The regulation of brain nucleoside utilization

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Abstract.

The homeostatic regulation of intracellular purine and pyrimidine pools has long been studied at the level of de novo nucleotide synthesis. However, brain maintains the proper qualitative and quantitative nucleotide balance by salvaging preformed nucleosides, imported from blood stream, rather than by de novo synthesis from simple precursors. The main salvage enzymes are the nucleoside-kinases, catalyzing the ATP mediated phosphorylation of nucleosides in their 5'-position. Salvaged nucleoside-monophosphates are then either further phosphorylated, or converted back to nucleosides by a set of 5'-nucleotidases. This poses the following problem: why are nucleosides produced from nucleoside-monophosphates, to be converted back to the same compounds at the expense of ATP? As discussed in this article, the quantitative and qualitative intracellular balance of brain purine and pyrimidine compounds is maintained i) by the intracellular interplay between the rates of nucleoside-kinases and 5'-nucleotidases, ii) by the relative rates of the inward and outward nucleoside transport through equilibrative and concentrative transport systems, iii) by the metabolic cross-talk between extracellularly exported nucleoside-triphosphate breakdown and the intracellular process of nucleoside-triphosphate salvage synthesis.

Keywords Substrate cycles. 5'-nucleotidases. cN-I. cN-II . cN-III. Nucleoside recycling

Introduction

The regulation of the pools of purine and pyrimidines in the brain, their linked enzymes, and their cross talk to other pathways, such as neurotransmitter pathways, are currently being investigated by members of the metabolomics community. Purine and pyrimidine metabolism and its modulation is central for the regulation of many cellular functions [1]. However, brain has a limited capacity to synthesize purine and pyrimidine rings de novo from simple precursors [2-4], and relies on the salvage of preformed purine and pyrimidine rings, mainly in the form of nucleosides, for the synthesis of nucleotides [5]. And in fact defects of the salvage enzymes are tightly linked to a number of degenerative and neuropsychiatric diseases [6]. This raises the following 2 intriguing questions: i) what is the origin of brain nucleosides? ii) How does brain maintain its purine and pyrimidine nucleoside (NS) pools in the proper qualitative and quantitative balance? As discussed in this article, intra and extracellular 5'-nucleotidases and related enzymes, such as nucleoside-kinase (NS kinase), adenosine deaminase (ADA), AMP deaminase (AMPD), purine nucleoside phosphorylase (PNP) and hypoxantine guanine phosphorybosil transferase (HPRT) play a major role in these aspects of brain metabolism.

Liver 5'-nucleotidases are the major source of brain nucleosides

Liver is the main site of nucleotide synthesis in mammals [7]. As outlined in Fig.1, purine and pyrimidine de novo pathways lead to the synthesis of the four main nucleotides, AMP, GMP, UMP, and CMP. It should be emphasized that i) the biosynthesis of the purine and pyrimidine rings from simple precursors in the liver requires the expenditure of considerable energy, in terms of ATP hydrolysis and ii) neither free bases nor NSs are intermediates [7]. Free nucleosides are produced in the liver through nucleoside monophosphate (NMP) dephosphorylation, as catalized by at least three cytosolic 5'-nucleotidases, [8-11]. NSs are then transported into the blood stream, and, after crossing the blood brain barrier, are finally transported into brain cytosol. In this cell compartment NSs are salvaged to their respective NMPs, mainly by the action of specific ATP dependent NS-kinases [5]. Remarkably, this "salvage synthesis" in the brain requires the expenditure of only 1 ATP molecule per molecule of NMP synthesized.

An "Equilibrative Nucleoside Transporter" (ENT) family and a "Concentrative Nucleoside Transporter" (CNT) family are responsible for the membrane transport of NSs in a variety of tissues [12]. The ENT system is bidirectional, and is driven by the NS gradient across the plasma

membrane [13]. The regulation of the ENT transport from liver to blood might play a central role in maintaining the qualitative and quantitative homeostasis of NSs. Indeed, NS plasma concentrations are strictly maintained at a low µM levels [14]. The CNT system mediates the inwardly directed transport of NSs, driven by sodium and proton electrochemical gradients [15] and is responsible for the membrane transport of NSs into the intracellular space [16]. Moreover, it is now well established that brain possesses several cytosolic 5'-nucleotidases, which counteract the activities of NS kinases [9].

