CMLS, Cell. Mol. Life Sci. 60 (2003) 786–793
1420-682X/03/040786-08
DOI 10.1007/s00018-003-2371-x

CMLS Cellular and Molecular Life Sciences

Research Article

The purine nucleoside cycle in cell-free extracts of rat brain: evidence for the occurrence of an inosine and a guanosine cycle with distinct metabolic roles

C. Barsotti, R. Pesi, F. Felice and P. L. Ipata*

Department of Physiology and Biochemistry, University of Pisa, Via Santa Maria 55, 56126 Pisa (Italy),
Fax + 39 050 502583, e-mail: ipata@dfb.unipi.it

Received 20 December 2002; received after revision 20 February 2003; accepted 27 February 2003

Abstract. The purine nucleoside cycle is a cyclic pathway composed of three cytosolic enzymes, hypoxanthine-guanine phosphoribosyltransferase, IMP-GMP specific 5′-nucleotidase, and purine-nucleoside phosphorylase. It may be considered a 'futile cycle', whose net reaction is the hydrolysis of 5-phosphoribosyl-1-pyrophosphate to inorganic pyrophosphate and ribose 1-phosphate. The availability of a highly purified preparation of cytosolic 5′-nucleotidase prompted us to reconstitute the purine nucleoside cycle. Its kinetics were strikingly similar to those observed when dialyzed extracts of rat brain were used. Thus, when the cycle is started by addition of inorganic phospate (Pi) and hypoxanthine or inosine (the 'inosine cycle'), steady-state levels of the intermediates are observed and the cycle 'turns over' as far as 5-phosphoribosyl-1-pyrophosphate is being consumed. In the presence of ATP, which acts both as an activator of IMP-GMP-specific 5′-nucleotidase and as substrate of nucleoside mono- and di-phosphokinases, no IDP and ITP are formed. The inosine cycle is further favored by the extremely low xanthine oxidase activity. Evidence is presented that ribose 1-phosphate needed to salvage pyrimidine bases in rat brain may arise, at least in part, from the 5-phosphoribosyl-1-pyrophosphate hydrolysis as catalyzed by the inosine cycle, showing that it may function as a link between purine and pyrimidine salvage. When the cycle is started by addition of Pi and guanine (the 'guanosine cycle'), xanthine and xanthosine are formed, in addition to GMP and guanosine, showing that the guanosine cycle 'turns over' in conjunction with the recycling of ribose 1-phosphate for nucleoside interconversion. In the presence of ATP, GDP and GTP are also formed, and the velocity of the cycle is drastically reduced, suggesting that it might metabolically modulate the salvage synthesis of guanyl nucleotides.

Key words: Purine salvage; PRPP homeostasis; purine nucleoside cycle; Rib1-P shuttle; nucleoside interconversion; rat brain.

Introduction

5-Phosphoribosyl 1-pyrophosphate (PRPP) may be considered as a 'high-energy' sugar phosphate, with a high potential of 5-phosphoribosyl transfer. A widely accepted tenet is that under normal conditions, the PRPP pool is maintained at a low level, to avoid excessive and unbalanced nucleotide synthesis. The regulation of PRPP synthesis by PRPP synthetase and its utilization by phosphoribosyltransferases are the major factors maintaining the intracellular level of PRPP [1]. However, an additional factor, the purine nucleoside cycle (PNC), should also be considered. The PNC, also named the 'oxypurine cycle,' is a cyclic pathway composed of three cytosolic enzymes:

* Corresponding author.

the hypoxanthine-guanine phosphoribosyltransferase (HGPRT), the purine-nucleoside phosphorylase (PNP), and the IMP-GMP-specific 5′-nucleotidase (cN-II). Because its net reaction is the hydrolysis of PRPP, PNC has been proposed to play a role in the metabolic regulation of intracellular PRPP levels [2, 3]. One of the products of PNC, ribose 1-phosphate (Rib1-P), is needed to salvage pyrimidine bases and to interconvert nucleosides [4, 5]. Moreover, due to the substrate specificity of the three enzymes of the cycle, PNC may function with either guanine or hypoxanthine compounds (figs. 1, 2). The two pathways will be called the 'guanosine cycle' and 'inosine cycle' respectively, throughout this paper. Both cycles have been reconstituted in vitro, by using commercial HGPRT and PNP and a highly purified preparation of cN-II, cloned and expressed in *Escherichia coli* [6]. Their kinetics were strikingly similar to those observed when crude extracts of rat brain were used as an enzyme source. Our results suggest that the two cycles may play additional metabolic roles. In those areas, such as brain, where purine salvage synthesis is particularly active [4, 7, 8], PRPP may indeed be the source of Rib 1-P needed for pyrimidine salvage and 5′-fluorouracil (5-FU) activation [9, 10]. Due to the presence a high guanase activity in rat brain [11], the guanosine cycle 'turns over' in conjunction with the recycling of Rib1-P for nucleoside interconversion first described by us in 1997 [5] and may play a role in modulating the salvage synthesis of guanyl nucleotides known to be important intracellular signals.

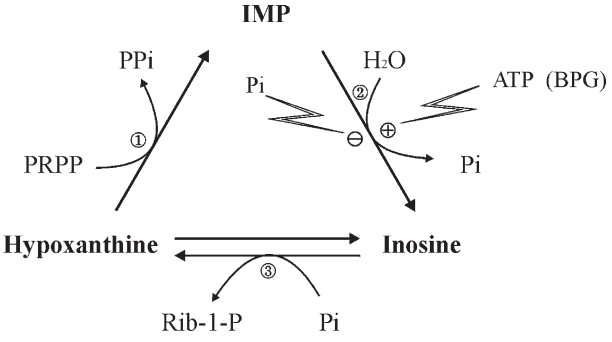

Figure 1. The inosine cycle. The enzymes participating in the cycle are: 1, HGPRT; 2, cN-II; 3, PNP. The net reaction is the hydrolysis of PRPP to Rib1-P and inorganic pyrophosphate (PPi). The allosteric activation by ATP and 2,3-bisphosphaglycerate (BPG), and inhibition by inorganic phosphate (Pi), modulate the cycle rate.

