The object of this work stems from our previous studies on the mechanisms responsible of ribose-1-phosphate- and 5-phosphoribosyl-1-pyrophosphate-mediated nucleobase salvage and 5-fluorouracil activation in rat brain (Mascia, L., Cappiello M., Cherri S., and Ipata, P. L. (2000) Biochim. Biophys. Acta 1472, 70–74; Mascia, L., Cotrufo, T., Cappiello, M., and Ipata, P. L. (1999) Biochim. Biophys. Acta 1472, 93–98). Here we show that when ATP at “physiological concentration” is added to dialyzed extracts of rat brain in the presence of natural nucleobases or 5-fluorouracil, adenine-, hypoxanthine-, guanine-, uracil-, and 5-fluorouracil-ribonucleotides are synthetized. The molecular mechanism of this peculiar nucleotide synthesis relies on the capacity of rat brain to salvage purine and pyrimidine bases by deriving ribose-1-phosphate and 5-phosphoribosyl-1-pyrophosphate from ATP even in the absence of added pentose or pentose phosphates. The levels of the two sugar phosphates formed are compatible with those of synthesized nucleotides. We propose that the ATP-mediated 5-phosphoribosyl-1-pyrophosphate synthesis occurs through the action of purine nucleoside phosphorylase, phosphopentomutase, and 5-phosphoribosyl-1-pyrophosphate synthetase. Furthering our previous observations on the effect of ATP in the 5-phosphoribosyl-1-pyrophosphate-mediated 5-fluorouracil activation in rat liver (Mascia, L., and Ipata, P. L. (2001) Biochem. Pharmacol. 62, 213–218), we now show that the ratio [5-phosphoribosyl-1-pyrophosphate]/[ATP] plays a major role in modulating adenine salvage in rat brain. On the basis of our in vitro results, we suggest that massive ATP degradation, as it occurs in brain during ischemia, might lead to an increase of the intracellular 5-phosphoribosyl-1-pyrophosphate and ribose-1-phosphate pools, to be utilized for nucleotide resynthesis during reperfusion.

It is well established that inosine and other ribonucleosides are transported into mammalian cells (1, 2) where they modulate the processes of nucleobase salvage and 5-fluorouracil (5-FUra) activation (3–8). The molecular mechanism relies on the enhancement of the cytosolic ribose-1-phosphate (Rib1-P) level, stemming from nucleoside phosphorylation and the subsequent synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP). Alternatively, Rib1-P may be converted into phosphorylated glycolytic intermediates via the pentose pathway (9). In previous papers, we have followed the time course of purine and pyrimidine ribonucleotides and 5-FUra-ribonucleotides biosynthesis in extracts of rat brain incubated with ATP and a nucleobase in the presence of Rib1-P or a Rib1-P-generating system such as inosine and P1 (10–13). During these studies we became aware that, after prolonged incubation, a small, but definite amount of nucleobases could be salvaged into their respective ribonucleotides even in the absence of Rib1-P or inosine (14). A related observation was the marked effect exerted by ATP in modulating the PRPP-mediated 5-FUra activation to cytotoxic 5-FUra-nucleotides (12) as catalyzed by rat liver orotate phosphoribosyltransferase (the PRPP pathway) (14, 15).

Taken together, these observations prompted us to undertake a study on the capacity of rat brain to salvage purine and pyrimidine bases by deriving Rib1-P and PRPP from ATP and to ascertain whether the salvage of purine bases might be modulated by ATP. In this paper, we show that: (i) massive ATP breakdown per se, as it probably occurs in rat brain during ischemia (16), may provide at least part of the Rib 1-P and of the PRPP needed to salvage uracil and purine bases, respectively; (ii) the [PRPP]/[ATP] ratio modulates the process of adenine salvage in rat brain extracts. We recall that in rat brain, which does not possess the de novo nucleotide synthesis, purine and pyrimidine salvage is particularly active (3, 13, 17).

EXPERIMENTAL PROCEDURES

Materials—[8-14C]Adenine (55 mCi/mmol), [8-14C]hypoxanthine (54 mCi/mmol), [8-14C]guanine (53.3 mCi/mmol), [2-14C]cytosine (51 mCi/mmol), [2-14C]uracil (54 mCi/mmol), [2-14C]5-FUra (55 mCi/mmol), adenosine deaminase, hypoxanthine-guanine phosphoribosyltransferase (HGPRT), PRPP, dithiothreitol, nucleobases, nucleosides, and nucleotides were from Sigma. Adenosine deaminase and HGPRT were diluted with 5 mM Tris-HCl buffer before use to contain 0.63 units/ml and 10 units/ml, respectively. Bacillus cereus adenosine phosphorolysis (0.14 units/ml) was prepared as described previously (18). 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. Three-month-old male Sprague-Dawley rats were killed according to the “Guiding Principles in the Care and Use of Animals” (DHREW, publication no. NIH 86-23). The brain was removed and kept frozen at −80 °C until needed. Storage time did not exceed 2 months.

