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5'-Nucleotidases, Nucleosides and their Distribution in the Brain: Pathological and Therapeutic Implications

Zsolt Kovács[1,*], Árpád Dobolyi[2], Katalin A. Kékesi[3,4] and Gábor Juhász[3]

[1]*Department of Zoology, University of West Hungary, Savaria Campus, Szombathely, Hungary;* [2]*Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University and the Hungarian Academy of Sciences, Budapest, Hungary;* [3]*Laboratory of Proteomics, Institute of Biology, Eötvös Loránd University, Budapest, Hungary;* [4] *Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary*

Abstract: Elements of the nucleoside system (nucleoside levels, 5'-nucleotidases (5'NTs) and other nucleoside metabolic enzymes, nucleoside transporters and nucleoside receptors) are unevenly distributed in the brain, suggesting that nucleosides have region-specific functions in the human brain. Indeed, adenosine (Ado) and non-Ado nucleosides, such as guanosine (Guo), inosine (Ino) and uridine (Urd), modulate both physiological and pathophysiological processes in the brain, such as sleep, pain, memory, depression, schizophrenia, epilepsy, Huntington's disease, Alzheimer's disease and Parkinson's disease. Interactions have been demonstrated in the nucleoside system between nucleoside levels and the activities of nucleoside metabolic enzymes, nucleoside transporters and Ado receptors in the human brain. Alterations in the nucleoside system may induce pathological changes, resulting in central nervous system (CNS) diseases. Moreover, several CNS diseases such as epilepsy may be treated by modulation of the nucleoside system, which is best achieved by modulating 5'NTs, as 5'NTs exhibit numerous functions in the CNS, including intracellular and extracellular formation of nucleosides, termination of nucleoside triphosphate signaling, cell adhesion, synaptogenesis and cell proliferation. Thus, modulation of 5'NT activity may be a promising new therapeutic tool for treating several CNS diseases. The present article describes the regionally different activities of the nucleoside system, demonstrates the associations between these activities and 5'NT activity and discusses the therapeutic implications of these associations.

Keywords: 5'-nucleotidases, human brain diseases, nucleoside system regionality, therapy.

1. INTRODUCTION

Adenosine and non-Ado nucleosides, such as Guo, Ino and Urd, regulate neuronal and glial functions in the brain [1-6] and participate in the modulation of different physiological (e.g., sleep and memory) and pathophysiological (e.g., epilepsy, Parkinson's disease and Alzheimer's disease) processes in the brain [2, 3, 7-13]. Thus, nucleoside derivatives, nucleoside uptake inhibitors and inhibitors of nucleoside metabolic enzymes have been used in drug development against various diseases, such as neurodegenerative disorders [9, 11, 14, 15].

Elements of the nucleoside system are unevenly distributed in the brain and are dependent on age and gender [11, 16-22]. Interactions have been demonstrated in the nucleoside system, such as the interaction of local nucleoside levels with the activities of nucleoside metabolic enzymes and the distribution of Ado receptors in the human brain [20] as well as the interaction of A_1 Ado receptor (A_1 receptor) density with equilibrative nucleoside transporter (ENT; ENT1 subtype) density [23] and 5'NT levels [24, 25]. Alterations in the nucleoside system including the level, distribution and/or activity of nucleosides, nucleoside metabolic enzymes and nucleoside transporters as well as Ado receptors may induce pathological changes, resulting in CNS diseases such as epileptic seizures, schizophrenia, sleep disorders, among others [26-40]. Indeed, several CNS diseases can be treated by modulation of the nucleoside system [9, 11, 40-52], suggesting that (i) a close correlation exists between regional differences in elements of the nucleoside system and their (patho)physiological functions; (ii) nucleosides have regionally different roles in the brain, which may be modulated by age and gender; (iii) changes in the 'purinome' (in which receptors, transporters, metabolic enzymes and ligands of nucleosides and nucleotides together generate purinergic signaling) [53] may evoke pathological consequences, resulting in different diseases in the CNS; and (iv) the nucleoside system may be a promising drug target to treat several CNS diseases [9-12, 54, 55].

5'-nucleotidases play a role in the intracellular and extracellular formation of nucleosides from their nucleoside monophosphates (NMPs) [56-59], cell adhesion, cell proliferation and synaptogenesis [60-68], as well as regeneration and neuronal development [69, 70]. Recently, it was demonstrated that 5'NTs may be promising drug targets against several CNS diseases such as epilepsy [68, 71-80].

In this review, we briefly discuss (i) 5'NTs in the CNS; (ii) nucleoside metabolism (with an emphasis on Ado, Ino, Guo and Urd), nucleoside transporters and nucleoside recep-

*Address correspondence to this author at the Department of Zoology, University of West Hungary, Savaria Campus, Szombathely, Károlyi Gáspár tér 4., 9700 Hungary; Tel: 0036 94/504 409; Fax: 0036 94/504 404;
E-mail: zskovacs@ttk.nyme.hu

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tors and their area-, age- and gender-dependence in the human brain; and (iii) the potential of the nucleoside system modulation in the development of pharmacological therapies with an emphasis on the relationships between 5'NTs and nucleoside levels, nucleoside transporters and nucleoside (Ado) receptors.

2. 5'-NUCLEOTIDASES

5'-nucleotidases (5'-ribonucleotide phosphohydrolases, EC 3.1.3.5) catalyze the conversion of (deoxy)nucleoside monophosphates into (deoxy)nucleosides and inorganic phosphates via hydrolysis of a phosphoric ester bond [81, 82]. Intracellularly and extracellularly, 5'NTs, which are integrant components of the endo- and ectonucleotidase enzyme cascade, catalyze the final step of nucleotide dephosphorylation (from NMPs to its corresponding nucleosides) [56-58, 83-86]. This process contributes to the maintenance of nucleoside/nucleotide levels and the balance necessary to ensure their physiological functions in the brain and modulates the nucleoside effects on brain tissue cells via nucleoside levels, nucleoside transporters and their receptors [82, 87]. In addition, the synaptic ectonucleotidase cascade system plays a role in the rapid termination of the activities of nucleoside di- and triphosphates on their receptors and in nucleoside recycling in the brain. Processes of nucleoside recycling consist of (i) intracellular synthesis of nucleoside triphosphates from nucleosides; (ii) release of nucleoside triphosphates into the extracellular space; (iii) extracellular degradation of nucleoside triphosphates into their corresponding nucleosides, which may exert their effects via nucleoside receptors (such as Ado receptors); and (iv) uptake of nucleosides into the cells, which contributes to the termination of nucleoside signaling via their receptors and the prompt re-utilization (salvage) of nucleosides. Consequently, intracellularly and extracellularly localized enzymatic chain reactions involving 5'NTs catalyzed reactions form a crosstalk between intracellular and extracellular nucleoside metabolism in the brain [56-59, 88-90].

Seven types of 5'NTs have been cloned, characterized [81, 91] and distinguished in cell extracts/micropunches using nucleotidase assays [92-94]. 5'-nucleotidases have been demonstrated in various tissues including brain tissue [81, 91, 95-99]. Five enzymes are cytoplasmic (cytosolic/soluble/cNs: cN-IA, cN-IB, cN-II, cN-III and cdN), one enzyme is mitochondrial (mdN) and one enzyme (e5'NT) is located on the outer side of the plasma membrane [81]. In this review article, we briefly discuss the main general features and role of 5'NTs. As (i) network of 5'NTs is complex (see the text below) and (ii) it is not clear whether results (derived from e.g., enzyme histochemistry) refer to only e5'NT, cNs or e5'NT plus cNs and so forth, it is sometimes impossible to ascribe an AMP hydrolyzing activity to a particular kind of 5'NT. Thus, when referring to generic 5'NTs in the text (and in the Table (**1**) without any further specification) we do not identify any specific 5'NT.

2.1. General Properties and Functions of 5'NTs

The ecto-5'-nucleotidase (aliases: e5'NT, CD73, ecto-5'-NT, eNT, NT5 and low K_m-5'-NT) gene is located on chromosome 6q14-q21 [81, 100]. e5'NT is ubiquitously expressed and present on both neurons and glial cells. It contains two glycoprotein subunits (the molecular mass of the subunit is 60-80 kDa) and is anchored to the plasma membrane by a glycosylphosphatidyl-inositol molecule [81, 82, 101]. Furthermore, the existence of its soluble extracellular form has also been demonstrated [82, 86, 101, 102]. The substrate specificity of e5'NT is broad, as it can hydrolyze both ribo- and deoxyribonucleoside monophosphates. However, e5'NT exhibits the highest affinity for Ado monophosphate (AMP) (low K_m-nucleotidase: K_m for AMP is 1-50 μM) [82, 86, 103-106]. Its optimal pH is approximately 6.8-9.0 depending on the type of tissue [81, 82]. Both Mg^{2+} and Mn^{2+} increase the activity of e5'NT [82, 103, 105], whereas Ado diphosphate (ADP) and Ado triphosphate (ATP) (and to a lesser extent Urd diphosphate/UDP, Urd triphosphate/UTP, Guo diphosphate/GDP, Guo triphosphate/GTP, cytidine (Cyd) diphosphate/CDP, Cyd triphosphate/CTP, concanavalin A and α,β-methyleneadenosine-5'-diphosphate (APCP)) inhibit e5'NT activity [82, 103, 105, 107]. Ecto-5'NT plays a role in (i) the extracellular generation of nucleosides [56-58, 86] and, as a consequence, nucleoside (e.g., Ado) receptor activation, neurotransmission modulation, and the regulation of learning and memory, sleep, psychomotor coordination, cardiac and renal function, platelet aggregation, nociception and so forth [7, 12, 82-84, 101, 108-116]; (ii) the termination of nucleoside di- and triphosphates activity on their receptors [63, 82, 117, 118]; (iii) the uptake of extracellular nucleosides [56-58]; (iv) T-cell activation (as a coreceptor) [119-121]; (v) cell-cell adhesion and cell proliferation [60, 62, 66, 121, 122]; (vi) synaptic malleability/arrangements and information processing, neuroglial/cell-matrix interactions and synaptogenesis [61, 63-68, 82]; (vii) neuronal regeneration and development [67, 70, 82, 86, 101]; (viii) the regulation of glial cell volume [123]; and (ix) epithelial ion and fluid transport, adaptation to hypoxia, ischemic preconditioning, vasodilatation and inflammation [124-127].