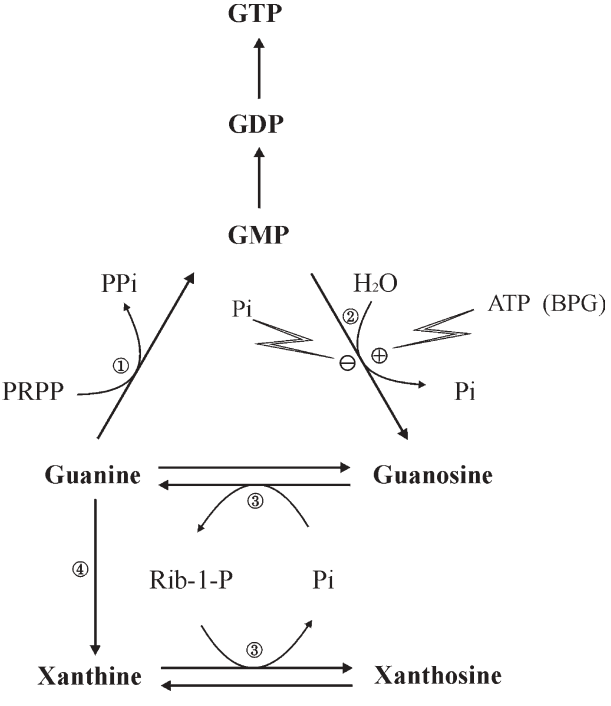

Figure 2. The 'Rib1-P shuttle'-linked guanosine cycle. The enzymes participating in this pathway are: 1, HGPRT; 2, cN-II; 3, PNP; 4, guanase. The guanosine cycle rate is limited by guanine deamination and GMP phosphorylation. The final products of the two linked pathways are GTP and xanthosine.

Materials and methods

Materials

[8-^{14}C]-adenine (55 mCi/mmol), [8-^{14}C]-guanine (53.3 mCi/mmol), [8-^{14}C]-inosine (460 mCi/mmol), [8-^{14}C]-hypoxanthine (54 mCi/mmol), [2-^{14}C]-uracil (54 mCi/mmol), [2-^{14}C]-5-FU (55 mCi/mmol), PNP, HGPRT, guanase, PRPP, 2,3-bisphosphoglycerate (BPG), dithiothreitol, bases, nucleosides, and nucleotides were from Sigma. Bovine recombinant cN-II was prepared as described previously [6]. Hi Safe II Scintillation liquid was purchased from Wallac. Polyethyleneimine (PEI)-cellulose-precoated thin-layer plastic sheets (0.1 mm thick) were purchased from Merck and prewashed once with 10% NaCl and three times with deionized water before use. All other chemicals were of reagent grade.

Preparation of rat brain extracts

Three-month-old male Sprague-Dawley rats (250 g) were sacrificed by decapitation. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies. The brain was removed and kept frozen at −80 °C until needed. Storage times did not exceed 3 months.

The brain was cut into small pieces, washed with cold saline and gently homogenized with a hand-driven Potter homogenizer in 3 vol of 100 mM Tris-HCl buffer, pH 7.4, with 20 mM KCl and 1 mM dithiothreitol. The homogenate was centrifuged at 4 °C at 39,000 g for 1 h. The supernatant fluid obtained was dialyzed overnight at 4 °C in dialysis bags against 10 mM Tris-HCl buffer, pH 7.4, supplemented with 1 mM dithiothreitol, and is referred to as crude extract. The preparation, which is devoid of

plasma membranes, nuclei, and mitochondria, proved to be extremely useful in studies on rat brain purine and pyrimidine salvage synthesis [4, 5] as well as in ATP breakdown studies under conditions mimicking anoxia [12]. Protein concentration was determinated by the Coomassie blue-binding assay, using bovine serum albumin as standard [13].

The inosine and guanosine cycles in rat brain

The standard reaction mixtures contained 5 mM Tris-HCl buffer, pH 7.4, 100 µM [8-^{14}C]-inosine (8000 dpm/nmol) (the inosine cycle), or 100 µM [8-^{14}C]-guanine (11,000 dpm/nmol) (the guanosine cycle), 1 mM PRPP, 8.3 mM $MgCl_2$, 4.5 mM BPG, 5 mM KH_2PO_4, and brain extract, 1.15 mg of protein/ml. Modifications of the standard incubation mixture are indicated in the figure and table legends. The reaction was started by addition of crude extract. At different time intervals, the reaction was stopped by rapidly drying portions of 10 µl of the incubation mixture on PEI-cellulose-precoated thin-layer plastic sheets, and a chromatogram was developed in n-propanol/NH_3/trichloroacetic acid (100%)/H_2O (75 : 0.7 : 5 : 20, v/v). In all separations, appropriate standards were used and detected as ultraviolet-absorbing areas which were excised and counted for radioactivity with 8 ml of scintillation liquid.