Metabolic regulation of intracellular adenosine in the brain

It has long been assumed that, in order to exert their functions, NSs should be released from the cells. For instance, Ado is produced intracellularly by AMP dephosphorylation, and is then transported across the cell membrane into the extracellular space. However, as discussed by Arch and Newsholme in 1978 in their seminal paper on Ado metabolism [17], the problem with this hypothesis is the localization of 5'-nucleotidase. Indeed, cytochemical, histochemical, and cell fractionation studies have unequivocally shown that 5'-nucleotidase activity is associated with plasma membrane in many districts, including brain, with its active site facing the extracellular milieu, and acts as an ecto-enzyme [18-21]. It was then hypothesized that the nucleotidase hydrolyzes exclusively extracellular NMPs, and that NSs are then transported into the cell. However, in 1988 Truong et al. described in rat heart a cytosolic 5'-nucleotidase, later named cN-I, whose kinetic and regulatory properties differ markedly from those of the plasma membrane associated 5'-nucleotidase [22]. AMP is the preferred substrate of CN-I, although its K_M value is the mM range, indicating a low affinity for AMP (vs. the broad specificity and the K_M value in the µM range of the membrane bound enzyme, named e-N) (23). However, CN-I. is potently activated by ADP, but not by ATP [24, 25]. ATP acts as a strong inhibitor of the membrane bound enzyme [20, 21, 26, 27]. Another important cytoplasmic 5'-nucleotidase, named cN-II, first identified by Itoh in 1967 [28], preferentially dephosphorylates IMP and GMP, and is highly sensitive to ATP activation [29-31]. A pyrimidine 5'-nucleotidase (cN-III), specific for UMP and CMP, is highly expressed in reticulocytes, and is assumed to participate in the process of erythrocyte maturation [11, 32]. The role of cN-I and cN-II, in the utilization of Ado and of its deamination product, inosine (Ino), in neurons and glia is illustrated in Fig 2. Brain Ado concentration is maintained at its lowest level among the other main nucleosides [33] by the relative extent of saturation of two cytosolic enzyme proteins: adenosine kinase (AdoK) (enzyme 1 of Fig. 2), adenosine deaminase (enzyme 4) and cN-I (enzyme 3). Because the K_m values for Ado of the kinase and the deaminase are around 0.2 µM and 50 µM, respectively [5], the kinase becomes saturated with Ado at low

micromolar concentration, and phosphorylates Ado to 5'-AMP at its V_{max}. The cell "senses" any further increase of Ado (either uptaken from the extracellular space, or generated by ATP breakdown), and irreversibly deaminates it to Ino. Contrary to Ado, Ino cannot be phoshorylated by a specific kinase, which is absent in mammals [34]. It must undergo prior phosphorolysis to hypoxanthine (Hyp) and ribose 1-phosphate (Rib 1-P) by PNP (enzyme 5 of Fig.2), HPRT (enzyme 6), and cN-I (enzyme 8), in order to be converted to IMP. Interestingly, contrary to GMP and AMP, IMP is not further phosphorylated in the brain [34]. However, as shown in Fig.1, it is the precursor of GMP and AMP.

AMP follows two distinct catabolic pathways in normoxic and anoxic/ischemic conditions

Fig.2 shows that cytosolic AMP breakdown to Ino may follow two distinct pathways. In the first route, where IMP-GMP preferring cN-II is involved, AMP undergoes deamination by adenylate deaminase (enzyme 7), followed by IMP dephosphorylation, catalyzed by cN-II (enzyme 8) (the "IMP pathway": AMP→ IMP → inosine [27]). In the second route AMP is dephosphorylated to Ado by the AMP preferring cytosolic 5'-nucleotidase cN-I (enzyme 3), and Ado is deaminated to Ino by ADA (enzyme 4) (the "adenosine pathway" : AMP → Ado → inosine [27]). The catalytic and regulatory properties of AMPD, cN-II, and cN-I, strongly suggest that the IMP pathway and the adenosine pathway are operative in normoxic and in anoxic/ischemic conditions, respectively. Both AMPD [35-39] and cN-II [29,40,41] are strongly activated by physiological (normoxic) ATP concentrations. Therefore, it is conceivable that in normoxic conditions, ATP directs AMP catabolism towards the IMP pathway, rather than the adenosine pathway, thus contributing in maintaining a low intracellular Ado level. Indeed, IMP, not Ado, accumulates, when brain cytosol is incubated with AMP in the presence of ATP at normoxic concentration [27, 42]. In anoxic/ischemic conditions ATP is drastically broken down [43], allowing Ado to be generated by the ADP activated cN-I. Indeed, Ado, not IMP, accumulates, when brain cytosol is incubated with AMP in the presence of ATP at anoxic/ischemic concentration [27, 42].

AMP modulates the energy metabolism in man

To date the increased cytosolic [AMP] (or the increased [AMP]/[ATP] ratio) is considered to be a signal of compromised energy status of the cell [44]. AMP "senses" this abnormal condition, and exerts a pivotal effect on the regulation of cellular and whole body metabolism. AMP interacts either directly with key catabolic regulatory enzyme proteins [45-47], or indirectly through an ATP activated protein kinase, named AMP kinase (AMPK) [44]. AMP binds to the allosteric sites of

muscle glycogen phosphorylase and 6-phosphofructokinase, and stabilizes their active conformation. During cellular stresses that deplete ATP, and consequently elevate AMP concentration, AMP specifically binds to one of the subunits of AMPK and switches off ATP consuming anabolic pathways, while switching on the ATP producing catabolic pathways, thus restoring the physiological ATP level. This molecular mechanism is operative in several mammalian districts, including brain [48-50] (Fig. 3).