The cytosolic 5'-nucleotidase IA (cN-IA, cN-I, AMP-specific 5'NT) and cytosolic 5'-nucleotidase IB (cN-IB, AIRP, cN-IA homologue: high homology between cN-IA and cN-IB was demonstrated) genes are located on chromosome 1p33-p34.3 and 2p24.3, respectively [96, 97]. Both cN-IA and cN-IB are ubiquitously expressed in human tissues [96, 97], and their optimum pH is between 6.5 and 7.0 [81, 82]. Cytosolic 5'-nucleotidase IA exhibits a high affinity for (deoxy)nucleoside monophosphates, but its preferred substrate is AMP (K_m 1.2-8.3 mM; cytosolic AMP specific 5'-nucleotidase, form A) [81, 82, 96, 128, 129]. This enzyme demonstrates a tetrameric structure (subunit molecular mass is 40-43 kDa) [81, 82, 129] and is activated by ADP and GTP [96, 128, 129]. Moreover, cN-IA is Mg^{2+} dependent [96, 129], does not show phosphotransferase activity [130], and may be inhibited by 5-ethynyl-2',3'-dideoxyuridine [131]. This enzyme plays a role in the increase of Ado levels under ischemic and hypoxic conditions [132] and controls (together with cN-II) the intracellular level/pools of NMPs and nucleosides [57-59, 81, 96, 133]. Cytosolic 5'-nucleotidase IB catalyzes the hydrolysis of AMP (cytosolic AMP specific 5'-nucleotidase, form B), and it may be activated via ADP [97]. Furthermore, 5-ethynyl-2',3'-dideoxyuridine may inhibit not only cN-IA but also cN-IB [81, 131].

Cytosolic 5'-nucleotidase II (cN-II, Ino monophosphate/IMP-Guo monophosphate/GMP specific NT, purine 5'NT, high K_m 5'NT, IMPase), which is localized on chromosome 10q24.32 [81, 134], is ubiquitously expressed in human tissues [81, 82, 135, 136] and has a tetrameric structure (the molecular mass of the subunit is 52-70 kDa) [82, 137-139]. Cytosolic 5'-nucleotidase II is Mg^{2+} dependent [139] and prefers 6-hydroxypurine (deoxy)nucleoside monophosphates, such as IMP and GMP (IMP-GMP specific 5'-nucleotidase; K_m for IMP is 0.1-0.6 mM) [82, 134, 139]. The optimal pH of cN-II is 6.0-7.0 [81, 82]. The activity of cN-II may be enhanced by (deoxy)ATP, GTP and ADP [82, 139-141]. Cytosolic 5'-nucleotidase II has a phosphotransferase activity from 6-hydroxypurine monophosphates (phosphate donors) to phosphate acceptors, such as Guo, (deoxy)Ino and nucleoside analogs, to form monophosphate derivatives [85, 133, 140-143], and plays a role in (i) purine nucleotide interconversion, (ii) the maintenance/modulation of intracellular 5-phosphoribosyl-1-pyrophosphate (PRPP) and purine nucleotide pools and (iii) the survival of cultured astrocytoma cells [81, 133, 144].

The cytosolic 5'-nucleotidase III (cN-III, P5'-NT, P5'N-1, PN-I, UMPH, UMPH-I) gene is localized on chromosome 7p14.3 [81, 98, 143]. Cytosolic 5'-nucleotidase III is widely expressed in mouse, rat and human tissues [98, 145, 146], is Mg^{2+} dependent [147], functions as a monomer (the molecular mass is 34 kDa) [98, 148], and hydrolyzes pyrimidine monophosphates [147]. However, cN-III shows the highest affinity for Cyd monophosphate (CMP) (K_m 10-150 μM) [81, 91, 147]. The optimal pH for cN-III is 7.5, and the activity of cN-III may be inhibited by nucleosides (e.g., Urd), phosphates and heavy metals [147]. Similar to cN-II, cN-III demonstrates phosphotransferase activity, where it transfers a phosphate from pyrimidine monophosphates (phosphate donors) to Urd, Cyd and deoxycytidine (dCyd) (phosphate acceptors) [147]. It has been shown that cN-III is involved in the degradation of RNA during erythrocyte maturation [147, 148] and the catabolism of pyrimidine (deoxy)nucleosides [81, 148]. Genetic mutations (deficiencies) of cN-III in reticulocytes cause hemolytic anemia [147, 148].

Cytosolic 5'(3')-deoxyribonucleotidase (cdN, PN-II, dNT-1, UMPH-2) is widely distributed in human, mouse and rat tissues [99, 145, 149-151]. Its gene has been localized on chromosome 17q25 [151]. Cytosolic 5'(3')-deoxyribonucleotidase exhibits a dimeric structure (the molecular mass of the subunit is approximately 23 kDa) [148, 150], is Mg^{2+} dependent [150], and prefers 2'- and 3'-monophosphates containing pyrimidine basis uracil (Ura) or thymine (Thy), such as 3'-deoxyuridine monophosphate (K_m for 2'- and 3'-monophosphates is approximately 0.3 mM) [81, 150]. However, lower nucleotidase activities of cdN have also been demonstrated for 5'-deoxynucleotides (e.g., 5'-deoxyuridine monophosphate, 5'-dUMP) and 5'-ribonucleoside monophosphates (e.g., UMP) [92, 150]. Its optimal pH is between 5.5 and 7.5 [81, 149, 150]. Phosphate, deoxyinosine (dIno) and deoxyuridine (dUrd) may inhibit cdN activity, whereas deoxyguanosine monophosphate (dGMP) and deoxythymidine monophosphate (dTMP) may increase cdN activity depending on different substrate molecules [81, 150]. Human erythrocyte cdN exhibits phosphotransferase activity [91, 152]. Both PMcH-U ((±)-1-trans-(2-phosphonomethoxycyclohexyl)uracil) and PMcP-U ((±)-1-trans-(2-phosphonomethoxycyclopentyl) uracil) inhibit cdN activity [92, 153]. The physiological function of cdN may be involved in the regulation of pyrimidine (deoxy)nucleotide levels [148, 154] as well as nucleotide recycling in dying cells [81, 155].

Genes of mitochondrial 5'(3')-deoxyribonucleotidase (mdN, dNT-2) are located on chromosome 17p11.2 [99, 151]. The expression of mdN has been demonstrated in mouse, rat and human tissues [99, 151, 156, 157]. It has a dimeric structure (the molecular mass of the subunit is 26 kDa) [99, 151, 157] and prefers pyrimidine monophosphates with Ura or Thy bases, such as 5'-dUMP (K_m 0.1 mM) [81, 153, 157]. Mitochondrial 5'(3')-deoxyribonucleotidase is Mg^{2+} dependent [157] and its pH optimum may be 5.0-5.5 [99, 153]. BPE-T (1-[2-deoxy-3,5,-O-(2-bromo-1-phosphono) ethylidene-β-D-threo-pentofuranosyl]thymine), PMcH-U, PMcP-U and DPB-T ((S)-1-[2'-deoxy-3',5'-O-(1-phosphono)benzylidene-β-D-threo-pentofuranosyl] thymine) inhibit mdN activity [92, 153]. In addition, mdN may be protective against excessive deoxythymidine triphosphate (dTTP) accumulation, which induces a mutagenic effect in mitochondrial DNA replication [91, 157].

Anti-viral and anti-cancer nucleoside analogs (i.e., prodrugs) may be transported to the cells via nucleoside transporters and activated (as a triphosphate) via phosphorylation by kinases [81, 153, 158-160]. Triphosphorylated nucleoside analogs exert their therapeutic effects mainly by termination of DNA chains via inhibition of DNA polymerases and incorporation into DNA. Thus, increased activity of 5'NTs (mainly cNs and indirectly, e5'NT) [81, 158, 159] may inhibit the activation of nucleoside analogs by dephosphorylation, which results in drug resistance. Dephosphorylated nucleoside analogs are not able to terminate DNA chains and may be transported into the extracellular space from the cells [81, 159]. The involvement of cN-I and cN-II in drug resistance has been previously demonstrated. For example, the phosphorylated form of the anti-viral nucleoside analogue 3'-azido-2',3'-dideoxythymidine (AZT) is the substrate of these enzymes [81, 96, 147, 153]. Thus, development of new 5'NT inhibitors and nucleoside analogs, which are weakly metabolized by 5'NTs, may improve the therapeutic efficacy of some anti-viral and anti-cancer nucleoside analogs by decreasing resistance [81, 91]. Nevertheless, cN-II may also have a role in drug activation (e.g., the anti-viral nucleoside analog 2',3'-dideoxyinosine, ddI and AZT) via its phosphotransferase activity [81, 140, 161]. In addition, cN-III and cdN may be involved in nucleoside analog resistance (cN-III and cdN) and activation (cN-III), and mdN may reduce the toxic side effects of activated (phosphorylated) nucleoside analogs in mitochondria [81, 147].

2.2. Structure-Activity Relationships

Considerable substrate specificity characterizes several 5′NTs. The preferred substrate is AMP for cN-I, IMP for cN-II, and pyrimidine nucleotides for cN-III. The phosphate moiety is present in all substrates and is important for its binding to the enzymes. Hydrolysis was dramatically reduced when modifications were introduced into the phosphate group. However, if the structure around the phosphor atom is not changed, but the oxygen connecting it with the

ribose moiety is converted into a carbon, the resulting non-hydrolyzable phosphates represent promising antagonists of 5′NTs [162]. Consequently, the sugar moiety appears to be less important for substrate binding since its modifications affect the rate of hydrolysis to a lesser degree. Indeed, deoxynucleotides are also hydrolyzed by 5′NTs [163], albeit with a much lower activity for e5'NT. The substrate specificity of 5′NTs is based on its recognition of the nucleobase. Not surprisingly, hydrogen-bond formation with the substituent in the 6-position of the purine ring and the hydrophobic attractions play major roles in the substrate specificity of 5′NTs [164]. The electron pair on N-1 is important in substrate binding of cN-II but is not a prerequisite for cN-I. However, hydrogen bonding with N-7 is not essential to substrate binding for either cN-I or cN-II [164] compared to Ado deaminase (ADA) [165]. Recent crystallographic approaches have provided a more detailed description of structure-activity relationships for 5′NTs. The structures of four of the seven human 5′NTs, cN-II, cN-III, mdN and e5'NT, have been previously described. Intracellular 5′NTs share three conserved motifs that have been identified in members of the haloacid dehalogenase superfamily of enzymes, suggesting divergent evolution from a common progenitor. The three motifs constitute the catalytic phosphate-binding site in these enzymes [166]. The first Asp in Motif I (DXDX[T/V]L) induces a nucleophilic attack on the phosphate of the NMP, and the second Asp donates a proton to the remaining nucleoside. This mechanism is thought to involve a stabilized pentacovalent phosphorane and a phosphoenzyme intermediate [167, 168], suggesting phosphotransferase activity, which has been demonstrated in cN-II and cN-III [137]. In cN-III and mdN the active site is located in a cleft between two different domains [157], in contrast, cN-II is a homotetrameric protein consisting of two identical dimers. The smallest active oligomerization state of the protein is dimer [137]. Unlike other 5′NTs, cN-II is allosterically activated by adenine/guanine nucleotides, which couple its activity to the metabolic state of the cell. This enzyme is also activated by millimolar concentrations of NaCl, KCl and LiCl, whereas inorganic phosphate demonstrated the opposite effect [137]. Activation of cN-II involved the transition of a catalytically essential Asp356. The substrate specificity of cN-II was determined by Arg202, Asp206 and Phe157 [169]. For cN-III, the structure and sequence analysis coupled with enzymatic characterization of several mutants revealed how cN-III achieved specificity for pyrimidine 5'NTs: the aromatic ring was stabilized by parallel pi-stacking interactions with Trp113 and His68 and by T-shaped stacking with Tyr114, as well as by polar contacts with side chains of Thr66 and Ser117. Two water molecules helped to stabilize the nucleotide binding by bridging it to protein residues Asp72 and His68 via hydrogen bonds [170].

