas and mechanisms.

2.4. The Distribution of Nucleoside Transporters in the CNS

The distribution of nucleoside transporters in the mammalian brain is uneven [42, 43, 46, 246]. The distributions of human and rat ENT1 transporters have been investigated in detail. In general, high levels of expression of ENT1 transporter protein were measured in the forebrain (cerebral cortex, thalamus and basal ganglia), the midbrain, and low levels (about four times less than in the cortex) in the lower brainstem (pons, medulla) in the human brain. In contrast, human ENT2 transporter is most abundant in the pons and the cerebellum, and 3-4 times less in the basal ganglia and the cerebral cortex [43]. In the rat brain, low ENT1 protein levels were found in the cerebellum and the hippocampus, but medium to high levels were shown in the thalamus and hypothalamus [246]. The distribution of the human ENT1 transporter in the brain correlates with the density of A_1 receptors [42, 43], which suggests a role of human ENT1 transporter-mediated transport in A_1 receptor-mediated neuromodulation [43]. In contrast, the distribution of the ENT2 transporter in the human brain does not correlate either with A_1 or A_{2A} receptor density [43]. ENT4 transporters are widely distributed in the brain [137, 138].

CNT1 transporters, CNT2 transporters and CNT3 transporters (subtypes of concentrative nucleoside transporters) are also widely distributed in the human brain. Among the different brain areas, relatively high expression of CNT1, CNT2 and CNT3 transporters was shown in the cerebral cortex, the cerebellum and the medulla oblongata respectively [44, 45]. In the rat brain, for example, the nucleus accumbens, and the dentate gyrus showed high levels of CNT2 transporter expression [46].

Our knowledge of nucleoside base transporters is still limited [132, 247]. However, functional data on nucleoside transporters in different brain regions suggest uneven distributions of nucleoside base transporters in the brain.

It is generally accepted that regional differences in the distribution of nucleoside transporters may reflect the specific functional significance of nucleosiderg neuromodulation in different brain regions. Further investigations are needed to understand this functional anatomical interrelationship.

2.5. The Distribution of Nucleoside Receptors in the CNS

The distribution of adenosine receptors in the CNS correlates with the physiological activity and importance of Ado in certain brain structures [35, 47, 48, 172]. Studies in rat, mouse, guinea pig and human brain, revealed that A_1 receptors are expressed at medium to high density in the cerebellar cortex, the cerebral cortex, the basal ganglia, the hippocampus and in some thalamic nuclei [42, 48, 248-252]. In human brain, high concentrations of A_1 receptors were measured in the frontal, visual, entorhinal and temporal cortices, in the cingulate gyrus and in the hippocampus (163-258 fmol/mg [^{3}H]cyclohexyl-adenosine/CHA binding) [42]. In the substantia nigra and the basal ganglia (caudate nucleus, putamen), the A_1 receptor density was from medium to high (84-101 fmol/mg CHA binding). In the thalamus, the ventral lateral nucleus, the mediodorsal nucleus and lateral dorsal nucleus exhibit high A_1 receptor density (102-144 fmol/mg CHA binding), but in the ventral posterior and ventral anterior nuclei, the A_1 receptor level is low (27 fmol/mg CHA binding). In the hypothalamus, there is a low A_1 receptor level in the mammillary body (55 fmol/mg CHA binding). The A_1 receptor density in the red nucleus, the superior colliculus, the inferior olive, the motor cortex and the nucleus of the solitary tract was low (36-53 fmol/mg CHA binding), but in the midbrain reticular formation it was high (101 fmol/mg CHA binding). The cerebellar A_1 receptor density is low to high depending on the layer (61-130 fmol/mg CHA binding). A medium density of A_1 receptor expression was shown in the spinal cord (central gray and cornu ventrale) [42]. The ischemia-sensitive hippocampus is one of the A_1 receptor rich regions [253], where the highest Ado release was found in response to electric stimulations [254] suggesting that hippocampal Ado neuromodulation could have significant functional importance. The purine uptake capacities, however, do not correlate with receptor density and release because the hippocampus has relatively low Ado uptake facility [42]. A_1 receptor density in the rat brain is similar to the density of this receptor in the human brain, but differences were shown in the cerebellar cortex [47]. The correlation between A_1 receptor density and 5'NTs is weak according to some authors [48] and high according to others [255].