In vitro reconstitution of the inosine and guanosine cycles

The standard incubation mixture contained, in a final volume of 110 µl, 100 µM [8-^{14}C]-inosine (8000 dpm/nmol), 1 mM PRPP, 8.3 mM $MgCl_2$, 4.5 mM BPG, 1 mM KH_2PO_4, 4.4 mU of PNP, 4.4 mU of HGPRT, 22 mU of cN-II in 5 mM Tris-HCl buffer, pH 7.4 (the inosine cycle). The guanosine cycle was reconstituted in the same experimental conditions with 100 µM [8-^{14}C]-guanine (11,000 dpm/nmol) and in the absence or presence of 22 mU of guanase. Modifications of the incubation mixtures are indicated in the figure and table legends. The reactions were started by addition of [8-^{14}C]-inosine or [8-^{14}C]-guanine. At different time intervals, the reactions were stopped and analyses were performed as described under 'The inosine and guanosine cycles in rat brain.'

Determination of PRPP levels during the inosine cycle

Crude extracts, containing 345 µg of protein, were incubated in a total reaction volume of 300 µl, in the presence of 100 µM inosine, 2 mM PRPP, 8.3 mM $MgCl_2$, 4.5 mM BPG, 5 mM KH_2PO_4, 5 mM Tris-HCl buffer, pH 7.4. The incubation was carried out at 37 °C. At different time intervals, a 30-µl portion was withdrawn, heated for 90 s at 100 °C, and centrifuged. PRPP was determined in the supernatant fluid according to King et al. [14], with minor modifications. The reaction mixtures contained, in a final volume of 50 µl, 2 mM [8-^{14}C]-hypoxanthine (8000 dpm/nmol), 68 mU of HGPRT, 20 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.4. The reaction mixtures were incubated at 37 °C for 1 h. Then, 10 µl was applied to a PEI-cellulose-precoated thin-layer plastic sheet and developed with 1.4 M LiCl. The marker compounds were detected with a UV lamp. The zones corresponding to IMP were excised and counted for radioactivity with 8 ml of scintillation liquid.

Results and discussion

The rat brain inosine cycle maintains a low intracellular PRPP level and provides Rib1-P needed for pyrimidine salvage and 5′-FU activation

The inosine cycle was reconstituted using highly purified enzyme preparations (fig. 3 A). At 100 µM inosine and 5 mM inorganic phosphate (Pi) initial concentrations, steady state levels of inosine and hypoxanthine were attained. When a rat brain extract was used as enzymes source (fig. 3 B), hypoxanthine, IMP, as well as inosine, attained steady-state levels, as occurs in vivo [15]. Strikingly, after about 20 min of incubation, hypoxanthine abruptly accumulated at the expense of IMP degradation. We explain these results as follows. While PRPP is being consumed, the intermediates are continuously recycled. But as soon as PRPP disappears, HGPRT becomes inactive. Then the cycle is interrupted, causing IMP degradation by cN-II, and hypoxanthine accumulation. These results appear particularly relevant in light of our recent observations, showing that the Lesh Nyhan syndrome is metabolically related not only to a deficiency of HGPRT, but also to a three- to fivefold increase in cN-II [16]. At 2 mM initial PRPP concentration, the steady-state period lasted about 40 min, indicating that at both 1 and 2 mM PRPP initial concentrations, the velocity of the cycle remained unchanged (fig. 3 C). Figure 3 C also shows the time course of PRPP breakdown as catalyzed by the inosine cycle. We cannot exclude that a small amount of PRPP is removed by the action of non-specific phosphatases and of the recently discovered PRPP-pyrophosphatase [17]. Hypoxanthine and inosine are the major metabolites accumulated. A thorough study of their precise metabolic fate is beyond the aim of the present work. Nevertheless, we notice that in the reconstituted cycle (fig. 3 A), once PRPP has been consumed, inosine is the major metabolite, as expected on the basis of the equilibrium constant of the PNP reaction, which favors inosine formation. In contrast, in the incubation mixture with rat brain extract, hypoxanthine is the major metabolite (fig. 3 B). Table 1 shows the requirements of the rat brain inosine cycle. The cycle responds to protein and inosine initial concentration, as well as to the allosteric activators of cN-II, ATP and BPG. This enzyme is also inhibited by Pi [18]. In fact, increasing the Pi concentration from 5 to 10 mM increases the duration of the steady period from 20 to 30 min (data not shown). We propose that modulation