Interestingly, the eukaryotic e5'NT but not the cytosolic 5′NT is structurally related to bacterial 5'NT enzymes in terms of sequence similarity. Monomeric bacterial 5'NT enzymes consist of distinct N-terminal metal binding and C-terminal substrate-binding domains, which together form the active site [171, 172]. The N- and C-terminal domains undergo extensive domain rotations relative to each other and thereby switch between the open and closed structural conformations [173]. Ecto-5'-nucleotidase is a dimeric extracellular glycoprotein with similar open and closed conformations to bacterial enzymes [121]. A crystal structure of the closed and open formations of human e'5NT have been obtained [174, 175]. Structural control of the domain movement may be responsible for the selectivity of monophosphate nucleotides [176].

3. REGIONAL DIFFERENCES AND CORRELATIONS IN THE NUCLEOSIDE SYSTEM OF THE HUMAN BRAIN

Age- and gender-modulated regional differences in the brain nucleoside system [11, 16-22] suggest (i) the existence of specific nucleoside pools in different brain areas, (ii) significant spatial differences in the nucleoside metabolic (anabolic and catabolic) network and signaling mechanisms induced by Ado and non-Ado nucleosides (i.e., Urd and Guo), (iii) fine and highly regulated modulation of different physiological processes in different human brain areas by nucleosides, (iv) the effect of nucleoside microenvironment on aging, which may be modulated by gender and (v) pathological consequences of changes in the nucleoside system, which may be related to the development of different CNS diseases, such as major depression, bipolar disorders, schizophrenia, Huntington's disease, Parkinson's disease and Alzheimer's disease as well as frontotemporal dementia [7, 50, 177-186].

3.1. Nucleoside Metabolism and Nucleoside Levels

Ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) consist of nucleotides that are synthesized from nucleosides and phosphate moieties. The major purine and pyrimidine ribonucleosides are Ado, Guo, Ino, and Cyd, Urd and thymidine (Thd) [187], which contain purine or pyrimidine bases that are connected to a pentose moiety. Nucleosides are obtained from food and are partially synthesized *de novo* in the liver, which may be transported into the brain by nucleoside transporters and anabolized into their corresponding nucleotides intracellularly [56-58, 133, 188-190]. Since limited *de novo* synthesis of nucleosides has been observed in the adult brain [191], salvage mechanisms have the predominant role in the preservation of purine and pyrimidine nucleosides and bases in the human brain (Fig. (**1**)). Hypoxanthine phosphoribosyltransferase (HGPRT; hypoxanthine-guanine phosphoribosyltransferase), Ado kinase (ADK), adenine (Ade) phosphoribosyltransferase (APRT), Cyd deaminase (CDA) and Urd-Cyd kinase (UCK) catalyze the conversion of (i) hypoxanthine (Hyp) to IMP and guanine (Gn) to GMP, (ii) Ado to AMP, (iii) Ade to AMP and (iv) Cyd and Urd to CMP and Urd monophosphate (UMP), respectively (Fig. (**1**)) [56-58, 189, 192-194]. The intracellular degradation of ATP and ADP by nucleoside tri- and diphosphate phosphatases followed by the catabolism of AMP occurs via two degradation pathways: AMP to IMP-Ino-Hyp (IMP pathway) or to Ado-Ino-Hyp (Ado pathway) (Fig. (**1**)) by AMP deaminase (AMPDA), cNs (cN-I and cN-II, the rate-limiting enzymes of intracellular Ado formation), ADA and purine nucleoside phosphorylase (PNP). Adenosine is also synthesized from S-adenosylhomocysteine (SAH) by adenosylhomocysteinase (SAHH, S-adenosylhomocysteine hydrolase) (Fig. (**1**)). The steady-state concentration of Ado is maintained by the simultaneously active cNs, ADK and ADA [56, 58, 195]; thus, an alteration

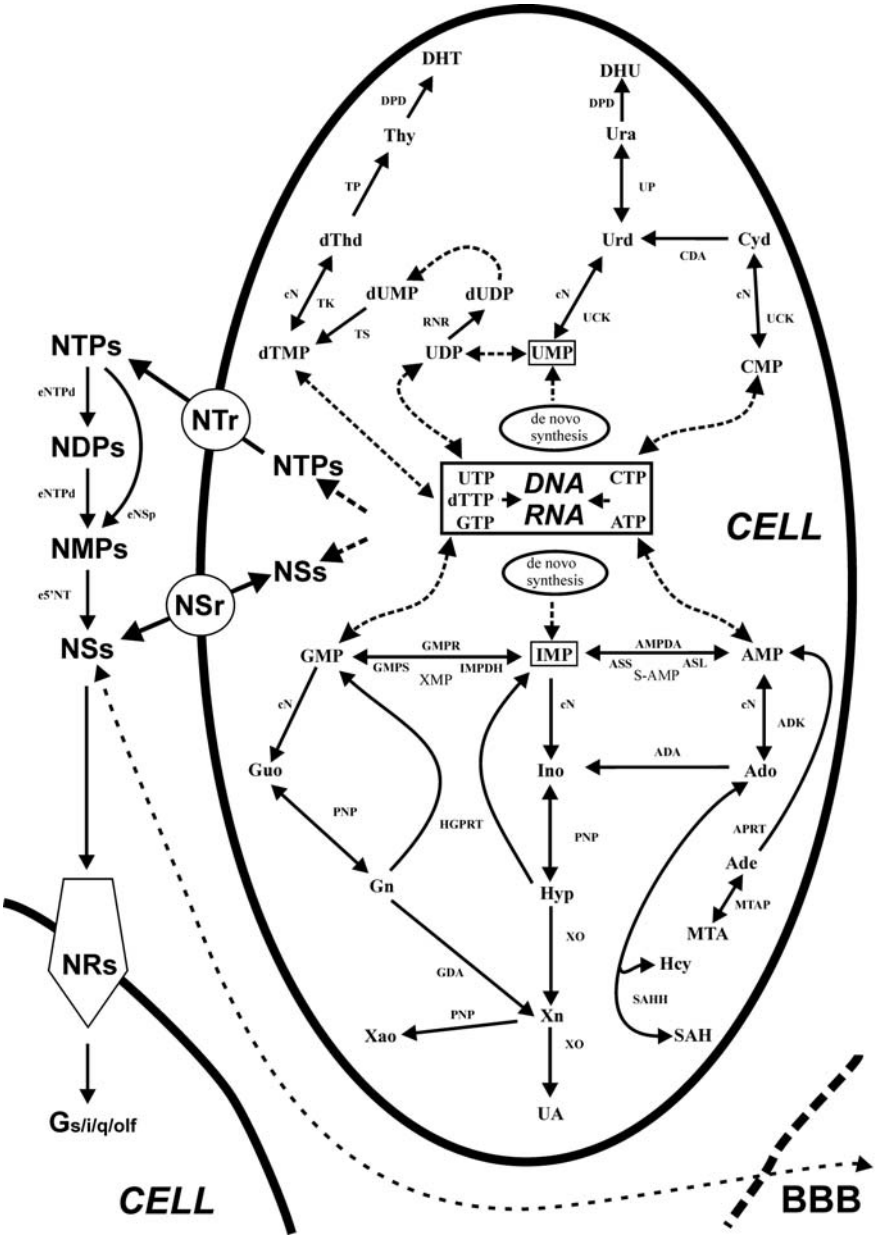

Fig. (1). Intracellular and extracellular metabolism of purine and pyrimidine nucleosides in the brain.

Abbreviations: cN: cytoplasmic 5'-nucleotidases; 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; ATP: adenosine triphosphate; BBB: blood brain barrier; CDA: cytidine deaminase; CMP: cytidine monophosphate; CTP: cytidine triphosphate; Cyd: cytidine; DHT: dihydrothymine; DHU: dihydrouracil; DPD: dihydropyrimidine dehydrogenase; dThd: deoxythymidine; dTMP: deoxythymidine monophosphate; dTTP: deoxythymidine triphosphate; dUMP: deoxyuridine monophosphate; e5'NT: ecto-5'-nucleotidase; eNSp: ecto-nucleoside pyrophosphatase diphosphohydrolase; eNTPd: ecto-NTP diphosphohydrolase; GDA: guanine deaminase; GMP: guanosine monophosphate; GMPR: GMP reductase; GMPS: GMP synthetase; Gn: guanine; $G_{s/i/q/olf}$: G proteins; GTP: guanosine triphosphate; 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; NDPs: nucleoside diphosphates; NMPs: nucleoside monophosphates; NRs: nucleoside receptors; NSr: nucleoside release (via nucleoside transporters); NSs: nucleosides; NTPs: nucleoside triphosphates; NTr: nucleotide release (via e.g. ATP channels and transporters as well as synaptic release); 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; UTP: uridine triphosphate; Xao: xanthosine; XMP: xanthosine monophosphate; Xn: xanthine; XO: xanthine oxidase; we didn't show intracellular nucleoside mono- and diphosphate kinases or nucleoside di- and triphosphate phosphatases.

in these enzymes' activity may change Ado levels and the effects of Ado on brain cells. Guanine-ribonucleotides may degrade via two pathways in the brain: the GMP-Guo-Gn-xanthine (Xn) pathway, which is catalyzed via cN-II, PNP and Gn deaminase (GDA) (Fig. (**1**)); or the GMP-IMP-Ino-Hyp-Xn pathway, which is regulated by GMP reductase (GMPR), cN-II, PNP and Xn oxidase (XO). Since uricase enzyme activity has disappeared from the mammalian brain during evolution, the final step of purine catabolism is the conversion of Xn into uric acid (UA) by XO in the human brain [95, 188, 196].