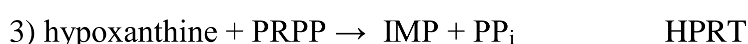
It may be speculated that fluctuations of AMP concentrations in cell cytosol, modulated by cN-I and AMPD, may influence AMPK activity [9, 51, 52]. Accordingly, silencing cN-I increases the [AMP], activates AMPK, and stimulates AMP downstream signaling [53] in human and mouse muscle. Conversely, overexpression of cN-I decreases AMPK activation [54]. These observations suggest that rodents may be useful models for the study of the role of AMP in energy metabolism in man. Metformin, the most widely used drug for type II diabetes [55], activates AMPK through inhibition of AMPD [56]. Taken together, these observations suggest that cN-I and AMPD, two key enzymes of the AMP catabolic pathways, may effectively modulate the energy metabolism in man.

cN-II as a regulator of the oxypurine cycle

Cytosolic PNP, HPRT, and cN-II (enzymes 5, 6, and 8 of Fig. 2), and the three intermediates, Hyp, Ino, and IMP, constitute the “oxypurine cycle” (OPC), also called purine nucleoside cycle first described by Barankiewicz in 1982 [57]. In an in vitro reconstituted cycle the rate of the IMP hydrolysis, modulated by ATP, acting as an activator of cN-II, and by Pi, acting as an inhibitor [41,58], was found to be the major determinant of the cycle’s rate [34]. The OPC turns over at the expense of PRPP, its net reaction being: PRPP + H_2O → ribose-1-phosphate + PP:

1) IMP + H_2O → inosine + P_i cN-II

2) inosine + Pi → hypoxanthine + Rib 1-P PNP


3) hypoxanthine + PRPP → IMP + PP_i HPRT

net reaction: PRPP + H_2O → Rib-1-P + PP_i

Excessive PRPP synthesis may lead to unbalanced purine nucleotide synthesis, and to clinical gout [59-61]. PRPP, a high energy sugar phosphate, with a high potential of ribose-5-phosphate transfer, is maintained at a low micromolar level [14]. It has therefore been hypothesized

that the OPC may serve as a metabolic regulatory system for PRPP pool homeostatic regulation. [34,41,62]. In addition, the OPC might contribute to maintaining the cytoplasmic homeostatic concentration of IMP, Ino and Hyp. Like Ino, Guo cannot be phosphorylated in its 5'- position, due to the absence of a specific kinase [63,64]. A similar substrate cycle, equally driven by cN-II, PNP, and HPRT, involves GMP, guanosine (Guo), and guanine (Gn) [34]. As shown in Fig. 4 , this "guanosine cycle" is linked to the apparent Guo deamination, resulting from the recycling of Rib-1-P through Guo phosphorolysis, Gn deamination, and xanthine ribosylation [27].

Finally, we emphasize that the absence of cytosolic Ado-phosphorylase in man [65]. prevents the existence of a substrate cycle involving AMP, Ado, and Ade.

The homeostatic regulation of uridine in brain

Uridine (Urd) is the predominant circulating pyrimidine in man [66]. Its plasma level is strictly maintained at 3-5 μM [14]. In the central nervous system it acts as the main precursor a of pyrimidine nucleotide salvage synthesis [66-68], and as an important synaptogenic nucleoside [69]. Moreover, Urd has found an important clinical application in the treatment of orotic aciduria [70]. The homeostatic regulation of Urd is maintained by the concerted action of three enzymes: uridine kinase (UrdK), catalyzing the first step of UTP and CTP salvage synthesis, uridine phosphorylase (UP), catalyzing the first step of Urd degradation, and cytosolic 5'-nucleotidase cN-III , catalyzing the hydrolytic dephosphorylation of UMP. cN-III plays a major role in the elimination of pyrimidine nucleosides formed from RNA degradation during erythropoiesis [71-75]. In brain it counteracts the activity of UrdK, thus contributing to Urd homeostasis through a seemingly "futile cycle" UMP→ Urd→ UMP (Fig. 5) whereby neuronal and glial cells maintain intracellular pools of pyrimidine compounds. A similar AMP→ Ado → AMP cycle is operative in liver cells [75].

Urd enters neuronal and glial cells via the Na^+-dependent, high affinity, CNT proteins or via ENT proteins [16]. The rationale of the molecular mechanism underlying metabolic Urd homeostasis mostly stands on the inhibition exerted on UrdK by UTP and CTP levels exceeding the physiological levels [67, 77-79]. Thus, at relatively low UTP and CTP levels, UrdK is fully active, and pyrimidine salvage synthesis outweighs catabolism. On the contrary, at relatively high UTP and CTP levels, the inhibition of UrdK channels Urd towards phosphorolysis. Urd, mainly generated by cN-II, is exported into bloodstream, and pyrimidine catabolism outweighs anabolism. Finally, ribose-1-phosphate, generated by Urd phosphorolysis, is transformed into PRPP, which is then used for purine salvage synthesis [67].