Preparation of Rat Brain Extracts—Rat brain was cut in small pieces. 5-FUra, 5-fluorouracil; Rib1-P, ribose-1-phosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; PEI, polyethyleneimine.
Experimental Procedures. — Crude extracts, containing 50–150 μg of protein, were incubated in a total reaction volume of 70 μl containing 1100 μM ATP, 498 nmol of MgCl₂, 5-fluorouridine 5'-monophosphate; 5, 5-fluorouridine 5'-diphosphate + 5-fluorouridine 5'-triphosphate.

Incubation Procedures — Crude extracts, containing 50–150 μg of protein, were incubated in a total reaction volume of 70 μl containing 5 mM Tris-HCl buffer, pH 7.4, 20 mM KCl and 1 mM dithiothreitol. The homogenate was centrifuged at 4 °C at 40,000 × 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 levels of the purine and pyrimidine salvage enzymes present in crude extract are reported in a previous paper (13).

**FIG. 1.** Time courses of ATP-mediated adenine salvage (A) and FRPP formation (B) catalyzed by rat brain crude extracts. A, the incubation mixture contained, in a final volume of 70 μl, 100–150 μg of protein, 70 nmol of [8-14C]adenine, 252 nmol of ATP, and 581 nmol of MgCl₂ in 5 mM Tris-HCl buffer, pH 7.4. •, adenosine; ▲, AMP; ●, ADP + ATP. B, [8-14C]Adenine was omitted in the reaction mixture, and FRPP was determined as described under “Experimental Procedures.” ○, PRPP. A bidimensional chromatographic analysis of the reaction mixture incubated for 60 min gave an ATP/ADP ratio of about 9:1.

**FIG. 2.** Time courses of ATP-mediated hypoxanthine salvage (A) and Rib1-P formation (B) catalyzed by rat brain crude extracts. A, the incubation mixture was as in Fig. 1 with the exception of [6-14C]hypoxanthine (70 nmol), which substituted [8-14C]adenine. •, inosine; ▲, IMP; ●, IDP + ITP. B, [8-14C]Hypoxanthine was omitted, and Rib1-P was determined as described under “Experimental Procedures.” ○, Rib1-P.

**FIG. 3.** Time courses of ATP-mediated uracil salvage (A) and 5-FUra activation (B) catalyzed by rat brain crude extracts. A, the incubation mixture was as described in Fig. 1 with the exception of [2-14C]Uracil (70 nmol), which substituted [8-14C]adenine. •, uridine; ▲, UMP; ●, UDP + UTP. B, [2-14C]Uracil was substituted with 70 nmol of [2-14C]5-FUra. •, 5-fluorouridine; ▲, 5-fluorouridine 5'-monophosphate; ○, 5-fluorouridine 5'-diphosphate + 5-fluorouridine 5'-triphosphate.

**FIG. 4.** Time course of ATP-mediated guanine salvage catalyzed by rat brain crude extracts. A, the incubation mixture was as described for Fig. 1 with the exception of [8-14C]guanine (70 nmol), which substituted [8-14C]adenine. ▲, GMP; ●, GDP + GTP; △, xanthine; ○, xanthosine; ●, xanthosine 5'-monophosphate.

**FIG. 5.** Effect of nucleobases on ATP-mediated purine and pyrimidine ribonucleotides (A) and ribonucleosides (B) synthesis in rat brain crude extracts. The standard incubation mixture contained, in a final volume of 60 μl, 100–150 μg of protein, 12 nmol each of [8-14C]adenine, [8-14C]hypoxanthine, [8-14C]guanine, [2-14C]cytosine, or [2-14C]Uracil, 216 nmol of ATP, 498 nmol of MgCl₂ in 5 mM Tris-HCl buffer, pH 7.4, in the presence of a mixture of 12 nmol each of the other four cold nucleobases. A, ○, total adenine nucleotides; ▲, total uracil nucleotides; ●, total hypoxanthine nucleotides; △, total guanine nucleotides. B, ○, adenosine; △, uridine; ●, inosine; △, xanthosine.