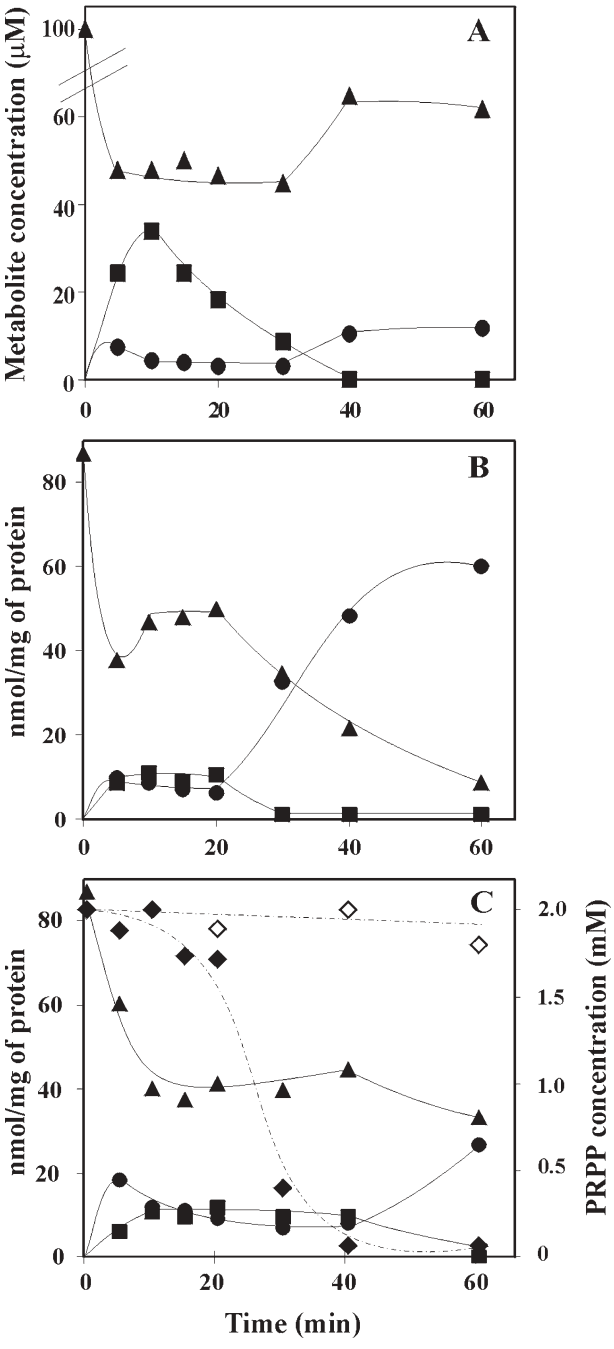

Figure 3. The inosine cycle as reconstituted in vitro (*A*) and as catalyzed by rat brain extract (*B*, *C*). ■, IMP; ●, hypoxanthine; ▲, inosine; ◆, PRPP; ◇, PRPP in the absence of rat brain extract. (*A*) the reaction mixture contained 100 μM [^{14}C]-inosine, 5 mM Pi, 1 mM PRPP, 4.5 mM BPG, 8.3 mM $MgCl_2$, 0.04 U/ml of PNP, 0.04 U/ml of HGPRT, and 0.2 U/ml of cN-II, in 5 mM Tris-HCl buffer, pH 7.4. (*B*, *C*) The complete reaction mixture contained 100 μM [^{14}C]-inosine, 1 mM PRPP (*B*) or 2 mM PRPP (*C*), 5 mM KH_2PO_4, 8.3 mM $MgCl_2$, 4.5 mM BPG, 5 mM Tris-HCl buffer, pH 7.4, and brain extract, 1.15 mg of protein/ml. Rat brain extract was added at zero time.

of cN-II by ATP and Pi might in turn regulate the inosine cycle flux. At a normal low [Pi]/[ATP] ratio, as found in well-oxygenated cells, cN-II is fully active and the velocity of the inosine cycle is maximal. As a consequence, the PRPP pool is maintained at a low level, thus avoiding unnecessary 'salvage' or 'de novo' nucleotide synthesis. During ischemia, the [Pi]/[ATP] ratio raises drastically [19, 20], the situation is reversed, and enough PRPP is made available for purine nucleotide salvage synthesis. This process, which in our opinion represents the real purine salvage, is favored by the increase in purine base levels occurring in ischemia [21] and by the increase in PRPP which accompanies massive ATP degradation, as occurs in rat brain during anoxia [12]. In in vivo conditions, the velocity of the inosine cycle might be influenced by the purine nucleotide cycle, in which IMP is a common intermediate [22], and by IMP dehydrogenase [23]. The other two components, inosine and hypoxanthine, should not leave the cycle. This is suggested (i) by the virtual absence of xanthine oxidase [22, 24] and inosine kinase activities [10, 25] and (ii) by the lack of further IMP phosphorylation in rat brain [12].

Among different rat organs tested, brain was found to be the most active (ca 42 nmol/mg of protein per minute), followed by testis (ca. 5), kidney and heart (ca. 2), and liver (ca. 1). The low activity of the liver extract should be ascribed to its high xanthine oxidase activity [26], even though the levels of HGPRT and PNP [4] as well as cN-II [27] are comparable with those of brain.

In previous papers, we have shown that in rat brain, uracil salvage and 5-FU activation are Rib1-P-mediated processes involving pyrimidine base (or base analog) ribosylation followed by multiple phosphorylation steps [9, 10]. In this pathway, uridine phosphorylase acts as an anabolic, rather than catabolic enzyme. Our results have

Table 1. Rat brain inosine cycle requirements.

Reaction mixture	Steady state duration (min)	Rate of PRPP disappearence (nmol/mg of protein per minute)
Complete system	20	42.3
2.3 mg of protein/ml	10	84,5
4.6 mg of protein/ml	5	168.5
0.575 mg of protein/ml	>100	<8,5
PRPP 2 mM	40	42.3
Minus BPG	30	28.2
Inosine 0.05 mM	60	14.1
ATP 5 mM instead of BPG 4.5 mM	20	42.3

The complete system contained 100 μM [^{14}C]-inosine, 1 mM PRPP, 5 mM KH_2PO_4, 8.3 mM $MgCl_2$, 4.5 mM BPG, 5 mM Tris-HCl buffer, pH 7.4, and brain extract, 1.15 mg of protein/ml. The reaction was initiated by addition of brain extract. The average rate of PRPP disappearance was calculated from the time of the hypoxanthine rise at the end of the steady-state period (see fig. 3C).