The nucleoside map of central nervous system

It has been hypothesized that NS transporters maintain the qualitative and quantitative balance of intracellular NTP pools, a major factor for the stability of the genetic information [67] and for the regulation of extracellular purine and pyrimidine molecular signals, generated from exported NTPs [80]. NS transporters, as well as purine and pyrimidine receptors, have a distinct regional distribution in CNS [81-85] suggesting that each brain district has its own specific NS pool. Kovacs et al. have measured the concentration of NSs and their metabolites in whole brain and spinal cord, and in 65 separate sites of the human CNS [33]. They found that NSs and their metabolites are unevenly distributed, thus providing the first NS map of CNS. Kovacs' data strongly suggest that, in addition to NS transport, also the relative expression and activities of both extra- and intracellular 5'-nucleotidases, and of enzymes of NS degradation may contribute to regulate the homeostatic NS levels in brain districts. NS degradation pathways are more active in glial cells, compared to neuronal cells [86]. Accordingly, the NS lowest concentrations were detected in cerebral white matter areas, where the glia/neuron ratio is relatively high.

A strict interconnection between neuronal and glial nucleotide and nucleoside metabolism modulates the brain information process [87,88]. ATP can be released as such, or coreleased with other transmitters by the presinaptic terminal, and stimulates P2 receptors of astroglia. Alternatively, released ATP can be degraded to adenosine, which acts on P1 receptors of astroglia (neuronal-glial signaling). Also astrocytes can release ATP, which can either stimulate neuronal P2 purinergic ionotropic receptors, or generate adenosine which acts on presinaptic P1 receptors (glial-neuronal signaling) [89].

Ectonucleotidases as a source of extracellular brain nucleosides: the brain nucleoside recycling

The dynamic interplay between NS-kinases, and 5'-nucleotidases (the "substrate cycle"), maintains the intracellular balance among the four main NTP pools. A second level of control comes from the modulated extracellular generation of NSs from NTPs. New insights into the importance of this important issue came from the recognition that intracellular ATP is stored inside small cytosolic exocytotic vesicles and granules in high millimolar concentration range [21] , and may be exported into the extracellular milieu from glial and neuronal cells [90,91], and from astrocytic cells [92,93]. ATP acts as an extracellular neuroactive ligand. After interacting with P2X and P2Y receptors [80], ATP signal is terminated by its catabolic breakdown to Ado, a neuroactive signal exerting an exceptional range of trophic functions in the central nervous system. After

interacting with a multiplicity of purinergic G-protein coupled P1 receptors, Ado signaling is terminated by its transport into brain cell cytosol [94-98].

The temporal patterns of the sequential production of ADP, AMP, and Ado in synaptosomes [99] and in astrocytes [100], and the time courses of all possible ATP catabolites formation by brain plasma membrane preparations [27] showed that in normal aerobic conditions extracellular ATP breakdown follows the so called “adenosine pathway” (ATP → ADP→ AMP→Ado) (Fig. 2). It is now well established that also cytosolic UTP can be released in the extracellular space to interact with P2Y receptors [101-104]. The UTP signal is inactivated by the ecto-nucleotidase cascade system, which converts UTP back to Urd. No specific Urd receptor has been clearly identified yet, however imported Urd acts as an essential precursor of phosphatdylcholine [105]. Interestingly, the feed-forward inhibition exerted by ATP on 5’-ectonucleotidase, the last enzyme of the ecto-nucleotidase cascade system, causes a considerable AMP and UMP accumulation, before Ado and Urd are produced [27, 104]. These observations strongly suggest that the function of the ecto-nucleotidase cascade system acting on extracellular ATP and UTP is the timely local inactivation of two important neurotrophic purines (ATP and Ado) and pyrimidines (UTP and Urd), respectively.

A second function of the ecto-nucleotidase cascade system comes from its metabolic cross-talk with the intracellular anabolic NTP salvage synthesis. In addition to ATP, other nucleotides for which a physiological function as extracellular messengers has not been identified (e.g. GTP, GDP, GMP, IMP, and CTP) [5, 104, 106, 107] are stored in cytosolic vesicles and/or granules. Moreover, contrary to Ado, no specific receptors have been definitely identified, purified, and cloned yet for Urd, Guo, Ino and Cyd (cytidine). Most likely, the neurotrophic actions of these nucleosides are mediated intracellularly, after their uptake [66, 108]. Nevertheless, UTP, GTP, and CTP are good substrates of brain ectonucleotidases and exert a competitive feed-forward inhibition of Urd, Guo, and Cyd generation from their respective nucleoside monophosphates, catalyzed by 5’-ectonucleotidase. The time courses of the intermediates formed are similar to those observed for the production of Ado from ATP. Taken together, these observations suggest that, apart from the well established modulation of the purinergic and pyrimidinergic agonists availability at their receptor sites, ectonucleotidases may serve the local recycling of NSs in the brain. Under physiological conditions, ectonucleotide-metabolizing enzymes play a tightly concerted actions with nucleotide and nucleoside transporters [109, 110]. It is therefore conceivable that, as shown in Fig. 6, a cross talk exists between the extra- and intracellular metabolism of purine and pyrimidine NSs in the brain. The extracellular space is the major site of NSs generation through three dephosphorylation steps of released triphosphates, whereas brain cytosol is the major site of

multiple phosphorylations of uptaken NSs s at their 5'-position. Modulation of both extracellular NSs generation by membrane bound ectonucleotidases, and intracellular nucleoside phosphorylation by cytosolic kinases might contribute to maintain the right purine and pyrimidine nucleotide balance in the brain. Finally, the local recycling of NSs avoids their dilution into bloodstream, with a considerable spatial-temporal advantage.