Extracellular nucleoside triphosphates (ecto-NTPs) may degrade into nucleoside diphosphates (NDPs), NMPs and nucleosides via an ectonucleotidase cascade system involving ecto-NTP diphosphohydrolases (eNTPd), ecto-nucleoside pyrophosphatase diphosphohydrolase (eNSp) and e5'NT (Fig. (**1**)) [56, 83-86, 197]. The extracellular levels of Ado are formed extracellularly from ATP and released from cells that are regulated by e5'NT, ecto-adenosine kinase (ecto-ADK) and ecto-adenosine deaminase (ecto-ADA) [56, 86, 101, 198-202].

Similarly to purine nucleotides, pyrimidine nucleotides (UTP; CTP; Thd triphosphate, TTP) are also intracellularly metabolized into their di- and monophosphate derivatives as well as nucleosides (Urd, Cyd and Thd) by nucleoside tri- and diphosphate phosphatases and cNs. The end products of pyrimidine nucleotide metabolism are dihydrothymine (DHT) and dihydrouracil (DHU) (Fig. (**1**)). Degradation of extracellular pyrimidine nucleotides into their corresponding nucleosides is catalyzed by eNTPd, eNSp and e5'NT [22, 56, 57, 189].

Several nucleoside metabolic enzymes, such as 5'NT, ADA, ADK, PNP and GDA, are unevenly distributed in the human brain [22, 203-207] (Table **1**). High activities of 5'NT, ADA, ADK, PNP and GDA have been previously demonstrated in several human brain areas (e.g., thalamus, hypothalamus and amygdala), whereas low levels have been detected in other brain areas (e.g., cerebellum and medulla oblongata). Most human brain areas demonstrate intermediate activity of 5'NT (e.g., parietal lobe, cingulate cortex, insula, caudate nucleus, putamen, pallidum (internal), thalamus (anterior), subthalamic nucleus, nucleus ruber, substantia nigra, amygdala and hypothalamus), ADA (e.g., gray matter of the frontal, occipital, orbital, parietal and temporal lobes; pons; putamen; hippocampus; caudate nucleus; globus pallidus; thalamus; and midbrain), ADK (e.g., cerebellum, temporal cortex and occipital cortex), PNP (e.g., caudate nucleus, medulla oblongata, white matter of the frontal lobe and gray matter of the temporal, parietal and frontal lobes) and GDA (e.g., parietal cortex, caudate nucleus, putamen, hippocampus and substantia nigra) (Table **1**). These results indicate spatial differences in the complex nucleoside metabolic network in relation to their functions in the CNS. Volonté and Ambrosi [53] have proposed that several elements of the purinergic (and likely the pyrimidinergic) system are tightly integrated with each other and modulate neuronal function together. For example, changes in one or more ele-

Table 1. Activity of Some Nucleoside Metabolizing Enzymes and their Distribution in the Human CNS.

	Activity level (5'NT: nmol/h/mg protein; ADA: nmol of ammonia/min/g of wet weight; ADK: nmol/min/g wet weight; PNP: substrate transformed (µmol)/min/g wet weight; GDA: substrate transformed (µmol)/min/mg protein) and distribution of nucleoside metabolic enzymes in the CNS				
	5'NT[1]	**ADA**[2]	**ADK**[3]	**PNP**[4]	**GDA**[5]
High	749-1123: temporal cortex, thalamus (medial and lateral), colliculus superior	387-579: white matter of frontal, orbital and temporal lobe	16.4-19.4: hypothalamus, pons, hind brain	223-261: pons, midbrain, thalamus, white and gray matter of occipital lobe, amygdala	12.9-19.2: thalamus, mamillary body
Intermediate	375-748: parietal lobe, cingulate cortex, insula, caudate nucleus, putamen, pallidum (internal), claustrum, thalamus (anterior), subthalamic nucleus, nucleus ruber, substantia nigra, amygdala, hypothalamus, midbrain (paramedian)	194-386: gray matter of frontal, occipital, orbital, parietal and temporal lobe; pons, putamen, hippocampus, caudate nucleus, globus pallidus, thalamus, midbrain, cerebellar white matter, white matter of parietal, cingulate and occipital lobe; corpus callosum	13.1-16.3: cerebellum, temporal cortex, corpus callosum, occipital cortex	183-222: caudate nucleus, white matter of cerebellum, medulla oblongata, white matter of frontal lobe, gray matter of temporal, parietal and frontal lobe; corpus callosum	6.5-12.8: parietal cortex, caudate nucleus, putamen, pons (basis), hippocampus, substantia nigra
Low	210-374: cerebellar cortex, lateral geniculate body, pallidum (external), centrum semiovale, corpus callosum, mamillary body, internal capsule	16-193: gray matter of cingulate cortex and cerebellum; hypothalamus, medulla oblongata, spinal cord	9.8-13.0: parietal lobe, frontal cortex	143-182: gray matter of cerebellum, white matter of temporal and parietal lobe, putamen, spinal cord	0.005-6.4: cerebellum, olivary nucleus, corpus callosum, lateral geniculate body

References: [1][204]; [2][205]; [3][207]; [4][206]; [5][203]; **Abbreviations:** 5'NT: 5'-nucleotidase; ADA: adenosine deaminase; ADK: adenosine kinase; GDA: guanine deaminase; PNP: purine nucleoside phosphorylase.

ments of the nucleoside system may result in an alteration(s) in its function. An increase or decrease in the extracellular and intracellular concentration of nucleosides may also change nucleoside uptake via their transporters and thereby alter nucleoside function via nucleoside receptors in the brain. Thus, regionality in nucleoside metabolic enzyme activity in different human brain areas, and their changes may reflect different roles of these enzymes in physiological functions and may cause pathophysiological processes in the brain.

It has been revealed that nucleoside concentrations are unevenly distributed in different human brain areas and that they may be age and gender dependent [16, 20-22]. On the basis of the extrapolation method [19], Kovács and colleagues prepared the first [57] nucleoside map of the human brain [20]. High Ado and/or Ino, Guo and Urd levels were calculated in the cochlear nuclei, vestibular nuclei, temporal and occipital cortices, cerebellar cortex, caudate nucleus and nucleus basalis (Table **2**), and the lowest concentrations were measured in the entorhinal cortex, zona incerta, substantia nigra, locus coeruleus, and habenula, among others (Table **2**). The highest and lowest nucleoside levels were measured in the cochlear nuclei and zona incerta, respectively. Moreover, both the nucleosides and their metabolites (e.g., Hyp, Xn and Ura) were unevenly distributed in the human brain [20].

Levels of Ino and Ado increased with age in the human frontal cortex, whereas lower Ado levels and higher Urd, Ino and Guo concentrations were measured in middle-aged and/or elderly female cortical samples when compared to middle-aged and older males [21]. Taken together, these findings indicated that (i) increased levels of Ado may result from increased activity of 5'NTs (mainly e5'NT and/or cN-I) according to age and that (ii) different human brain 5'NTs activities may exist between females and males, consistent with findings obtained from previous animal studies [118, 208-213]. These results suggested that (i) age and gender may modulate nucleoside levels and nucleoside metabolic enzymes activities and that (ii) the nucleosides may have a role in aging processes in the brain and in decreasing the effects of excitotoxic insults on the female brain [21].

3.2. Nucleoside Transporters

Nucleoside transporters control the levels of nucleosides required to maintain the nucleoside balance in the extracellular and intracellular space and to exert their functions in brain tissue. Nucleosides are transported via the membranes of brain cells by two types of nucleoside transporters [15, 54, 214]. The ENT family consists of four ENT transporter types (ENT1-ENT4), which achieve bidirectional facilitated (equilibrative) diffusion depending on extracellular/intracellular concentration gradient [15, 56]. Equilibrative S-(4-nitrobenzyl)-6-thioinosine (NBTI)-insensitive ENT types ('ei'; e.g., ENT2) are inhibited by μM concentrations of NBTI, whereas NBTI-sensitive ENT types ('es'; e.g., ENT1) are inhibited by three orders of magnitude lower levels (nM concentration) of NBTI. Different types of ENT transporters can transport purines and pyrimidines (e.g., ENT1-ENT3) and their bases (e.g., ENT2 and ENT3). The concentrative nucleoside transporter (CNT) family consists of six CNT transporter types (N1-N6), which transport nucleosides unidirectionally and in a sodium/proton-dependent manner (symport of sodium ions or sodium ions and protons with nucleosides into the cells) in the brain. Concentrative nucleoside transporters are classified on the basis of their transported nucleosides (e.g., CNT3 transports purines and pyrimidines, whereas CNT1 transports pyrimidines, Ino and Ado) and sodium/proton transport coupling [15, 23, 215-218]. Nucleoside analogs with anti-viral and anti-tumor effects are transported via both ENT and CNT transporters [160, 214].

The uneven distribution of nucleoside transporters has also been demonstrated in the human brain [23, 215-218], which showed different expression patterns of ENT1-ENT4 transporters (e.g., high ENT1, intermediate ENT3 and low ENT2 and ENT4 transporter density was demonstrated in the frontal cortex) with the exception of the thalamus, where ENT transporters are expressed at uniformly intermediate levels (Table **3**). The distribution of CNT1 transporters was uniform, but the expression of CNT2 and CNT3 transporters were uneven in the human brain (Table **3**). Relatively high expression of CNT2 and CNT3 transporters was demonstrated in the cerebellum, putamen, hippocampus, medulla oblongata and pituitary gland, whereas intermediate/low activities were demonstrated in other brain areas, including the amygdala; frontal, occipital and temporal lobe; substantia nigra; thalamus; spinal cord; cerebellum; caudate nucleus; putamen; and nucleus accumbens (Table **3**). This uneven distribution of nucleoside transporters may reflect the different roles of nucleoside transporter types in neuromodulation at functionally different brain areas [23, 215-218].