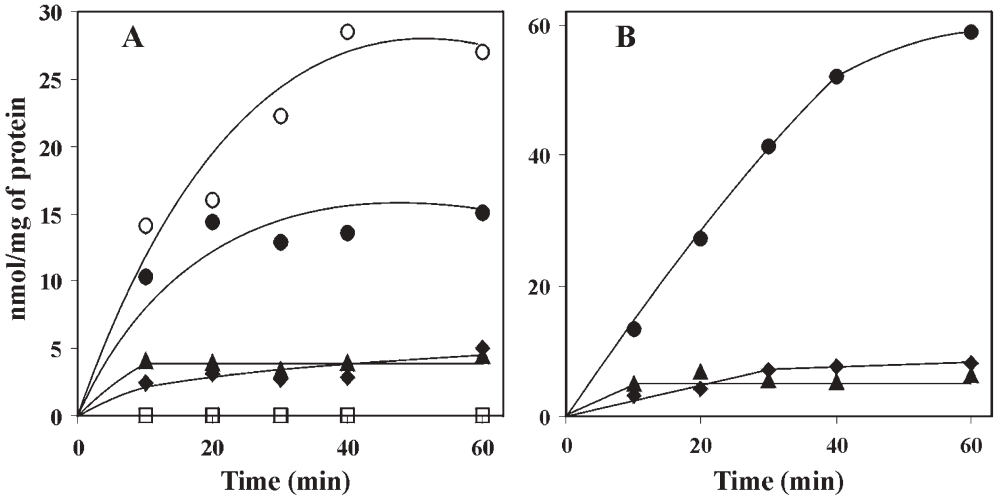

Figure 4. Inosine cycle-mediated uracil salvage (*A*) and 5′-FU activation (*B*). ■, 5′-fluorouridine; ▲, 5′-FUMP; ◆, 5′-FUDP + 5′-FUTP. No uridine- or 5′-fluorouridine-nucleotides were formed in the absence of ATP. (*A*) The reaction mixtures contained 100 μM hypoxanthine, 1 mM PRPP, 5 mM KH_2PO_4, 8.3 mM $MgCl_2$, 4.5 mM BPG (open symbols) or 5 mM ATP (filled symbols), 5 mM Tris-HCl buffer, pH 7.4, brain extract, 1.15 mg of protein/ml, and 1 mM [^{14}C]-uracil. Rat brain extract was added at zero time. ●, uridine; ▲, UMP; ◆, UDP + UTP; □, uridine formed in the absence of hypoxanthine. (*B*) the reaction mixture contained 100 μM hypoxanthine, 1 mM PRPP, 5 mM KH_2PO_4, 8.3 mM $MgCl_2$, 5 mM ATP, 5 mM Tris-HCl buffer, pH 7.4, brain extract, 1.15 mg of protein/ml, and 1 mM [^{14}C]-5′-FU. Rat brain extract was added at zero time.

been elegantly confirmed by Cao et al. [28] in a uridine phosphorylase gene knockout cell model. We now propose that Rib1-P needed to ribosylate uracil or 5-FU might well arise from PRPP breakdown, as catalyzed by the inosine cycle. This idea is strongly supported by the results presented in figure 4A, showing that uracil is readily converted into uridine by rat brain extracts, provided hypoxanthine is present in the reaction mixture. In our experimental conditions, hypoxanthine starts the inosine cycle, and thus Rib1-P formation. In the presence of ATP, uridine and 5′-fluorouridine are further phosphorylated to their respective ribonucleotides (figs. 4A, B). One might speculate that the inosine cycle may act as a linkage between PRPP mediated purine salvage and Rib1-P-mediated pyrimidine salvage, as illustrated in figure 5. We observe that an adenosine cycle, similar to the inosine cycle, cannot be operative, due to the absence of adenosine phosphorylase in mammals [29].

The 'Rib1-P shuttle'-linked guanosine cycle modulates the intracellular pool of guanine compounds

Figure 6 compares the kinetic features of the reconstituted guanosine cycle with those of the guanosine cycle, as catalyzed by rat brain extract. The kinetics of the reconstituted system (fig. 6A, B) become comparable with those of the brain extract (fig. 6C), provided an additional enzyme, guanase, is added to PNP, HGPRT, and cN-II. Guanase has been recently cloned from rat brain [30] and an early ob-

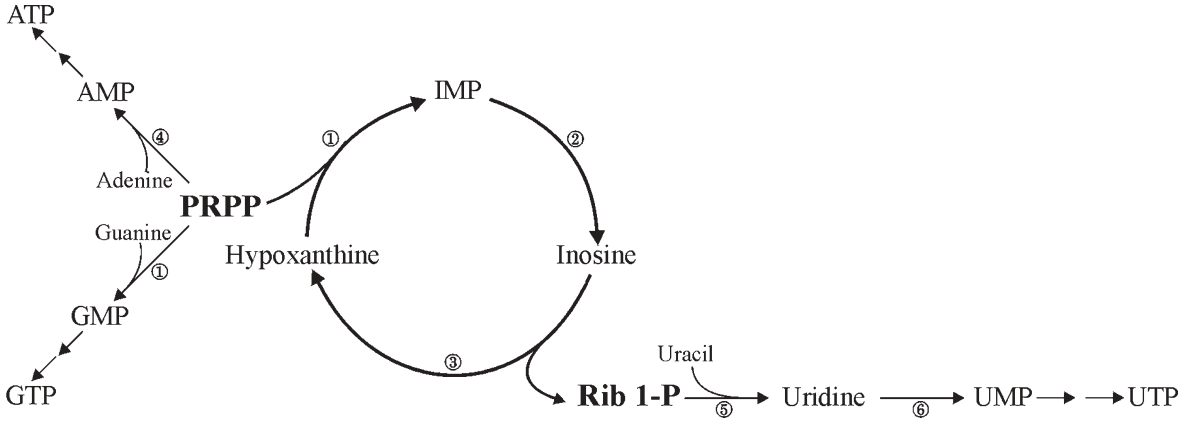

Figure 5. The central position of the inosine cycle between PRPP-mediated purine salvage and Rib1-P-mediated pyrimidine salvage. 1, HGPRT; 2, cN-II; 3, PNP; 4, adenine phosphorybosyl-transferase; 5, uridine phosphorylase; 6, uridine kinase. Note that IMP, in contrast to GMP, AMP and UMP, is not further phosphorylated.