Conclusions

Brain nucleotide synthesis depend on the availability of preformed NSs and on the activities of the salvage pathway enzymes. Brain nucleoside homeostasis is maintained by the modulation of NS transport [16] and by the degree of saturation of key enzymes by their substrates or competitive inhibitors [69, 105], rather by the allosteric control of key enzyme activities. To date, it is safe to state that, apart from the well established modulation of ATP, ADP and Ado (the purinergic agonists), UTP and UDP (the pyrimidinergic agonists) at their respective receptors [95], ectonucleotidases may also serve the local reutilization of NSs in the brain. After their production in the extracellular space, NSs are transported into neurons and glia, and converted to NTPs via a set of purine and pyrimidine salvage enzymes. Finally, an aliquot of NTPs are transferred into cytosolic vesicles and /or granules, and released to the extracellular space.

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ABBREVIATIONS

Ado	=	Adenosine
ADA	=	Adenosine deaminase
AdoK	=	Adenosine kinase
AMPD	=	AMP deaminase
AMPK	=	AMP activated protein kinase
cN-I	=	AMP preferring 5'-nuclotidase
cN-II	=	IMP-GMP preferring 5'-nucleotid**a**se
cN-III	=	Uridine-Cytidine preferring 5'-nucleotidase
CNT	=	Concentrative nucleoside transporters
Cyd	=	Cytidine
ENT	=	Equilibrative nucleoside transporters
Gua	=	Guanine
Guo	=	Guanosine
Hyp	=	Hypoxanthine
HPRT	=	Hypoxanthine-guanine phosphoribosyltransferase
Ino	=	Inosine
NDP	=	NucleosideDiPhosphate
NMP	=	NucleosideMonoPhosphate
NTP	=	NucleosideTriPhosphate
NS	=	Nucleosides
NS kinase	=	Nucleoside kinase
PNP	=	Purine nucleoside phosphorylase
Pi	=	Inorganic phosphate
PPi	=	Pyrophosphate

PRPP = Phosphoribosyl Pyrophosphate

OPC = OxyPurine Cycle

Rib 1-P = Ribose 1-phosphate

Ura = Uracil

Urd = Uridine

UrdK = Uridine kinase

UP = Uridine phosphorylase

Xan = Xanthosine

Xao = Xanthine

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Figure legends

Fig. 1. Diagram of the metabolic pathways for the de novo synthesis of purine nucleotides nucleosides, starting from PRPP (**A**) and of pyrimidine nucleotides and nucleosides starting from glutamine (**B**) in the liver. IMP is the common precursor of AMP and GMP, and UMP is the precursor of CMP. In both pathways free nucleosides are neither precursors, nor intermediates.

Nucleosides are produced by nucleoside-monophosphate dephosphorylation catalyzed by cytosolic 5'-nucleotidases: 1) succinyl-AMP synthetase; 2) succinyl-AMP lyase; 3) IMP dehydrogenase; 4) GMP synthetase; 5) nucleoside-monophosphate kinase; 6) nucleoside-diphosphate kinase; 7) CTP-synthetase.

Fig. 2. Utilization of adenosine and inosine for the salvage synthesis of purine nucleotides in brain. Enzymes 3 and 1 constitute the AMP → adenosine → AMP substrate cycle, which regulates the homeostatic balance of adenine nucleotides. The enzymes participating in this pathways are : 1) adenosine kinase; 2) adenylate kinase; 3) 5'-nucleotidase cN-1; 4) adenosine deaminase; 5) purine nucleoside phosphorylse; 6) hypoxanthine guanine phosphoribosyl transferase; 7) AMP deaminase; 8) 5'-nucleotidase cN-II. IMP is not further phosphorylated, however it is converted to AMP and GMP by the same pathways reported in Fig. 1.

Fig. 3. The role of cytosolic 5'-nucleotidase cN-I and AMP deaminase in regulating energy metabolism. Normally, energy demand increases cytosolic ADP concentration (enzyme 1), and consequently the flow of adenylate kinase towards AMP (enzyme 2). Binding of AMP activates muscle glycogen phosphorylase (enzyme 3), muscle and liver 6-phosphofructokinase (enzyme 4) and AMPK-P, the phosphorylated form of AMP kinase (AMPK, enzyme 5). 5'-Nucleotidase and AMP deaminase (enzymes 6 and 7, respectively) catalyze the entry steps of AMP catabolism, thus modulate the energy cell metabolism (modified from reference [9]).