3.3. Nucleoside Receptors

Four known G-protein-coupled Ado receptor (P1 receptors) subtypes (A_1, A_{2A}, A_{2B} and A_3) have been shown in neurons and glial cells [9, 23, 219, 220]. A_1 and A_3 receptors inhibit adenylate cyclase (AC) activity via G_i-proteins, thereby inhibiting cyclic AMP (cAMP)/protein kinase A (PKA)-evoked signaling processes. A_1 receptors also stimulate phospholipase C (PLC) and can increase and decrease the activity of K^+ and Ca^{2+} channels, respectively. Consequently, this receptor subtype may inhibit synaptic transmission and hyperpolarize neurons, which can result in neuroprotective effects. Furthermore, A_1 receptors are involved in the regulation of sleep, cognition and memory and decrease cell metabolism (homeostatic functions). Importantly, both Ado and AMP are full agonists of A_1 receptors [221]. A_{2A} and A_{2B} receptors stimulate AC via G_S- (A_{2A} and A_{2B}) and/or G_{olf}- proteins (A_{2A}) and facilitate neurotransmitter release. A_{2A} receptors can modulate the processes of sleep, motor activity, cognition and memory, and both A_{2A} and A_{2B} receptors mediate vasodilatation. Moreover, A_3 receptors are involved in neuroinflammation [1, 9, 13, 220, 222]. G_q proteins can couple to both A_{2B} and A_3 receptors and stimulate PLC activity. A novel Ado receptor (A_4) and receptors of Urd, Guo and Ade (UrdR, GuoR, and AdeR, respectively) have also been postulated [223-230].

The expression level of Ado receptors is also region specific in the human brain [23, 25, 220, 231] (Table **4**), which may reflect the different effects of Ado in brain structures under physiological conditions. Thus, alterations in Ado receptor density may result in pathological processes [24, 25, 50, 183, 232]. High expression of A_1 and/or A_{2A} and A_3

Table 2. Levels and Distribution of Nucleosides in the Human CNS.

	Concentration (pmol/mg wet weight) and distribution of nucleosides in the CNS			
	Ado[1]	**Ino**[1]	**Guo**[1]	**Urd**[1]
High	15.9-23.9: cochlear nuclei, vestibular nuclei, cerebellar cortex, supraoptic nucleus, flocculo-nodular lobe	107.7-161.5: cochlear nuclei, spinal trigeminal nucleus	17.7-26.4: cochlear nuclei; temporal and occipital cortex; caudate nucleus, nucleus basalis, medial geniculate body, amygdala	44.1-66.2: cochlear nuclei; temporal and occipital cortex; cerebellar cortex, amygdala, spinal central gray, spinal cord (ventral horn)
Intermediate	8.0-15.8: spinal cord (ventral and dorsal horn), amygdala, temporal and prefrontal cortex, caudate nucleus, mediodorsal thalamic nucleus	53.9-107.6: frontal, temporal, somatosensory, prefrontal, cingulate and occipital cortex; caudate nucleus, substantia innominata, nucleus basalis, nucleus accumbens, reticular formation (medulla oblongata), amygdala, cerebellar nuclei, spinal cord (ventral and dorsal horn), mediodorsal thalamic nucleus, spinal cord (white matter)	8.9-17.6: insular, prefrontal, entorhinal, cingulate and somatosensory cortex; white matter (cerebral and cerebellar), nuclei of diagonal band, substantia innominata, lateral geniculate body, hippocampus, nucleus accumbens, cerebellar nuclei, mediodorsal thalamic nucleus, spinal cord (ventral and dorsal horn)	22.1-44.0: cerebral and cerebellar white matter; somatosensory, prefrontal, cingulate, insular and entorhinal cortex; hippocampus, caudate nucleus, globus pallidus externa, anterior nuclei (thalamus), substantia nigra, inferior colliculus, nucleus accumbens, locus coeruleus, inferior olive, reticular formation (medulla oblongata), cerebellar nuclei, mediodorsal thalamic nucleus, spinal cord (white matter), spinal cord (dorsal horn)
Low	1.4-7.9: frontal, somatosensory, cingulate and entorhinal cortex; hippocampus, nuclei of diagonal band, septum, globus pallidus externa, ventral lateral nucleus, habenula, pulvinar, zona incerta, preoptic area, paraventricular nucleus, dorsomedial nucleus (hypothalamus), lateral hypothalamic area, substantia nigra, inferior colliculus, locus coeruleus, dorsal vagal nuclei, nucleus accumbens, spinal central gray	29.8-53.8: entorhinal and parahippocampal cortex; hippocampus, nuclei of diagonal band, habenula, pulvinar, zona incerta, paraventricular nucleus, substantia nigra, inferior colliculus, locus coeruleus	4.1-8.8: septum, habenula, pulvinar, zona incerta, paraventricular nucleus, lateral hypothalamic area, substantia nigra, superior colliculus, inferior colliculus, locus coeruleus, spinal cord (white matter)	15.7-22.0: ventral anterior nucleus, zona incerta, preoptic area, motor facial nucleus

References: [1][20]; **Abbreviations:** Ado: adenosine; Guo: guanosine; Ino: inosine; Urd: uridine.

receptors has been demonstrated in several human brain areas (e.g., in the parietal, temporal and occipital cortices, caudate nucleus; putamen; globus pallidus; nucleus accumbens; and hippocampus), whereas other brain areas showed intermediate or low expression of A_1, A_{2A} and A_3 receptors (e.g., in the frontal cortex, thalamus, medulla oblongata, midbrain and pons). A_{2B} receptors are uniformly distributed in the human brain (Table **4**).

Age-related changes in Ado receptor distribution have been shown in the brains of both animals and humans. Since A_1 receptor density was decreased and A_{2A} receptor activity was increased by age, the excitatory(A_{2A})/inhibitory(A_1) effects/balance may also shift toward excitation via A_{2A} receptors with aging [182, 233-240]. Consequently, increased Ado levels in the aged human brain may increase the release of neurotransmitters, which may have both positive (e.g., increasing acetylcholine release in dementia) and negative (e.g., increasing the risk of excitotoxicity) effects [12, 118, 241, 242].

3.4. Correlations Between Elements of the Nucleoside System

Interactions have been demonstrated between (i) regional differences in the nucleoside concentrations and the distribution of nucleoside metabolic enzyme activities and Ado receptor expression [20], (ii) NBTI binding site and the density of ADA immunoreactive neurons [243], (iii) ENT1 transporters and A_1 receptor density [23], (iv) 5'NT levels and A_1 receptor density [24, 25] and (v) ADA and A_1 receptor functionality [201] in the nucleoside system. In contrast, the distribution of ENT2 transporters has not shown a correlation with either A_1 or A_{2A} receptor density in the human brain [23]. In this review, we discuss the association of 5'NTs with nucleoside levels, nucleoside transporters and Ado receptors.

The nucleoside metabolic enzyme activity may be related to the uneven distribution of nucleosides in the human brain [20, 203-207] (Table **1** and **2**). Intermediate or high 5'NT activity and/or low or intermediate ADA/ADK activity may increase the concentration of Ado, Ino, Guo and Urd

Table 3. Relative Density of Nucleoside Transporters and their Distribution in the Human CNS.

Relative density and distribution of nucleoside transporters in the CNS				
	ENT transporters			
	ENT1[1]	**ENT2**[1]	**ENT3**[2]	**ENT4**[3]
High	frontal and parietal cortex	midbrain, pons, cerebellum	occipital and temporal lobe, corpus callosum, medulla oblongata, putamen	temporal lobe, paracentral gyrus, amygdala, caudate nucleus, hippocampus, medulla oblongata, putamen
Intermediate	temporal and occipital cortex, thalamus, midbrain, caudate nucleus, putamen, globus pallidus	medulla oblongata, thalamus	frontal lobe, paracentral gyrus, pons, hippocampus, nucleus accumbens, thalamus, spinal cord, cerebellum (right)	parietal and occipital lobe, pons, cerebellum (right), corpus callosum, thalamus, pituitary gland, spinal cord, substantia nigra, nucleus accumbens
Low	medulla oblongata, pons, cerebellum, hippocampus	frontal, occipital, temporal and parietal cortex; hippocampus, caudate nucleus, putamen, globus pallidus	parietal lobe, cerebellum (left), amygdala, caudate nucleus, substantia nigra, pituitary gland	frontal lobe, cerebellum (left)
	CNT transporters			
	CNT1 (N2/cit)[4]	**CNT2** (N1/cif)[4]	**CNT3** (N3/cib)[5]	
High	Uniform distribution	cerebellum, putamen, hippocampus, medulla oblongata	hippocampus, medulla oblongata, pituitary gland	
Intermediate		amygdala, cerebral cortex, frontal, occipital and temporal lobe; substantia nigra, thalamus, spinal cord	frontal, parietal and occipital lobe; corpus callosum, cerebellum, amygdala, caudate nucleus, putamen, thalamus, temporal lobe, paracentral gyrus, pons, substantia nigra, nucleus accumbens, spinal cord	
Low				

References: [1][23]; [2][215]; [3][216]; [4][217]; [5][218]; **Abbreviations:** CNT transporters: concentrative nucleoside transporters; ENT transporters: equilibrative nucleoside transporters

Table 4. Relative Density of Ado Receptors and their Distribution in the Human CNS.

	Relative density and distribution of Ado receptors in the CNS			
	A_1[1]	A_{2A}[2,3]	A_{2B}[4]	A_3[4]
High	parietal, temporal, and occipital cortex; caudate nucleus, putamen (medial), anterior nuclei (thalamus), medial nuclei (thalamus)	caudate nucleus, putamen, globus pallidus, nucleus accumbens	Uniform distribution	cerebellum, hippocampus
Intermediate	frontal, and cingulate cortex; nucleus accumbens, entorhinal cortex, hippocampus, amygdala, thalamic reticular nuclei, pulvinar, medial geniculate body	frontal, temporal, parietal and occipital cortex; thalamus, hippocampus, medulla oblongata, midbrain, pons, cerebellum		other brain areas
Low	globus pallidus, substantia innominata/nucleus basalis, ventral posterior nuclei (thalamus), lateral geniculate body, superior colliculus, inferior colliculus, substantia nigra, pons, medulla oblongata, spinal cord, cerebellar cortex, cerebellar nuclei			

References: [1][25]; [2][23]; [3][231]; [4][220]; **Abbreviations:** $A_1/A_{2A}/A_{2B}/A_3$: $A_1/A_{2A}/A_{2B}/A_3$ subtype of adenosine receptors; Ado: adenosine

(Fig. (**1**)), such as in the caudate nucleus and temporal cortex in humans. In addition, high 5'NT, PNP and GDA activities may correspond with low Guo and Ino levels, such as in the zona incerta. Low 5'NT and ADA activity and intermediate ADK activity may also correspond with high Ado levels, such as in the cerebellar cortex. In addition, low 5'NT, PNP and GDA activities may enhance the levels of Ino and Guo in the human cerebellum. Thus, (i) the activities of only several nucleoside metabolic enzymes have been established in several human brain areas (e.g., occipital cortex), and (ii)

although we have sufficient comparable information of the nucleoside metabolic enzyme activities in different human brain areas, we do not have data on the nucleoside levels (e.g., in the parietal cortex) (Table **1** and **2**); however, we can conclude that regionally different activities of nucleoside metabolic enzymes may generate regionally altered nucleoside concentrations in the human brain. Moreover, different nucleoside metabolism and neuron/glia ratios have been demonstrated in neurons and glial cells and in human brain areas, respectively [244-249]. These results suggest that spatial differences in nucleoside distribution may result from (i) different neuron/glia ratios and different neuronal/glial nucleoside metabolisms, (ii) spatially organized nucleoside metabolisms and (iii) different activities of nucleoside metabolic enzymes in functionally different human brain areas [20]. Importantly, changes in the glia/neuron ratio may cause regional alterations of nucleoside metabolism and nucleoside levels [20], which may evoke different CNS diseases, such as major depressive disorder, bipolar disorders, Huntington's disease, Alzheimer's disease, schizophrenia and frontotemporal dementia [177-181].