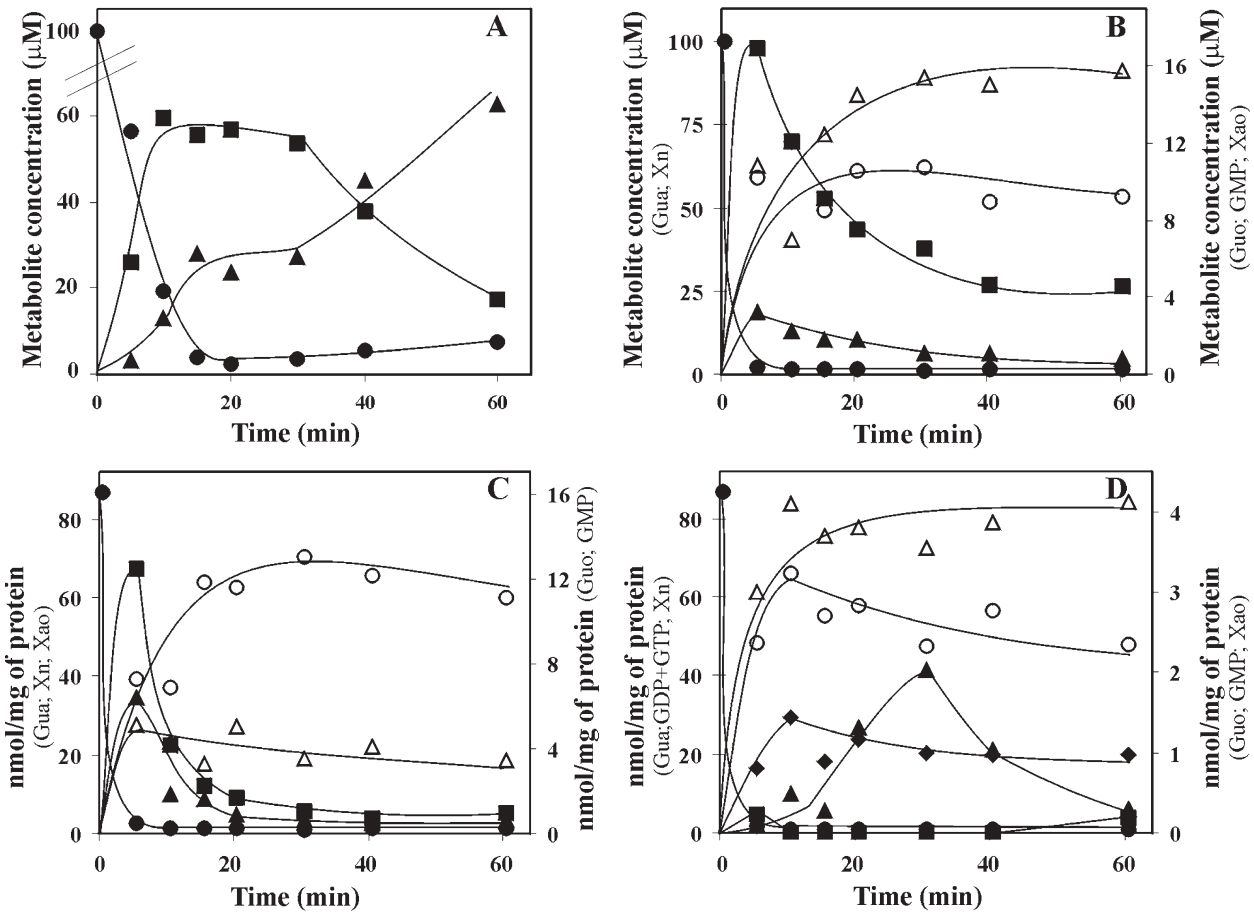

Figure 6. The guanosine cycle as reconstituted in vitro (*A*, *B*) and as catalyzed by rat brain extracts (*C*, *D*). ■, GMP; ●, guanine (Gua); ▲, guanosine (Guo); ◆, GDP + GTP; ○, xanthine (Xn); △, xanthosine (Xao). (*A*, *B*) The reaction mixtures contained 100 μM [^{14}C]-guanine, 1 mM PRPP, 5 mM KH_2PO_4, 8.3 mM $MgCl_2$, 4.5 mM BPG, 0.04 U/ml of PNP, 0.04 U/ml of HGPRT, 0.2 U/ml of cN-II, in 5 mM Tris-HCl buffer, pH 7.4. (*B*) 0.02 U/ml of guanase was added. (*C*, *D*) The reaction mixtures contained 100 μM [^{14}C]-guanine, 1 mM PRPP, 5 mM KH_2PO_4, 8.3 mM $MgCl_2$, 5 mM Tris-HCl buffer, pH 7.4, brain extract, 1.15 mg of protein/ml, and 4.5 mM BPG (*C*) or 5mM ATP (*D*).