Fig. 4. Utilization of guanosine for the salvage synthesis of guanine nucleotides. Enzymes 5, 1, and 2 constitute the GMP→ guanosine → guanine "oxypurine cycle", which regulates the balance of guanine nucleotide pool. The enzymes participating in the pathway are: 1) purine nucleoside phosphorylase; 2) hypoxanthine guanine phosphoribosyl phosphotransferase; 3) nucleoside-monophosphate kinase; 4) nucleoside-diphosphate kinase; 5) 5'-nucleotidase cN-II; 6) guanase. The concerted action of enzymes 1 and 6 lead to xanthosine deamination, thus bypassing the absence of xanthosine deaminase in man.

Fig. 5. Utilization of uridine for the salvage synthesis of uracil nucleotides. The enzymes participating in the pathway are: 1) uridine kinase; 2) 5'-nucleotidase cN-III; 3) nucleoside-monophopsphate kinase; 4) nucleoside-diphosphate kinase; 5) cytidine deaminase; 6) CTP synthetase; 7) uridine phosphorylase. Enzymes 1 and 2 constitute the UMP → uridine → UMP substrate cycle which regulates the homeostatic balance of uracil nucleotides.

Fig. 6. Cross talk between extracellular mileu and cell cytosol for nucleoside recycling in brain. Nucleoside triphosphates are released from cytosolic vesicles into the extracellular space, and broken down to their respective nucleosides by the ecto-nucleotidase cascade system. Nucleosides are then taken up by neurons and glial cells, to be phosphorylated in their 5'positions via specific nucleoside-kinases. The nucleoside monophosphates are further phosphorylated to nucleoside triphosphates by non specific kinases. The enzymes participating in the nucleoside brain recycling process are: 1) nucleoside kinase; 2) 5'-nucleotidase 3) nucleoside monophosphate kinase; 4) nucleoside diphosphate kinase; 5) ecto-triphosphohydrolase; 6) ecto-diphosphohydrolase; 7) ecto-5'-nucleotidase. The recycling process is modulated by several factors, including nucleoside transport, the relative rates of cytosolic nucleoside kinases and 5'-nucleotidases, and the feed forward inhibition exerted by extracellular nucleoside triphosphates on ecto-5'-nucleotidase, the last enzyme of the extracellular enzyme cascade system.