The density of A_{2A} receptors in the human brain is high in the basal ganglia (nucleus caudatus, putamen, nucleus accumbens, globus pallidus). It is medium to low in several other brain areas such as cerebral cortex, thalamus, amygdala, hippocampus, substantia nigra, locus coeruleus and cerebellum [49, 50, 52]. The expression of A_{2A} receptors was similar in the brain of rodents [52, 248, 251, 256]. A_{2B} receptor and A_3 receptor density is generally low in the human and rat brain [143, 251, 257]. Our knowledge about the AdeR is limited, but it seems that this receptor type is widely distributed in the brain [158].

It has been suggested that Ado participates in several different physiological and pathophysiological processes, such as cognition, sleep, memory, pain, epilepsy, depression, schizophrenia, Alzheimer's, Parkinson's and Huntington's diseases, ischemia and drug abuse via its actions on A_1 receptors and A_{2A} receptors [1, 14, 73, 161, 163, 182, 199, 258, 259]. In general, Ado levels in different brain areas show correlations with the distribution of adenosine receptors [41, 42, 49]. For example, low or moderate Ado concentrations in the human cerebral cortex and hippocampus [41] well correlate with the A_1 receptor expression in these brain regions [1, 42, 47]. The caudate nucleus shows moderate Ado levels and high density of A_1 receptors and A_{2A} receptors in relation to modulatory function of Ado in the brain. Similarly, high Urd and/or Ino, Guo and Ado levels were measured in the caudate nucleus, the vestibular nuclei, the cochlear nuclei, the medial geniculate body and in the spinal trigeminal nucleus Fig. (**3**), suggesting the involvement of