Functional relationships were revealed between different elements of the nucleoside system and its function under physiological and pathophysiological (e.g., hypoxic/ischemic) conditions. Intracellular levels of nucleoside triphosphates (NTPs) were approximately 0.2-10.0 mM, whereas the concentrations of the nucleosides were at least three orders of magnitude lower in the human brain. Thus, a small decrease in NTPs levels and/or changes in enzymatic activity of the nucleotide/nucleoside metabolic systems may cause a dramatic alteration in the nucleoside levels [20, 250-255]. Extracellular nucleoside triphosphates (such as ATP and UTP) competitively inhibit the activity of e5'NTs, causing a delay in the onset of nucleoside production [58, 118, 256-258]. Thus, the increased extracellular nucleotide levels (e.g., ATP) in the synaptic cleft (e.g., under hypoxic/ischemic conditions) may cause an accumulation of NMPs (e.g., AMP) to a greater extent compared to nucleosides (e.g., Ado). The decrease in nucleoside triphosphate levels may result in relief of e5'NT inhibition and thereby elevate nucleoside levels and nucleoside transport into the brain cells anabolized into nucleoside mono-, di- and triphosphates derivatives [58, 84, 85, 257]. During normoxic conditions (at 3.6 mM ATP levels) [133], extracellular nucleotides (e.g., ATP) are mainly catabolized into nucleosides (e.g., Ado) since the level of e5'NT inhibitor nucleoside triphosphates are lower than under ischemic conditions. Under these conditions, intracellular ATP is catabolized into Ino via IMP (IMP pathway; AMPDA and cN-II may be activated by ATP) rather than into Ado, which maintained low cytoplasmic Ado levels [57]. Decreased levels of intracellular ATP under ischemic conditions (less than 2 mM ATP levels) [59] may slow down the nucleoside recycling system [88, 133]. In addition, AMP is also degraded into Ino, but it is degraded via the Ado pathway since the degradation of ATP decreases the activation of AMPDA and cN-II [57, 59, 133] (Fig. (**1**)). In addition, under these circumstances, the K_m value for IMP increases from micromolar to millimolar [140]. As a consequence of the increased Ado levels, Ado may be released into the extracellular space via ENT transporters and contribute to the protection of brain tissue cells via A_1/A_2 receptor activation (e.g., reduction of ischemic injury by vasodilatation and inhibition of glutamate release) [1, 13, 79, 118, 259-262].

A tight interplay between extracellular ATP metabolism (involving AMP catabolism by e5'NT), activation/inhibition of Ado receptors and function of nucleoside transporters and nucleotide/neurotransmitter release has also been demonstrated in rat hippocampal glutamatergic nerve terminals [115, 263]. Low frequency nerve stimulation may cause the release of a small amount of ATP into the synaptic cleft, thereby resulting in low extracellular concentrations of Ado from the degradation of ATP via the ectonucleotidase cascade system and the release of Ado via ENT transporters. Subsequently, Ado may decrease neurotransmitter (and ATP) release via the activation of A_1 receptors [115, 264]. The greater activation of A_1 receptors compared to A_{2A} receptors by Ado (derived mainly via nucleoside transporters) may be associated with the tight proximity of A_1 receptors and nucleoside transporters in synapses [115, 263]. Indeed, the human ENT1 transporter distribution correlated with the density of A_1 receptors in the brain [23, 265], which also strengthened the correlation between human ENT1 transporter-mediated transport processes and A_1 receptor-mediated neuromodulation [23]. However, high frequency stimulation also increased the amount of ATP released from the cells, which enhanced the extracellular levels of Ado via ectonucleotidases resulting in (i) a stronger activation of A_{2A} receptors compared to A_1 receptors and (ii) an A_{2A} receptor-evoked increase of neurotransmitter and ATP release as well as ENT transporter uptake activity [115, 263, 266]. Increased activation of A_{2A} receptors by high Ado levels (derived from extracellular ATP metabolism) may be due to the close proximity of A_{2A} receptors and e5'NT, whereas decreased activation of A_1 receptors may be due to limited Ado availability to A_1 receptors via suppression of Ado spreading in synapses by the A_{2A} receptor-evoked increase in Ado uptake [115, 263].

An increase in the expression and/or synthesis and activity of e5'NT may enhance neuromodulation by increasing Ado levels via Ado receptors [1, 267, 268]. Thus, the e5'NT activity may be functionally coupled with an activation of Ado receptors [81]. However, only a poor correlation has been demonstrated between A_1 receptor agonist binding sites and e5'NT activity [24, 25, 269]. Intermediate/high activity of 5'NT, intermediate/high A_1 receptor density, and low/intermediate A_{2A} receptor expression have been observed in the temporal cortex and several thalamic nuclei in the human brain, whereas brain areas with intermediate 5'NT activity, such as in the parietal lobe, putamen and caudate nucleus, showed high A_1 and intermediate/high A_{2A} receptor expression. In addition, low levels of 5'NT activity were correlated with low A_1 and/or A_{2A} receptor density in the human cerebellum and lateral geniculate body [23, 25, 204, 231] (Table **1** and **4**).

Although the Ado uptake inhibitor dipyridamole showed no effect on the activity of e5'NT and e5'NT inhibition by anti-5'-nucleotidase IgG did not evoke an alteration in the Ado uptake into astrocytes [258], there may be a relationship between e5'NT activity and Ado transporters [270-272]. A close association between e5'NT and nucleoside transporters was demonstrated in vascular endothelial cells, heart cells and other cell types [270, 273], which indicated vectorial

transport of Ado via nucleoside transporters [258, 271]. Transport of Ado from AMP by e5'NT into the cells was more rapid compared to Ado in free solution. Consequently, e5'NT-catalyzed hydrolysis of AMP may contribute to Ado uptake [270, 271]. Taken together, e5'NT may function as an integral part of the nucleoside transport process [270, 271], and activity of e5'NT may be linked to Ado translocation in brain cells. Indeed, the ectonucleotidase system and nucleoside transporters may form a cycle for the control of vascular tone in vascular cells (release of ATP, ATP catabolism to Ado, termination of Ado action/vasodilatation by the uptake of Ado) after hypoxia [273]. High 5'NT activity may be attributed to intermediate ENT1 and high ENT3 and ENT4 expression in the human temporal cortex, whereas enhanced 5'NT activity in the thalamus showed intermediate ENT transporter (ENT1-ENT4) activity [23, 204, 215, 216] (Table **1** and **3**). Importantly, the cerebellum exhibited low 5'NT activity and low ENT1, ENT3 and ENT4 density but high ENT2 density, which indicates the main nucleoside transporter type(s) differs among functionally different human brain areas.

Modulatory effects of 5'NTs on the purinergic/nucleosidergic system and their physiological/pathophysiological effects are dependent not only on their distribution/activity in the brain, but also on (i) the availability, proximity and distribution of nucleosides, nucleoside transporters and nucleoside receptors in different brain areas and (ii) on relationships between nucleoside system elements. However, alterations not only in the nucleoside levels but also in other elements of the nucleoside system, which may modulate nucleoside functionality, such as 5'NTs, may affect the physiological roles (e.g., cognition and memory, motor control, emotions, sensory information processing) of nucleosides [12, 265, 274-278] and cause pathophysiological conditions and diseases (e.g., Alzheimer's disease, Parkinson's disease and Huntington's disease) [12, 279] in the human CNS.

4. CONCLUSIONS: THERAPEUTIC TOOLS AND PERSPECTIVES

4.1. Therapeutic Tools Against CNS Diseases Based on the Nucleoside System

Drugs that modulate the nucleoside system are widely used, for example, (i) as anti-cancer and anti-viral therapies and treatment for gout (e.g., nucleoside metabolic enzyme inhibitors and/or synthetic nucleosides), (ii) as coronary vasodilators (e.g., nucleoside transport inhibitors), (iii) as vasodilators, and (iv) to treat cardiac arrhythmias, acute renal failure, carcinomas, rheumatoid arthritis and asthma (e.g., Ado receptor agonists and antagonists) [11, 13, 15, 38, 54, 158, 280-286]. Modulation of the adenosinergic system is also effective against several brain disorders, such as multiple sclerosis [287, 288], in which specifically affected brain regions are not known. Thus, the identification of brain regions in which changes in one or more elements of the nucleoside system may be associated with these diseases is likely impracticable. However, considering the regionally different physiological roles of nucleosides in the brain, regional differences in the nucleoside system and its changes in affected brain areas may be related to the development of CNS diseases as previously discussed [11]. Moreover, regional differences were demonstrated in the nucleoside system of human brain areas implicated in several CNS diseases in which different drugs effects on the nucleoside system are or may be used later in treatment of the following CNS diseases (Table **5**): movement disorders such as Parkinson's disease and Huntington's disease (A_1 receptor agonists, A_{2A} receptor agonists/antagonists and nucleoside transporter inhibitors) [13, 55, 183, 289-296], drug addiction and alcoholism (A_{2A} receptor antagonists and nucleoside transporter inhibitors) [13, 297-299], pain and migraines (A_1 receptor agonists, A_{2A} receptor antagonists, ADK inhibitors, recombinant e5'NT and nucleoside transporter inhibitors) [13, 54, 109, 283, 297, 300-302], mania and anxiety (A_1 receptor antagonists; ADK, ADA or XO inhibitors, nucleoside transporter inhibitors) [13, 303-305], schizophrenia (A_{2A} receptor agonists, ADK, ADA or XO inhibitors and nucleoside transporter inhibitors) [184, 299, 306-309], epilepsy (A_1 receptor agonists, A_{2A} receptor antagonists, ADK and XO inhibitors and nucleoside transporter inhibitors) [13, 46, 50, 51, 54, 302, 310-316], sleep disorders (A_1 receptor agonists, A_{2A} receptor agonists/antagonists and nucleoside transport inhibitors) [13, 54, 283, 297, 299] and Alzheimer's disease (A_{2A} receptor antagonists and nucleoside transport inhibitors) [186, 317, 318]. In addition, Urd and/or Guo and Ino may also have therapeutic potential against epilepsy, pain, anxiety, schizophrenia, sleep disorders, ischemic injury, multiple sclerosis, Alzheimer's disease and Parkinson's disease, among others [3, 6, 79, 319-335]. Thus, elements of the nucleoside system are promising drug targets for the treatment of brain disorders, such as epilepsy, schizophrenia, Alzheimer's, Huntington's and Parkinson's diseases. However, mechanisms underlying the regional differences in the nucleoside system in specific areas of the human brain implicated in CNS diseases have not yet been elucidated (e.g., thalamic relay nuclei, periaqueductal gray, rostroventromedial medulla, and thalamic reticular and relay nuclei, tuberomamillary nucleus, and so forth, are involved in the physiological processes of pain and sleep). Thus, (i) to reveal the precise links between the regional differences of the nucleoside system, which are modulated by age and gender, and brain diseases, (ii) to obtain evidence that changes in the nucleoside system are a consequence of mechanisms underlying brain diseases and (iii) to develop effective and safe therapeutic strategies against brain diseases based on the nucleoside system, future studies on the nucleoside system focused on all of the affected human brain areas are required.