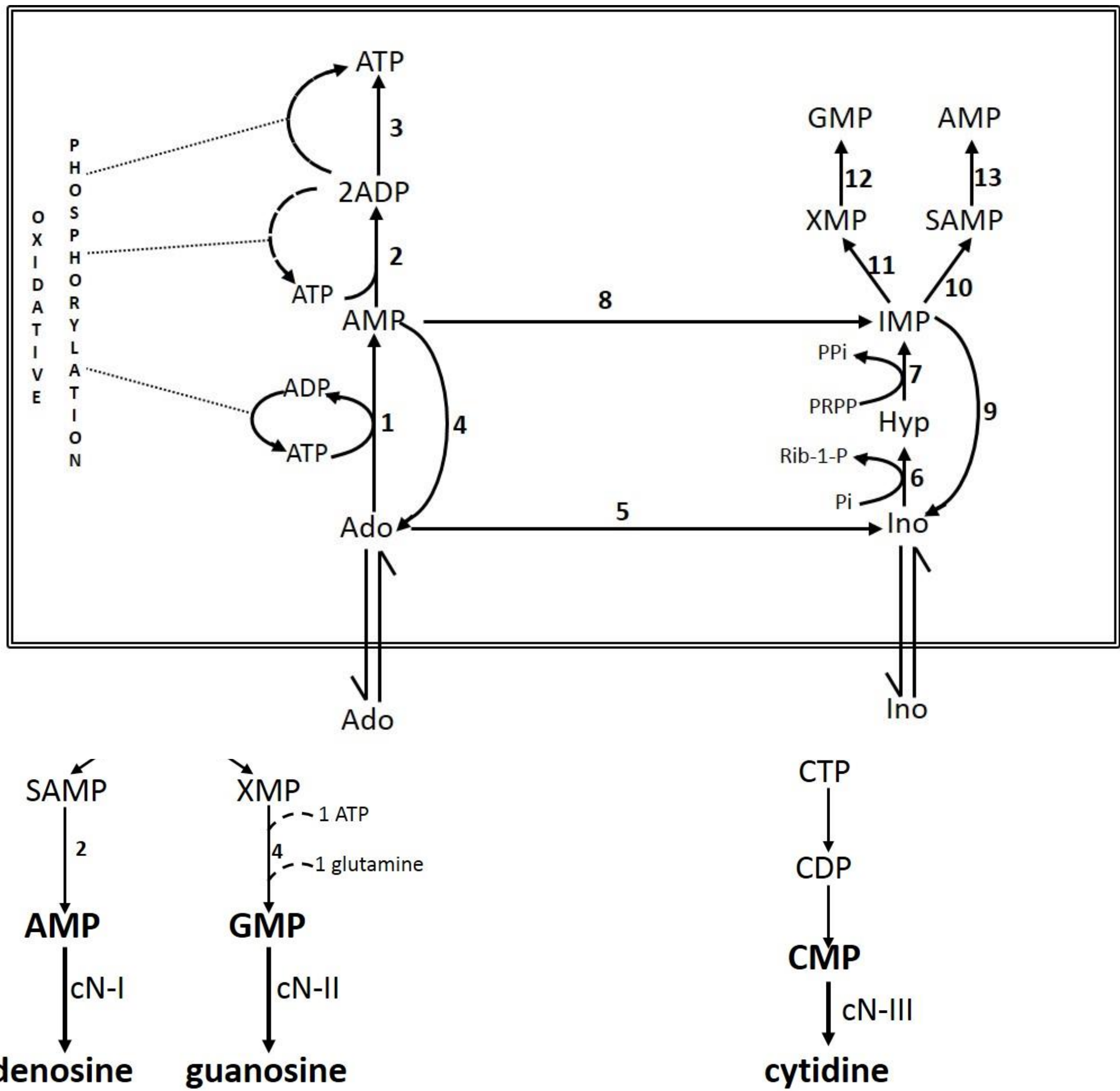

Figure 1

Figure 2

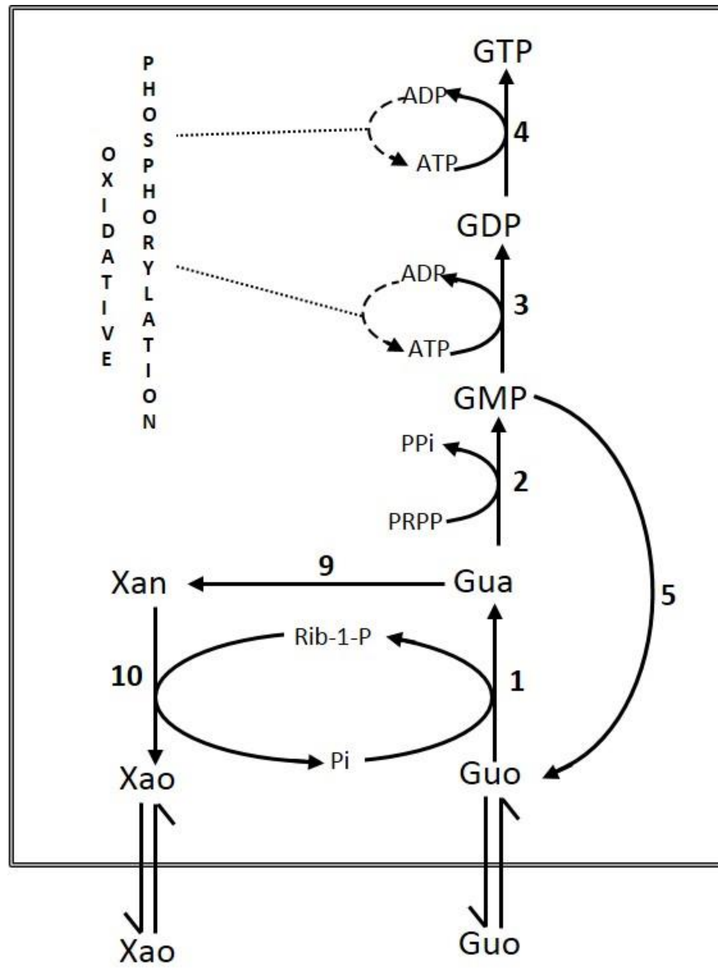

Figure 3