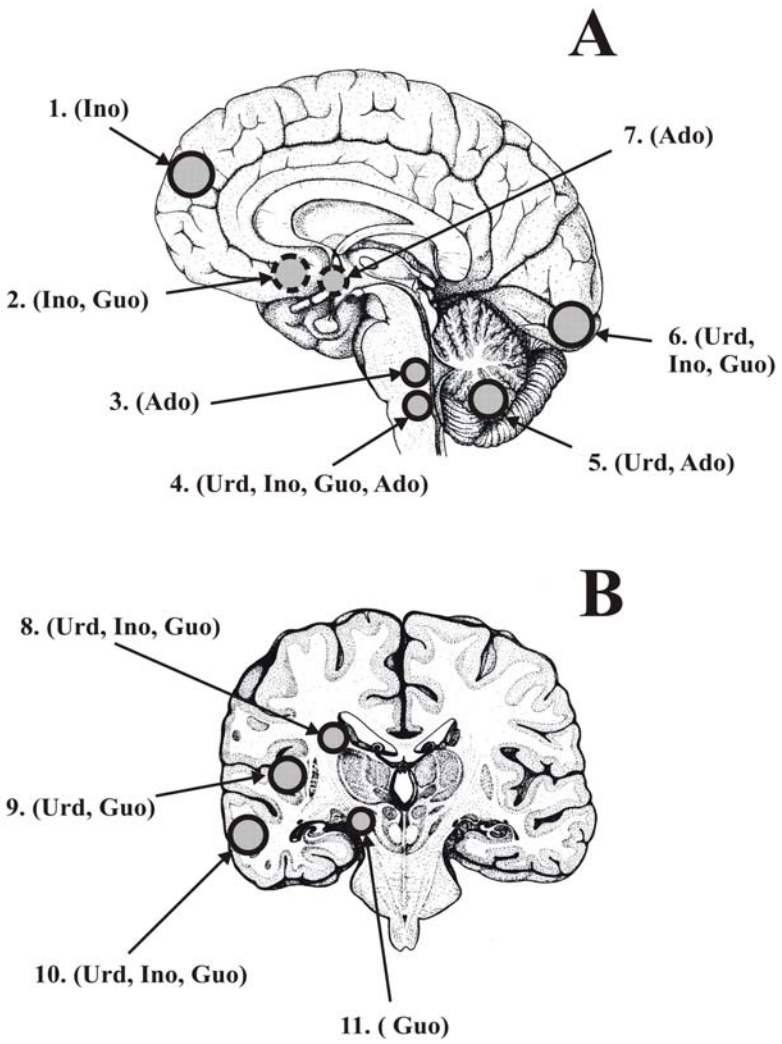

Fig. (3). Human brain areas containing the highest nucleoside levels
A mediansagittal section (Part A) and frontal section (Part B) of the human brain. Human brain areas with high nucleoside levels are labeled with gray oval circles. Abbreviations: 1: Frontal cortex; 2: Substantia innominata, nucleus basalis; 3: Vestibular nuclei; 4: Cochlear nuclei; 5: Cerebellar cortex; 6: Occipital cortex; 7: Supraoptic nucleus; 8: Caudate nucleus; 9: Insular cortex; 10: Temporal cortex; 11: Medial geniculate body; Ado: adenosine; Guo: guanosine; Ino: inosine; Urd: uridine; Based on data published by Kovács *et al.* [41]

nucleosides in the modulation of some of the sensory and motor information processing in the brain [41, 260]. All of these results suggest that not only Ado but also Urd, Ino and Guo might have a role in brain functions.

Experimental evidence supports a wide range of nucleoside functions in the brain. However, our knowledge about the metabolism of nucleosides and distribution of nucleoside transporters and nucleoside receptors in several brain areas under different circumstances is still incomplete. Thus, the determination of the physiological and pathophysiological functions of nucleosides in the brain requires the simultaneous measuring of (i) nucleoside levels, (ii) expression levels of nucleoside receptors and transporters and (iii) the activity of nucleotide/nucleoside metabolic enzymes in different brain areas. Experimental animals showed different nucleoside levels and nucleoside metabolism activities compared with human brain. In addition, differences were demonstrated in nucleoside transporter and nucleoside receptor expression. Therefore, we think that making of functional anatomical nucleoside brain maps in experimental animals, particularly in the case of model animals of different human CNS diseases, is important (i) to reveal of the functions of nucleosides in the brain areas and (ii) to develop novel, safe and effective drugs to treat CNS diseases such as epilepsy, depression, schizophrenia, Alzheimer's and Parkinson's diseases.

3. AGE DEPENDENCE OF THE NUCLEOSIDE SYSTEM

3.1. Age-Dependent Differences in Levels of Nucleosides in the Brain

The brain tissue concentrations of nucleosides show age-dependent alterations. Ado concentration in all investigated brain areas was 2-3 times higher in adult (36 weeks) rats than in juvenile (7 weeks) ones (Table **6**) [37], suggesting age-dependent changes in nucleoside metabolism [59, 90, 102, 261-264]. However, hippocampal Ado levels in 6-week and 24-month-old rats were only slightly different [265]. It has also been shown that the levels of Ino and Ado in the frontal cortex and Ino and Urd in the white matter are age-dependent in the human brain (Table **6**) [63].

3.2. Age-Dependent Changes in Nucleoside Metabolic Enzyme Activity, Distribution of Nucleoside Transporters and Receptor Distribution

The alteration of nucleoside concentrations by age [40, 59, 63, 263, 266] could reflect changes in the metabolic enzyme activity because some of the nucleoside metabolism enzymes are age-dependent. Age dependence of RNR, 5'NTs, ADA, TS, ADK, HGPRT, APRT, uridine phosphorylase (UP) and UCK have been demonstrated [56, 59, 90,102, 242, 261-263, 267, 268]. Activity of ADA decreases with age, e.g., in the superior colliculus, the cortex, the hippocampus and in the cerebellum in the rat brain [262]. However, ADA activity increases in the hypothalamus with age, indicating that hypothalamic salvage of purines has increasing importance [262]. RNR activity was decreased with age in the rat cortex and the cerebellum [102].