4.2. Modulation of 5'-Nucleotidase Activity: A New Promising Therapeutic Tool for the Treatment of Several CNS Diseases

Since e5'NT-generated nucleoside levels may be sufficient to exert modulatory effects via stimulation of their receptors and provide nucleoside uptake into the cells, an alteration of e5'NT activity may be involved in the progression of disorders in the brain and, as a consequence, modulation of e5'NT activity may be a potential target for the development of drugs effective against several CNS diseases.

It has been demonstrated that the activities of 5'NTs are altered under different pathological conditions. Increased cN-II (in Lesch-Nyhan patients with HGPRT deficiency) and

Table 5. Regional Differences in the Nucleoside System in Human Brain Areas: Therapeutic Implications for Therapy in CNS Diseases.

Regional differences in the nucleoside system: therapeutic implications			
Regional differences in the nucleoside system in brain areas implicated in CNS diseases	**Diseases**	**Drugs name: (pre)clinical, licensed or potential therapeutic application**	**Ref.**
Thalamus, motor cortex, caudate nucleus, putamen, globus pallidus, substantia nigra: - different distribution of nucleosides (e.g., intermediate levels of Ado, Ino and Urd and high levels of Guo in caudate nucleus; low Ado, Ino and Guo and intermediate Urd levels in the substantia nigra) and their metabolic enzymes (e.g., intermediate and low activity of PNP in the caudate nucleus and putamen, respectively; intermediate 5'NT and GDA activity in the substantia nigra) as well as nucleoside transporters (e.g., high ENT4, intermediate and intermediate/low ENT1 and CNT3 expression respectively, as well as low ENT2 and ENT3 density in the caudate nucleus; intermediate and low ENT3, ENT4, CNT2 and CNT3 density in the substantia nigra) - high (A_1 and A_{2A}: caudate nucleus, globus pallidus and putamen) or intermediate/intermediate to low (A_1 and A_{2A}: thalamus) density of Ado receptors; low density of A_1 receptors in the globus pallidus and substantia nigra	- Parkinson's disease and Huntington's disease	- A_1 receptor agonists (in Parkinson's disease) - A_{2A} receptor agonists and/or antagonists (in Huntington's disease; e.g., N^6-(4-hydroxybenzyl)adenine riboside) - A_{2A} receptor antagonists (e.g., Istradefylline/ KW6002 and Preladenant in Parkinson's disease) - nucleoside transporter inhibitors (e.g., N^6-(4-hydroxybenzyl)adenine riboside)	[11, 13, 20, 23, 55, 183-185, 203, 204, 206, 215-218, 231, 289-296]
Nucleus accumbens, prefrontal cortex: - intermediate (Ino, Guo and Urd) and low (Ado) levels of nucleosides in the nucleus accumbens; intermediate nucleoside (Ado, Ino, Guo and Urd) concentrations in the prefrontal cortex - intermediate (ENT3 and ENT4) and intermediate/low (CNT3) density of nucleoside transporters in the nucleus accumbens - intermediate A_1 receptor expression and high A_{2A} receptor density in the nucleus accumbens	- drug/alcohol addiction	- A_{2A} receptor antagonists - nucleoside transporter inhibitors	[11, 13, 20, 23, 184, 215, 216, 218, 231, 297-299]
No/little data in the specific areas of nociceptive circuitry; e.g., intermediate Ino, Guo and Urd and low Ado levels in the **somatosensory cortex**	- pain and migraine	- A_1 receptor agonists (e.g., GW-493838) - A_{2A} receptor antagonists - ADK inhibitors (e.g., GP-3269) - recombinant e5'NT - nucleoside transporter inhibitors	[11, 13, 20, 54, 109, 283, 297, 300-302]
Amygdala, prefrontal and cingulate cortex, locus coeruleus: - high (Guo and Urd) and intermediate (Ado and Ino) nucleoside levels in the amygdala; intermediate to low nucleoside (Ado, Ino, Guo and Urd) levels in the prefrontal and cingulate cortices; low Ado, Ino and Guo whereas intermediate Urd levels in the locus coeruleus - intermediate/high activities of 5´NT and PNP in the amygdala and cingulate cortex; intermediate activity of ADA in the cingulate cortex - high ENT4 and low ENT3 transporter expression in the amygdala; intermediate/low CNT2 and CNT3 transporter density in the amygdala - intermediate A_1 receptor density in the cingulate cortex and amygdala	- mania, anxiety	- A_1 receptor antagonists (e.g., FR194921 in anxiety disorders) - ADK, ADA or XO inhibitors (e.g., allopurinol in mania) - nucleoside transporter inhibitors	[11, 13, 20, 23, 204-207, 215-218, 231, 303-305]
Prefrontal cortex, nucleus accumbens, mediodorsal thalamic nucleus, hippocampus, entorhinal cortex, amygdala: - intermediate level of nucleosides (Ado, Ino, Guo and Urd) in most of these brain regions (except e.g., amygdala: high Guo and Urd levels; entorhinal cortex and hippocampus: low Ado and Ino concentrations) - amygdala: intermediate 5'NT and high PNP activity; hippocampus: intermediate ADA and GDA activity - high (ENT4: amygdala and hippocampus; CNT2 and CNT3: hippocampus), intermediate (ENT3: hippocampus and nucleus accumbens; ENT4: nucleus accumbens), intermediate to low (CNT2 and CNT3: amygdala; CNT3: nucleus accumbens) and low (ENT1 and ENT2: hippocampus; ENT3: amygdala) nucleoside transporter density	- schizophrenia	- A_{2A} receptor agonists - ADK, ADA or XO inhibitors (e.g., allopurinol) - nucleoside transporter inhibitors (e.g., dipyridamole)	[11, 20, 23, 184, 203-206, 215-218, 220, 231, 299, 306-309]

(Table 5) contd….

Regional differences in the nucleoside system: therapeutic implications			
Regional differences in the nucleoside system in brain areas implicated in CNS diseases	**Diseases**	**Drugs name: (pre)clinical, licensed or potential therapeutic application**	**Ref.**
- high (A_{2A} receptor: nucleus accumbens; A_3 receptor: hippocampus), intermediate (A_1 receptor: nucleus accumbens, amygdala, hippocampus and entorhinal cortex) and intermediate/low (A_{2A} receptor: hippocampus) expression of Ado receptors			
Hippocampus: - low Ado and Ino levels, intermediate Guo and Urd concentrations - intermediate ADA and GDA activity - high (ENT4, CNT2 and CNT3) and low (ENT1 and ENT2) expression of nucleoside transporters - high (A_3), intermediate (A_1) and intermediate/low (A_{2A}) Ado receptor density	- epilepsy	- A_1 receptor agonists - A_{2A} receptor antagonists - ADK inhibitors (e.g., 5'-iodotubercidin and GP-3269) - XO inhibitors (e.g., allopurinol) - nucleoside transporter inhibitors (e.g., dipyridamole)	[11, 13, 20, 23, 46, 50, 51, 54, 203, 205, 216-218, 231, 302, 311-316]
Preoptic area (hypothalamus), nucleus basalis, tegmental nuclei (pons), lateral hypothalamus: - high to low nucleoside levels (intermediate Ino and high Guo levels in the nucleus basalis; low Ado and Guo level in the lateral hypothalamic area; low Ado and Urd level in the preoptic area) - low ADA, intermediate 5'NT and high ADK activity in the hypothalamus - low A_1 receptor density in the nucleus basalis	- sleep disorders	- A_1 receptor agonists - A_{2A} receptor agonists/antagonists - nucleoside transport inhibitors	[11, 13, 20, 54, 204, 205, 207, 283, 297, 299]
Nucleus basalis, hippocampus, frontal, parietal and temporal cortex: - intermediate to high nucleoside levels (Ado, Ino, Guo and Urd) in most of these brain regions (except e.g., hippocampus: low Ado and Ino level; frontal cortex: low Ado levels) - high and intermediate 5'NT (temporal cortex and parietal lobe, respectively) and low and intermediate ADK (frontal cortex, parietal lobe and temporal cortex, respectively) activity; intermediate ADA (hippocampus, frontal, parietal and temporal lobe), PNP (frontal, parietal and temporal cortex) and GDA (hippocampus and parietal cortex) activity - high (ENT1: frontal and parietal cortex; ENT3: temporal lobe; ENT4: temporal lobe and hippocampus; CNT2 and CNT3: hippocampus) and intermediate to low (e.g., ENT1, ENT2 and ENT3 in the hippocampus) nucleoside transporter density - high (A_1: temporal and parietal cortex; A_3: hippocampus), intermediate (A_1: hippocampus and frontal cortex), intermediate/low (A_{2A}: hippocampus, frontal, parietal and temporal cortex) and low (A_1: nucleus basalis) Ado receptor density	- Alzheimer's disease	- A_{2A} receptor antagonists - nucleoside transport inhibitors (e.g., propentofylline)	[11, 20, 23, 186, 203-207, 215-218, 220, 231, 317, 318]