servation on its distribution throughout mouse and rat forebrain has been confirmed [11, 31]. The rapid decay of [^{14}C]-guanine was accompanied by the appearance of a series of radioactive compounds. Thus, in addition to GMP and guanosine, the other two intermediates of the cycle, xanthine and xanthosine were also formed (fig. 6C). These results may be explained by assuming that in rat brain, the guanosine cycle 'turns over' in conjunction with the recycling of Rib1-P for purine nucleoside interconversion, otherwise called by us the 'Rib1-P shuttle' [5]. In this pathway, Rib1-P needed to ribosylate xanthine stems from the 'turning over' of the cycle. Therefore, the amount of xanthosine formed may be taken as an index of the velocity of the guanosine cycle (table 2). Only trace amounts of [^{14}C]-uric acid could be detected. ATP has a dual effect on the guanosine cycle, being not only an activator of cN-II, but also a substrate of nucleoside mono- and di-phosphokinases. Thus, in the presence of ATP at physiological concentration [32, 33], GDP and GTP were formed, in addition to xanthine, guanosine, GMP, and xanthosine (fig. 6D). Phosphorylation of GMP reduces the velocity of the guanosine cycle by subtracting one of its intermediates, as shown by the decrease in xanthosine formed when ATP substituted BPG as cN-II activator (fig. 6D, table 2). Table 2 also shows that brain was by far the most active, among a series of rat tissues tested. An additional GMP-leaving route due to the action of GMP reductase [34] might also be considered to occur in vivo. Our results suggest that the guanosine cycle and the Rib1-P shuttle, working in conjunction, might modulate the synthesis of guanyl nucleotides in those areas, such as brain, which strongly depend on the salvage pathway for nucleotide synthesis. Guanine nucleotides in the form of GDP, GTP, and cyclic GMP are becoming increasingly recognized in neuronal signal pathways, so that regulation of their synthesis could alter cellular signaling. Moreover, we propose that, at least in part, xanthosine present in serum [35] might originate from the guanosine cycle-linked Rib1-P shuttle.

Finally, we emphasis that an artifactual presence of ecto 5′-nucleotidase in our cell-free brain extract, even when

Table 2. Effect of ATP on the level of xanthosine synthesized through the guanosine cycle and the Rib1-P shuttle.

	Xanthosine (nmol/mg of protein after 60 min incubation)			GDP + GTP (nmol/mg of protein after 60 min incubation)	
	Minus activators	+ BPG	+ATP	Minus ATP	+ ATP
Brain	5.70	18.48	4.13	0.00	20.04
Heart	0.82	1.02	0.56	0.00	11.71
Liver	0.55	0.66	0.52	0.00	6.80
Kidney	0.92	1.72	0.54	0.00	7.60
Testis	1.17	2.10	0.56	0.00	9.17

The standard reaction mixture contained 100 μM [^{14}C]-guanine, 1 mM PRPP, 5 mM KH_2PO_4, 8.3 mM $MgCl_2$, 4.5 mM BPG, 5 mM Tris-HCl buffer, pH 7.4, and rat organ extract, 1.15 mg of protein/ml. The reaction was initiated by addition of extract.

obtained with a gentle homogenization procedure, cannot be excluded a priori. However, as pointed out by Torrecilla et al. [36], in the presence of ATP as low as 10 μM, this enzyme would not have functional importance related to the metabolism of purine nucleoside monophosphates.

Acknowledgements. This work was supported by C.N.R. Target Project 'Biotecnologie' and by the Italian MURST National Interest Project 'Molecular mechanisms of cellular and metabolic regulation of polynucleotides, nucleotides and analogs.'