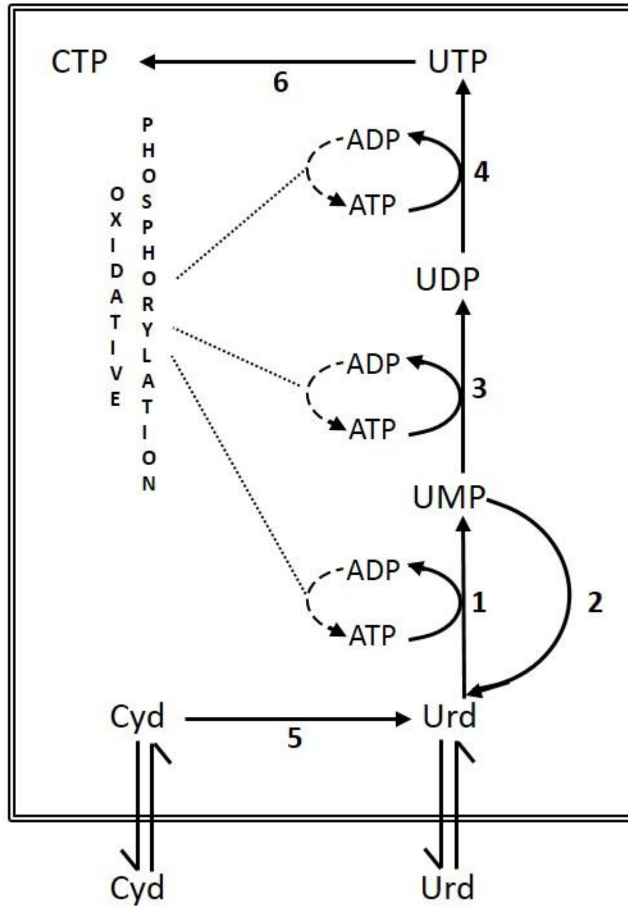

Figure 4

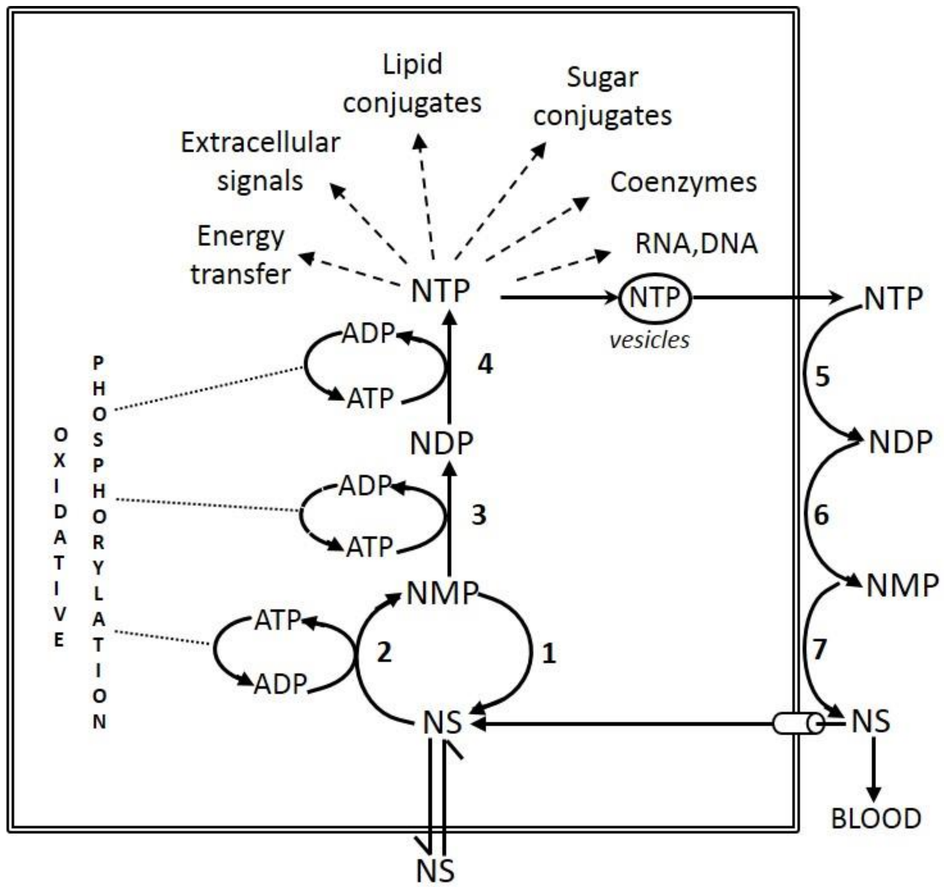

Figure 5

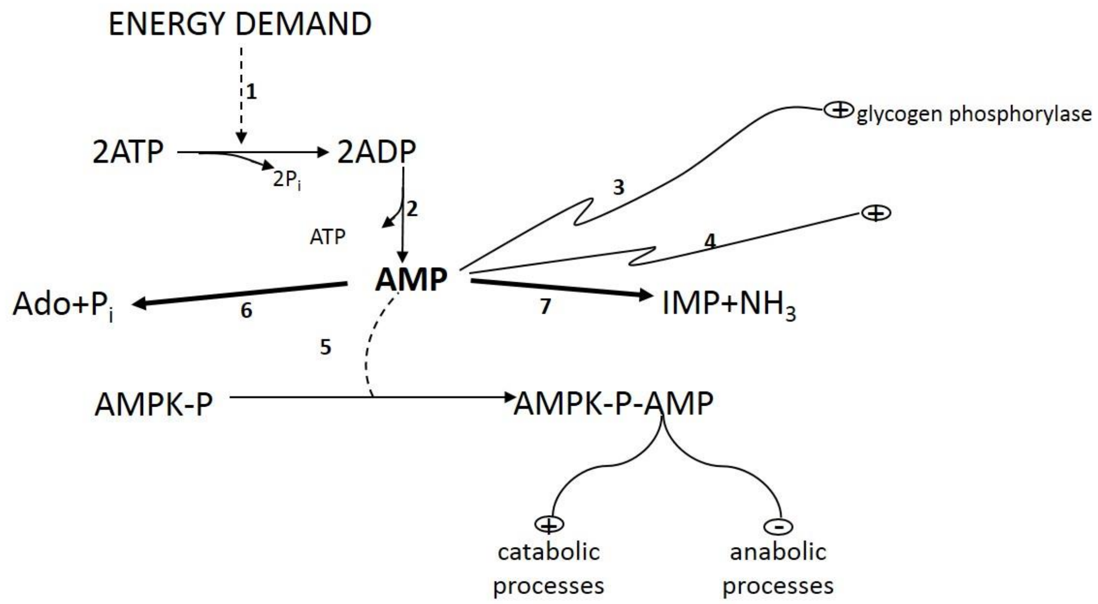

Figure 6