Abbreviations: A_1 receptor/A_{2A} receptor/A_{2B} receptor/A_3 receptor: $A_1/A_{2A}/A_{2B}/A_3$ subtype of adenosine receptors; ADA: adenosine deaminase; ADK: adenosine kinase; Ado: adenosine; CNT transporters: concentrative nucleoside transporters; ENT transporters: equilibrative nucleoside transporters; GDA: guanine deaminase; Guo: guanosine; Ino: inosine; PNP: purine nucleoside phosphorylase; Urd: uridine; XO: xanthine oxidase

e5'NT (in nucleotidase-associated pervasive developmental disorders) activity are associated with neurological symptoms, such as seizures and ataxia [35, 37, 38, 87, 336-338]. Increased serum 5'NT (enhanced AMP hydrolysis by e.g., glycosylphosphatidyl-inositol anchored and mainly soluble extracellular form of e5'NT) [82, 200] activity have also been shown in a chronic animal model of electroconvulsive shock [339], in patients with epilepsy and in clozapine-treated schizophrenic patients [72, 73] and in pentylenetetrazol-induced seizures [74, 75]. Moreover, increased e5'NT activity has also been demonstrated in the rat striatum after chronic clozapine treatment [340], in different rat models of epilepsy evoked by administration of pentylenetetrazol, kainic acid and pilocarpine [76-78] and in a rat model of Parkinson's disease [71]. Furthermore, 3-mercaptopropionic acid induced seizures, which increased e5'NT activity in the rat cerebellum [341]. The hippocampus of temporal lobe epileptic patients also showed enhanced e5'NT activity [68]. In addition, the pilocarpine-induced increase in e5'NT activity may be prevented by anti-epileptic drugs (phenytoin, carbamazepine) [342]. These results suggest that e5'NT may play a role in (i) sprouting control, (ii) the modulation of epileptic activity and (iii) blockade of spontaneous seizures by the production of the inhibitory neuromodulator Ado [72, 77, 343] and (iv) reactive synaptogenesis in temporal lobe epileptic patients [68]. Focal ischemia also increased the activ-

ity of cerebroprotective e5'NT, which may increase the levels of neuroprotective Ado [63, 118, 126, 344, 345]. Rosuvastatin increased the extracellular levels of Ado via an enhancement of e5'NT activity and, as a consequence, increased the vasodilatator response to ischemia [346]. Upregulation of e5'NT activity was also demonstrated in models of brain damage, such as the cortical stub injury [347, 348]. Thus, increased activity of e5'NT after brain injury, epilepsy and other conditions may be an adaptive response that increases the levels of extracellular Ado and enhances its neuroprotective/anti-epileptic/anti-epileptogenetic effects via A_1 receptors [1, 13, 79, 349]. In addition, modulation of e5'NT activity (e.g., by activators of e5'NT such as methotrexate) [350] may be a potential treatment used to improve cognitive and motor deficits, chronic inflammation associated with neurodegenerative diseases [114, 351], and febrile illness [352] via A_1 receptors. Moreover, interferon-β (IFN-β) upregulated e5'NT expression, which may have a role in the beneficial effects of IFN-β treatment in patients with multiple sclerosis via increased Ado levels-evoked enhancement of endothelial barrier function, suggesting the important neuromodulatory role of e5'NT in brain inflammation [126, 353-355]. However, both Ado and non-Ado nucleosides (such as Urd and Guo) continuously form intra- and extracellularly from corresponding triphosphates and are recycled/transported by nucleoside transporters [57, 59, 88], which exhibit anti-epileptic/neuroprotective effects [39, 322, 325, 356-358]. For example, activity of the ectonucleotidase cascade system increased after quinolinic acid-evoked seizures in rat hippocampal slices [359]. Both Guo and GMP via metabolism to Guo by e5'NT showed anti-convulsant effects in rats [360]. In addition, pentylenetetrazol kindling increased GMP hydrolysis in the rat hippocampus [78]. Furthermore, recombinant e5'NT exerted anti-nociceptive effects in mice via the hydrolysis of AMP and A_1 receptor activation [361]. Thus, application of recombinant e5'NT may be a therapeutic tool used to treat chronic pain [109, 300, 361, 362] and may likely serve as a treatment approach in several other CNS diseases in which increased levels of Ado may have beneficial effects, such as epilepsy. Consequently, elucidation of the precise role of 5'NTs and changes in its activity in the pathomechanism of brain disease may provide an opportunity for the application of 5'NT modulation as a promising therapeutic tool against several CNS diseases.

Inhibitors of e5'NT, such as APCP, 3,3′,4′,5,7-pentahydroxyflavon (quercetin), 1-amino-4-[4-fluoro-2-carboxyphenylamino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate (PSB-0952), sodium nitroprusside (SNP), and 1-amino-4-[2-anthracenylamino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate (PSB-0963) as well as anti-CD73 antibodies may potentially treat several diseases in the CNS [62, 124, 144, 254, 280, 363-368]. Indeed, APCP and quercetin reduced the proliferation of human glioma cells (U138MG cell line) by inhibition of e5'NT activity and, as a consequence, decreased the extracellular levels of the glioma cell proliferation/adhesion stimulator Ado [62, 367, 369, 370]. Decreasing e5'NT activity may also serve as a promising therapeutic target in melanoma, which could be metastatic to the brain [371]. In addition, inhibition of overexpressed cN-II may increase the cytotoxic effects of anti-viral and anti-cancer nucleoside analogs [159, 372]. However, APCP also showed convulsant effects in rats [373], and decreased activity of 5'NTs was demonstrated in brain sample homogenates obtained from patients with Alzheimer's disease [374]. These results suggest that the therapeutic application of 5'NTs inhibitors may be limited due to a reduction in the neuroprotective/anti-epileptic effects of nucleosides, such as Ado, Urd and Guo.

Taken together, the available data suggest that application of drugs effective on nucleoside metabolic enzymes, such as 5'NTs, nucleoside transporters and Ado receptors (Table **5**), may represent effective and safe therapeutic approaches against different CNS diseases via the re-establishment of the fine regulation of different physiological functions by the nucleosidergic system, which may be altered under pathological conditions in the human brain.

Since 5'NTs (and nucleoside transporters) are responsible for the activation of Ado receptors by increasing the extracellular Ado level, 5'NTs play a role in the regulation of physiological functions of nucleosides such as Ado. However, widespread distribution of Ado receptors has been demonstrated in several organs of human body and nucleoside system may be modulated by factors such as age and gender. Consequently, modulation of 5'NT activity by synthetic inhibitor/activator drugs (i) may cause an alteration in Ado-dependent physiological processes in different organs of the human body, (ii) may evoke not only beneficial effects but also unwanted side effects and (iii) may cause pathophysiological changes and diseases in the CNS. Thus, additional investigations should explore the potential use of 5'NTs modulation in the treatment of different human diseases.

Further studies are required (i) to understand the links between unevenly distributed nucleoside levels and the distribution of nucleoside metabolic enzymes activity, nucleoside transporters and Ado receptors under physiological conditions in the human brain, (ii) to reveal changes in the nucleoside system induced by different diseases in affected human brain areas, (iii) to obtain evidence for the precise role of different elements of the nucleoside system in the progression of human CNS diseases and (iv) to develop useful and safe pharmacological strategies based on the nucleoside system without (or with minimal) side effects against CNS diseases. To achieve this goal, the generation and comparison of functional anatomical brain maps of the nucleoside system containing information on (i) extracellular nucleoside levels, (ii) intracellular/tissue nucleoside levels, (iii) activity of intracellular nucleoside metabolic enzymes, (iv) activity of enzymes in ectonucleotidase cascade system, (v) expression of nucleoside transporters and (vi) nucleoside receptor density based on standard research/methodological conditions and simultaneous measurement of the same (age, gender, cause of death, etc.) samples from brain areas implicated in different brain diseases are required not only in healthy and diseased human brains but also in experimental animal models of different human CNS diseases.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

5'-dUMP	=	5'-deoxyuridine monophosphate
5'NT	=	5'-nucleotidase
A_1 receptor/ A_{2A} receptor/ A_{2B} receptor/ A_3 receptor	=	$A_1/A_{2A}/A_{2B}/A_3$ subtype of Ado receptors
AC	=	Adenylate cyclase
ADA	=	Adenosine deaminase
Ade	=	Adenine
ADK	=	Adenosine kinase
Ado	=	Adenosine
ADP	=	Adenosine diphosphate
AMP	=	Adenosine monophosphate
AMPDA	=	AMP deaminase
APCP	=	β-methyleneadenosine-5'-diphosphate
ATP	=	Adenosine triphosphate
AZT	=	3'-azido-2',3'-dideoxythymidine
cdN	=	Cytosolic 5'(3')-deoxyribonucleotidase
CDP	=	Cytidine diphosphate
CMP	=	Cytidine monophosphate
cN	=	Cytoplasmic nucleotidase
cN-IA	=	Cytosolic 5'-nucleotidase IA
cN-IB	=	Cytosolic 5'-nucleotidase IB
cN-II	=	Cytosolic 5'-nucleotidase II
cN-III	=	Cytosolic 5'-nucleotidase III
CNS	=	Central nervous system
CNT transporters	=	Concentrative nucleoside transporters
CNT1/CNT2/ CNT3 transporters	=	CNT1/CNT2/CNT3 subtype of concentrative nucleoside transporters
CTP	=	Cytidine triphosphate
Cyd	=	Cytidine
e5'NT	=	Ecto-5'-nucleotidase
ENT transporters	=	Equilibrative nucleoside transporters
ENT1/ENT2/ ENT3/ENT4 transporters	=	ENT1/ENT2/ENT3/ENT4 subtype of equilibrative nucleoside transporters
GDA	=	Guanine deaminase
GDP	=	Guanosine diphosphate
GMP	=	Guanosine monophosphate
GMPR	=	GMP reductase
Gn	=	Guanine
GTP	=	Guanosine triphosphate
Guo	=	Guanosine
HGPRT	=	Hypoxanthine-guanine phosphoribosyltransferase
Hyp	=	Hypoxanthine
IFN-β	=	Interferon-β
IMP	=	Inosine monophosphate
Ino	=	Inosine
mdN	=	Mitochondrial 5'(3')-deoxyribonucleotidase
NBTI	=	S-(4-nitrobenzyl)-6-thioinosine
NDP	=	Nucleoside diphosphate
NMP	=	Nucleoside monophosphate
NTP	=	Nucleoside triphosphate
PLC	=	Phospholipase C
PMcH-U	=	((±)-1-trans-(2-phosphonomethoxycyclohexyl)uracil)
PMcP-U	=	((±)-1-trans-(2-phosphonomethoxycyclopentyl)uracil)
PNP	=	Purine nucleoside phosphorylase
Thd	=	Thymidine
Thy	=	Thymine
UDP	=	Uridine diphosphate
Ura	=	Uracil
Urd	=	Uridine
UTP	=	Uridine triphosphate
Xn	=	Xanthine
XO	=	Xanthine oxidase

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