It has been shown that the activity of 5'NTs increases with age [59], specifically in sleep regulatory areas [56] and hippocampus [263]. Regional differences in age-dependent increases in 5'NTs might reflect the changes in glia-neuron distribution and the differential changes of 5'NT activity in glia cells and neurons during development [269]. HGPRT and APRT activity increased with age [242, 267], which suggests the importance of salvage purine metabolism in brain development. Therefore, the elevation of 5'NT activity and purine nucleoside salvage intensity as well as the decrease in ADA activity with age could increase the Ado and Ino concentrations and decrease Hyp level, as we measured in elderly people [63].

The effect of age on nucleoside transporter distribution is unknown, but it has been shown that adenosine receptor distribution in the CNS changes with age [57, 62, 270]. It was observed that adenosine receptor density [230] and Ado levels [37] increase during aging in correlation with a decrease in ADA activity. Age-related decreases in A_1 receptor levels were detected in several brain areas of different species, such as cortex and hippocampus [62,264,271-274]. Levels of A_{2A} receptors increase in the cerebral cortex and the hippocampus with age, which could lead to an imbalance between inhibitory A_1 receptor and excitatory A_{2A} receptor mediated processes [27, 270, 271, 275] and could shift the excitatory/inhibitory balance in brain cells and enhance excitation in elderly people. In addition, the number of neurons and the neuron/glia ratio decrease with age [276], which can also contribute to the increased Ado concentrations in cerebral cortex in elderly people. In spite of the fact that Ado is considered to be an inhibitory neuromodulator, an elevated Ado level increases the risk of excitotoxicity and the sensitivity to excitatory influences in aging brain [1, 63, 180].

The age-dependence of nucleoside metabolism, receptor density distribution, neuron/glia ratio and Ado concentration suggest that nucleosides are important factors in functional changes in the aging brain, such as declines in learning and memory and the changes in the sleep-wakefulness cycle. Increases in neuroprotective Ino and Urd levels with age in the human brain cortex and white matter [63] could compensate for the cytotoxic effect of increased concentrations of Ado to some extent [24, 277-279]. It has been shown that a nucleotide-nucleoside mixture (Ino, GMP, Cyd, Urd and Thd) as a food additive reduces ageing and memory problems [199, 280], suggesting that endogenous nucleoside metabolism might contribute to senile dementia and depression [197,280].

4. GENDER DEPENDENCE OF THE NUCLEOSIDE SYSTEM

4.1. Gender-Dependent Differences in Levels of Nucleosides and Their Metabolites in the Brain

Gender differences in human brain nucleoside levels were shown in cortex and white matter samples of female and male brains (Table **6**) [63]. Guo level was higher while the concentration of Ado was lower in cortices of old women (see Table **6**) compared to old men. Levels of Urd, Guo and Ino were higher but Ado concentration was lower in cortical samples of middle-aged women (see Table **6**) than men. The level of Ino was higher in white matter samples from middle-aged women and white matter samples from old women while level of Urd and Guo was higher only in white matter samples from middle-aged women.

4.2. Gender-Dependent Changes in Nucleoside Metabolic Enzyme Activity, Distribution of Nucleoside Transporters and Receptor Distribution

5'-ecto-nucleotidase activity has gender differences in the brain of rats [61, 281] and the neuron/glia ratio shows also gender differences [282]. Our knowledge about the gender dependence of nucleoside transporter and receptor distribution in the brain is very limited. The mRNA level of the ENT1 is significantly higher in male than female rat brains [60]. No significant gender differences in A_1 receptor distribution were found in the rat and human brain [274, 283]. In fact, several brain functions, in which the nucleoside neuromodulation is involved, change with age and gender [284-288]. The structure and synaptic morphology of some brain areas and brain hypoxic tolerance have gender differences [289, 290]. The different nucleoside levels possibly protect the female brain against the increased excitatory action of enhanced A_{2A} receptor density [63].

It has to be noted that our knowledge of the gender-dependence of nucleoside system is very limited. These results suggest that nucleosides may serve as an important factor in gender-dependent processes in the brain. However, to reveal the role of nucleosides in the brain areas of different

genders, further comparative investigations are needed on (i) nucleoside levels, (ii) the activity of nucleoside metabolic enzymes, (iii) the expression levels of nucleoside transporters and nucleoside receptors in the several brain areas of men and women and in animals.