**Determination of ATP-derived Rib1-P and PRPP—Crude extracts, containing 1100 μg of protein, were incubated in a total reaction volume of 500 μl in the presence of 3.6 mM ATP, 8.3 MgCl₂, 5 mM Tris-HCl...**
buffer, pH 7.4. The incubation was carried out at 37 °C. At different time intervals, 70–90 μl portions were withdrawn, heated for 90 s at 100 °C, and centrifuged. The supernatant fluid was used to determine Rib 1-P and PRPP.

Rib 1-P was determined according to Ipata and Camici (18). Briefly, 25 μl of the above supernatant fluid was added to 20 μl of 10 mM [8-14C]adenine (8,000 dpm/nmol) in 5 mM Tris-HCl buffer, pH 7.4, 10 μl of diluted commercial adenosine deaminase, and 5 μl of the adenosine phosphorylase preparation. The reaction mixtures were incubated at 37 °C for 2 h. Then, 10–μl portions were applied to a PEI-cellulose precoated thin-layer plastic sheet and developed with deionized water. The marker compounds were detected with a UV lamp. The zones corresponding to inosine were excised and counted for radioactivity with 8 ml of scintillation liquid.

PRPP was determined according to King et al. (19), with minor modifications. Briefly, 25 μl of the above supernatant fluid was added to 10 μl of 10 mM [8-14C]hypoxanthine (8,000 dpm/nmol) in 5 mM Tris-HCl buffer, pH 7.4, 2 μl of the diluted HGPRT, 1 μl of 100 mM dithiothreitol, 2 μl of 2 mM MgCl₂, and 10 μl of Tris-HCl, pH 7.4. The reaction mixtures were incubated at 37 °C for 10 min. 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.

Protein Concentration—Protein concentration was determined by the Coomassie Blue binding assay using bovine serum albumin as standard (20).

RESULTS AND DISCUSSION

It is a widely accepted tenet in neurobiology that under normal conditions intracellular ATP levels are rigorously protected. However, in such events as ischemia or anoxia, massive ATP breakdown occurs. Many reports have demonstrated a substantial recovery of rat brain adenine nucleotides levels following ischemic episodes (16, 21–27). Moreover, a considerable increase in intracellular nucleosides and nucleobases, important precursors of nucleotide resynthesis following reperfusion, has been reported (16, 28). This paper deals with two different, although related, aspects of ATP metabolism in rat brain.

First, we have shown that PRPP, in addition to Rib 1-P, may be synthesized during massive ATP breakdown and utilized to salvage purine bases (Figs. 1–5). Second, we have shown that the [PRPP]/[ATP] ratio is an important factor in modulating the purine salvage process in rat brain (Figs. 6 and 7). Essentially, the rationale of our experimental approach was the following. Because in rat brain adenine, hypoxanthine and guanine salvage are PRPP-dependent processes (3), we reasoned that any ATP-mediated purine ribonucleotide biosynthesis would necessarily imply that PRPP was synthesized during ATP breakdown. Likewise, because uracil salvage and 5-FUra activation are Rib 1-P-dependent processes in rat brain (12,
breakdown, was utilized to ribosylate hypoxanthine, whereas PRPP was utilized to phosphoribosylate hypoxanthine through the action of purine nucleoside phosphorylase and HGPRT activities present in rat brain, respectively. Because no inosine kinase activity is present in mammalian tissues (30), phosphorylation of inosine can be excluded a priori. We cannot exclude the possibility that a small portion of IMP might have arisen by the action of the phosphotransferase activity of cytosolic 5'-nucleotidase (31) as well as that some inosine might have arisen from IMP dephosphorylation. We noticed, however, that the amount of free Rib1-P formed in the absence of added nucleobase (Fig. 2B) was in good correlation with that of total inosine synthesized (Fig. 2A). A chromatographic analysis of the UV absorbing products of ATP breakdown clearly showed that IMP was formed before adenosine. Thus, IMP was already present in the reaction mixture after 10 min of incubation, whereas adenosine and hypoxanthine appeared after 20 and 60 min, respectively. ATP and ADP completely disappeared only after 2 h of incubation.

**ATP-mediated Uracil Salvage and 5-FUra Activation**—When crude extract of rat brain was incubated in the presence of either [14C]uracil (Fig. 3A) or [14C]5-FUra (Fig. 3B) and ATP, uridine and 5-fluorouridine were synthesized as intermediates of uracil- and 5-FUra-ribonucleotides synthesis, respectively (reactions 12 and 13 in Fig. 8) in accordance with the existence of high uridine phosphorylase and uridine kinase activities in rat brain (13) and with our previous results showing that 5-FUra activation occurs through the Rib1-P-mediated ribosylation of the pyrimidine analog followed by multiple phosphorylation steps (“the Rib1-P pathway”) (12).