1 Becker M. A. (2001) Phosphoribosylpyrophosphate synthetase and the regulation of phosphoribosylpyrophosphate production in human cells. Progr. Nucleic Acids Res. Mol. Biol. **69:** 115–147
2 Barankiewicz J., Gelfand E. W., Issekutz A. and Cohen A. (1982) Evidence for active purine nucleoside cycles in human mononuclear cells and cultured fibroblasts. J. Biol. Chem. **257:** 11597–11600
3 Berman P. A. and Human L. (1990) Regulation of 5-phosphoribosyl 1-pyrophosphate and of hypoxanthine uptake and release in human erythrocytes by oxypurine cycling. J. Biol. Chem. **265:** 6562–6568
4 Cappiello M., Mascia L., Scolozzi C., Giorgelli F. and Ipata P. L. (1998) In vitro assessment of salvage pathways for pyrimidine bases in rat liver and brain. Biochim. Biophys. Acta. **1425:** 273–281
5 Giorgelli F., Bottai C., Mascia L., Scolozzi C., Camici M. and Ipata P. L. (1997) Recycling of α-D-ribose 1-phosphate for nucleoside interconversion. Biochim. Biophys. Acta **1335:** 16–22
6 Allegrini S., Pesi R., Tozzi M. G., Fiol C. J., Johnson R. B. and Eriksson S. (1997) Bovine cytosolic IMP/GMP-specific 5′-nucleotidase: cloning and expression of active enzyme in *Escherichia coli*. Biochem. J. **328:** 483–487
7 Nelson D. L. and Cox M. M. (2000) Lehninger Principles of Biochemistry, Freeman, New York
8 Mascia L., Cappiello M., Cherri S. and Ipata P. L. (2000) In vitro recycling of alpha-D-ribose 1-phosphate for the salvage of purine bases. Biochim. Biophys. Acta. **1474:** 70–74
9 Mascia L. and Ipata P. L. (2001) Activation pathways of 5-fluorouracil in rat organs and in PC12 cells. Biochem. Pharmacol. **62:** 213–218
10 Barsotti C. and Ipata P. L. (2002) Pathways for α-D-ribose utilization for nucleobase salvage and 5-fluorouracil activation in rat brain. Biochem. Pharmacol. **63:** 117–122
11 Davies L. P. and Taylor K. M. (1979) Rat brain guanine deaminase; correlation with regional levels of cyclic GMP phosphodiesterase. J. Neurochem. **33:** 951
12 Barsotti C., Tozzi M. G. and Ipata P. L. (2002) Purine and pyrimidine salvage in whole rat brain: utilization of ATP-derived ribose-1-phosphate and 5-phosphoribosyl-1-pyrophosphate generated in experiments with dialyzed cell-free extracts. J. Biol. Chem **277:** 9865–9869
13 Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254
14 King M. T., Passonneau J. V. and Veech R. L. (1990) Radiometric measurement of phosphoribosylpyrophosphate and ribose 5-phosphate by enzymatic procedures. Anal. Biochem. **187:** 179–186
15 Curto R., Voit E. O., Sorribas A. and Cascante M. (1997) Validation and steady-state analysis of a power-law model of purine metabolism in man. Biochem. J. **324:** 761–775
16 Pesi R., Micheli M., Jacomelli G., Peruzzi L., Camici M., Garcia-Gil M. et al. (2000) Cytosolic 5′-nucleotidase hyperactivity in erythrocytes of Lesch-Nyhan syndrome patients. Neuroreport **11:** 1827–1831
17 Kawaguchi T., Veech R. L. and Uyeda K. (2001) Regulation of energy metabolism in macrophages during hypoxia: roles of fructose 2,6-bisphosphate and ribose 1,5-bisphosphate. J. Biol. Chem. **276:** 28554–28561
18 Pesi R., Turriani M., Allegrini S., Scolozzi C., Camici M., Ipata P. L. et al. (1994) The bifunctional cytosolic 5′-nucleotidase: regulation of the phosphotransferase and nucleotidase activities. Arch. Biochem. Biophys. **312:** 75–80
19 Leach R. M., Sheehan D. W., Chacko V. P. and Sylvester J. T. (1998) Effects of hypoxia on energy state and pH in resting pulmonary and femoral arterial smooth muscles. Am. J. Physiol. **275:** L1051–L1060
20 Corbett R. J., Laptook A. R., Nunnally R. L., Hassan A. and Jackon J. (1988) Intracellular pH, lactate, and energy metabolism in neonatal brain during partial ischemia measured in vivo by 31P and 1H nuclear magnetic resonance spectroscopy. J. Neurochem. **51:** 1501–1509
21 Phillis J. W., O'Regan M. H., Estevez A. Y., Song D. and VanderHeide S. J. (1996) Cerebral energy metabolism during severe ischemia of varying duration and following reperfusion. J. Neurochem. **67:** 1525–1531
22 Schultz V. and Lowenstein J. M. (1976) Purine nucleotide cycle: evidence for the occurrence of the cycle in brain. J. Biol. Chem **251:** 485–492
23 Hall H. I. and Wyrick S. D. (1996) Cytoxicity of [(5,6-dichloro-9a-n-propyl-2,3,9,9a-tetrahydro-3-oxo-1 fluoren-7-yl)oxy]acetic acid, an agent known to reduce brain edema. Biomed. Pharmacother. **50:** 19–23
24 Markley H. G., Faillace L. A. and Mezey E. (1973) Xanthine oxidase activity in rat brain. Biochim. Biophys. Acta **309:** 23–31
25 Mascia L., Cotrufo T., Cappiello M. and Ipata P. L. (1999) Ribose 1-phosphate and inosine activate uracil salvage in rat brain. Biochim. Biophys. Acta **1472:** 93–98
26 Pritsos C. A. (2000) Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system. Chem. Biol. Interact. **129:** 195–208
27 Itoh R. and Tamada K. (1991) Determination of cytoplasmic 5′-nucleotidase which preferentially hydrolyses 6-hydroxypurine nucleotides in pig, rat and human tissues by immunotitration. Int. J. Biochem. **23:** 461–465
28 Cao D., Russell R. L., Zhang D., Leffert J. J. and Pizzorno G. (2002) Uridine phosphorylase (–/–) murine embryonic stem cells clarify the key role of this enzyme in the regulation of the

pyrimidine salvage pathway and in the activation of fluoropyrimidines. Cancer Res. **62:** 2313–2317
29 Stone T. W and Simmonds H. A. (1991) Purine: Basic and Clinical Aspects. Kluwer, Dordrecht
30 Paletzki R. F. (2002) Cloning and characterization of guanine deaminase from mouse and rat brain. Neuroscience **109:** 15–26
31 Berger S. J., Carter J. G. and Lowry O. H. (1985) Distribution of guanine deaminase in mouse brain. J. Neurochem. **44:** 1736–1740
32 Hisanaga K., Onodera H. and Kogure K. (1986) Changes in levels of purine and pyrimidine nucleotides during acute hypoxia and recovery in neonatal rat brain. J. Neurochem. **47:** 1344–1350
33 Traut T. W. (1994) Physiological concentrations of purines and pyrimidines. Mol. Cell. Biochem. 140: 1-22
34 Deng Y., Wang Z., Ying K., Gu S., Ji C., Huang Y et al. (2002) NADPH-dependent GMP reductase isoenzyme of human (GMPR2) expression, purification, and kinetic properties. Int. J. Biochem. Cell Biol. **34:** 1035–1050
35 Niwa T., Takeda N. and Yoshizumi H. (1998) RNA metabolism in uremic patients: accumulation of modified ribonucleosides in uremic serum. Kidney Int. **53:** 1801–1806
36 Torrecilla A., Marques A. F. P., Buscalioni R. D., Oliviera J. M. A., Teixeira N. A., Atencia E. A. et al. (2001) Metabolic fate of AMP, IMP, GMP and XMP in the cytosol of rat brain: an experimental and theoretical analysis. J. Neurochem. **76:** 1291–1307