5. CONCLUSIONS

A great deal of evidence suggests that the levels of nucleosides in the brain are area-, age- and gender-dependent, reflecting the involvement of nucleosides in functional changes in different brain areas. The sophisticated functional roles of nucleosides are supported by the regionally-different distribution of nucleoside transporters and receptors and nucleoside metabolic enzymes in the CNS. The changes in nucleoside levels in some of the brain areas may be involved in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease or senile dementia. However, there are insufficient data about nucleoside functions in the brain, so additional investigations are needed to discover the functions of nucleosides in particular brain areas, such as the cochlear nuclei. Further functional studies on non-adenosine nucleosides in the CNS are also required. Our knowledge of the effect of gender and age differences on brain nucleoside content is very limited so studies on concentrations of nucleosides and expression levels of nucleoside transporters and nucleoside receptors in several brain areas in relation to age and gender should be continued.

Some of the ADK inhibitors, adenosine uptake inhibitors and nucleoside derivatives are used in treatment of CNS diseases in spite of their side effects. In addition, nucleoside transporters and nucleoside receptors are widely distributed in several organs, and the transport and metabolism of nucleoside derivative drugs are modulated by age and gender. Therefore, the available data suggest that nucleoside system is a promising drug target for developing gender- and age-specific treatments for CNS diseases.

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ABBREVIATIONS

5'NT	=	5'-nucleotidases
A_1 receptor	=	A_1 subtype of adenosine receptors
A_{2A} receptor	=	A_{2A} subtype of adenosine receptors
A_{2B} receptor	=	A_{2B} subtype of adenosine receptors
A_3 receptor	=	A_3 subtype of adenosine receptors
A_4 receptor	=	A_4 subtype of adenosine receptors
ADA	=	Adenosine deaminase
Ade	=	Adenine
AdeR	=	Adenine receptor
ADK	=	Adenosine kinase
Ado	=	Adenosine
AMP	=	Adenosine monophosphate
AMPDA	=	AMP deaminase
APRT	=	Adenine phosphoribosyltransferase
ATP	=	Adenosine triphosphate
CHA	=	[^{3}H]cyclohexyl-adenosine
cN	=	Cytoplasmic 5'-nucleotidases
CNS	=	Central nervous system
CNT transporters	=	Concentrative nucleoside transporters
CNT1/CNT2/ CNT3 transporters	=	CNT1/CNT2/CNT3 subtype of concentrative nucleoside transporters
CSF	=	Cerebrospinal fluid
Cyd	=	Cytidine
Cyt	=	Cytosine
dAdo	=	Deoxyadenosine
dCyd	=	Deoxycytidine
dUrd	=	Deoxyuridine
EC	=	Extracellular
"ei" transporters	=	Equilibrative, NBTI insensitive type of ENTs
ENT transporters	=	Equilibrative nucleoside transporters
ENT1/ENT2/ENT3/ ENT4 transporters	=	ENT1/ENT2/ENT3/ENT4 subtype of equilibrative nucleoside transporters
"es" transporters	=	Equilibrative, NBTI sensitive type of ENTs
GDA	=	Guanine deaminase
GMP	=	Guanosine monophosphate
Gn	=	Guanine
Guo	=	Guanosine
HGPRT	=	Hypoxanthine phosphoribosyltransferase (hypoxanthine-guanine phosphoribosyltransferase)
Hyp	=	Hypoxanthine
IC	=	Intracellular
IMP	=	Inosine monophosphate
Ino	=	Inosine
MTA	=	5'-deoxy-5'-methylthioadenosine
MTAP	=	5'-deoxy-5'-methylthioadenosine phosphorylase
NBTI	=	S-(4-nitrobenzyl)-6-thioinosine

PNP = Purine nucleoside phosphorylase

RNR = Ribonucleotide reductase

SAH = S-adenosylhomocysteine

SAHH = Adenosylhomocysteinase (S-adenosylhomocysteine hydrolase)

Thd = Thymidine

Thy = Thymine

TS = Thymidylate synthetase

UA = Uric acid

UCK = Uridine-cytidine kinase

Ura = Uracil

Urd = Uridine

Xn = Xanthine

XO = Xanthine oxidase

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