**ATP-mediated Guanine Salvage**—Utilization of [14C]guanine during ATP breakdown appears to be rather complex. Fig. 4 shows that a series of radioactive compounds was formed including guanine-nucleotides, xanthine, xanthosine, and XMP. Changes in the specific radioactivities of metabolites to ascertain their order of formation have not been analyzed. Such experiments would be helpful in the future. The appearance of large amounts of xanthine and xanthosine in the course of reaction is indicative of strong guanine deaminase and purine nucleoside phosphorylase activities and of recycling of Rib1-P in the nucleoside interconversion (32). The activity of xanthine oxidase in rat brain is very low (33). Because xanthine is not used as substrate by mammalian HGPRT (34), and we are not aware of the existence of any nucleoside kinase acting on xanthosine, the small amount of XMP formed might be ascribed to the phosphoribosyltransferase activity of rat brain cytosolic 5'-nucleotidase (35).

**ATP-mediated Cytosine Salvage**—Strikingly, cytosine was not utilized at all during ATP breakdown (results not shown), showing that the base is not deaminated to uracil and confirming the absence of cytidine phosphorylase and cytosine phosphoribosyltransferase activities in rat brain (13).

As shown in Fig. 5, the patterns of the ATP-mediated salvage of each radioactive nucleobase is not significantly altered by the presence of all other cold nucleobases.

**Modulation of Adenine Salvage by the [PRPP]/[ATP] Ratio**—The intracellular ATP level in mammalian tissues is in the mM range (e.g. 3.3 mM in rat brain (36)), whereas that of PRPP in mammalian tissues is in the mM range (37). Most likely, the [PRPP]/[ATP] ratio must be maintained at low values to avoid excessive de novo or “salvage” purine nucleotide synthesis. And in fact PRPP synthetase superactivity, a rare abnormality in man, leads to purine overproduction, gout, and urolithiasis (38). The results presented in Fig. 6 and 7 suggest that the [PRPP]/[ATP] ratio might modulate the process of adenine salvage. The maximal amount of adenine salvaged at a fixed
initial PRPP concentration was a function of ATP levels (Fig. 6). Moreover, the rate of salvaged adenine nucleotides degrada
tion observed after prolonged incubation increased sharply
by decreasing the initial ATP concentration. These observa-
tions further suggest that the ATP level might be an impor-
tant factor in modulating the PRPP-mediated process of purine base
salvage. As expected, at fixed “physiological” rat brain ATP con-
centration (36), the maximal amount of adenine salvaged
was a function of initial PRPP levels (Fig. 7).

An artificial presence of ecto-5’-nucleotidase in our brain
extract cannot be excluded a priori. In our view, the presence of
this enzyme does not invalidate the results presented here,
which can be extrapolated to an in vivo situation because, as
pointed out by Torrecilla et al., in the presence of an ATP
concentration as low as 10 μM, this enzyme would not have
functional importance related to the metabolism of AMP
(40, 41).

In conclusion, our results suggest that rat brain has the
capacity to salvage the main natural purine and pyrimidine
bases, except cytosine, and to activate 5-FUra by deriving
Rib1-P and PRPP from ATP. In our opinion, this process rep-
resents a vital mechanism for the brain to achieve reperfusion-recovery of certain
nucleotide pools lost during hypoxia. In addition, our results
suggest that the ratio [PRPP]/[ATP] plays a major role in
modulating the salvage process of purine nucleotides.

REFERENCES
1. Plageman, P. G. W., Wohlhuter, R. M., and Woffendin, C. (1988) Biochim. 
Biophys. Acts 947, 405–431
2. Griffith, D. A., and Jarvis, S. M. (1996) Biochim. Biophys. Acts 1286, 153–181
3. Mascia, L., Cappiello M., Cherri, S., and Ipata, P. L. (2000) Biochim. Biophys. 
Acts 1474, 3585–3589
4. Goto, A. M., Belkhode, M. L., and Touster, O. (1969) Cancer Res. 29, 807–811
5. Peters, G. J., Laureusse, E., Levy, A., and Pinedo, H. M. (1987) Eur. J. Cancer 
Clin. Oncol. 23, 1869–1881
6. Teruk, M., Chiba, Y., Tamaiosa, O., and Okada, S. (1986) J. Pharmacobiodynam. 9, 962–969
7. Betz, R. E., and Hunter-Lazlo, M. J. (1985) Cancer Lett. 28, 263–271
8. Schwartz, P. M., Mirr, R. D., Hyde, C. M., Turek, P. J., and Handschumacher 
R. E. (1985) Biochem. Pharmacol. 34, 3585–3589
9. Jurkowitz, M. S., Litsky, M. L., Browning, M. J., and Hohl, C. H. (1998) J. Neurochem. 71, 535–548
10. Mascia, L., Turchi, G., Bemi, V., and Ipata, P. L. (2000) Biochim. Biophys. Acts 
1324, 45–50
11. Mascia, L., Cotrufo, T., Cappiello, M., and Ipata, P. L. (1999) Biochim. Biophys. 
Acts 1472, 93–98
12. Mascia, L., and Ipata, P. L. (2001) Biochem. Pharmacol. 62, 213–218
13. Cappiello, M., Mascia, L., Scolozzi, C., Giorgelli, F., and Ipata, P. L. (1998) 
Biochim. Biophys. Acts 144, 235–239
14. Barsotti, C., and Ipata, P. L. (2002) Biochem. Pharmacol. 63, 117–122
15. Grem, J. L. (1996) in Cancer Chemotherapy and Bother. Principles and 
Practise (Chabner, B. A., and Longo, D. L., eds) pp. 149–211, Lippincott-Raven, 
New York
16. Phillips, J. W., O’Regan, M. H., Estevez, A. Y., Song, D., and VanderHeide, S. J. 
(1996) J. Neurochem. 67, 1525–1531
17. Alloso, J., and Watts, R. W. (1988) Adv. Exp. Med. Biol. 65B, 21–26
18. Ipata, P. L., and Camici, M. (1981) Anal. Biochem. 112, 151–153
19. King, M. T., Passonneau, J. V., and Veech, R. L. (1990) Anal. Biochem. 187, 
179–186
20. Bradford, M. M. (1976) Anal. Chem. 947, 405–431
21. Sims, N. R., and Pulsinelli, W. A. (1987) J. Neurochem. 49, 1367–1374
22. Nordstrom, C. H., Rehncrona, S., and Siesjo, B. K. (1976b) J. Neurochem. 30, 
479–486
23. Ljunggren, B., Ratcheson, R. A., and Siesjo, B. K. (1974) Brain Res. 73, 
291–307
24. Pulsinelli, W. A., and Duffy, T. E. (1983) J. Neurochem. 40, 1500–1503
25. Crumrine, R. C., and LaManna, J. C. (1991) J. Cereb. Blood Flow Metab. 11, 
272–282
26. Folbergrova, J., Zhao, Q., Katsura, K., and Siesjo, B. K. (1995) Proc. Natl. 
Acad. Sci. U. S. A. 92, 5057–5061
27. Paschen, W., Olah, L., and Mies, G. (2000) J. Neurochem. 75, 1675–1680
28. Kaseta, P., Kohoyashi, K., and Hossmann, K. A. (1974) J. Neurochem. 23, 
417–425
29. Sonoda, T., Ishizuka, T., I shi jima, S., Kita, K., Ahmad, I., and Taiti bana, M. 
(1998) Biochim. Biophys. Acts 1387, 32–40
30. Stone, T. W., and Simmonds, H. A. (1993) in Purines: Basic and Clinical 
Aspects, pp. 8–22, Kluver Academic Publishers, Dordrecht
31. Pest, R., Turriani, M., Allegri, S., Scolozzi, C., Camici, M., and Ipata, P. L. 
(1994) Arch. Biochem. Biophys. 312, 75–80
32. Giorgelli, F., Battai, C., Mascia, L., Scolozzi, C., Camici, M., and Ipata, P. L. 
(1997) Biochim. Biophys. Acts 1335, 16–22
33. Markley, H. G., Faillace, L. A., and Mezey, E. (1973) Biochim. Biophys. Acts 
369, 23–32
34. Flaks, J. G. (1963) Methods Enzymol. 6, 144–154
35. Marques, A. F. P., Teixeira, N. A., Gambaretto, C., Siliero, A., and Siliero, 
M. A. G. (1998) J. Neurochem. 71, 1241–1250
36. Hisanaga, K., Onodera, H., and Kogure, K. (1986) J. Neurochem. 47, 
1344–1350
37. Trosz, T. W. (1994) Mol. Cell. Biochem. 140, 1–22
38. Becker, M. A., Losman, J. M., Wilson, J., and Simmonds, H. A. (1986) Biochim. 
Biophys. Acts 882, 168–176
39. Torrecilla, A., Marques, A. F. P., Buseaionii, R. D., Oliviera, J. M. A., Teixeira, 
N. A., Alenca, E. A., Siliero, M. A. G., and Siliero, A. (2001) J. Neurochem. 
76, 1291–1307
40. Mallol, J., and Borel, J. (1988) Neurochem. Int. 12, 61–67
41. Orford, M. B., and Saggerson, E. D. (1996) J. Neurochem. 67, 795–804
