Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 2829–2833, May 1983
Biochemistry

Evidence for a substrate cycle between AMP and adenosine in isolated hepatocytes

(AMP deaminase/5′-nucleotidase/adenosine kinase/coformycin/5-iodotubercidin)

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Communicated by Christian de Duve, January 24, 1983

ABSTRACT **The effect of adenosine on the metabolism of prelabeled adenine nucleotides was investigated in isolated hepatocytes. Adenosine caused an ≈2-fold increase in the ATP content of the cells. This effect was in part counteracted by an increased rate of adenine nucleotide catabolism that could be explained by a stimulation of both AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) and the cytoplasmic 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) because of the increased concentration of ATP. The unexpected finding that labeled adenosine was formed immediately after the addition of the unlabeled nucleoside could be explained by the trapping effect of adenosine. An accumulation of labeled adenosine was observed also in the presence of 5-iodotubercidin, a potent inhibitor of adenosine kinase (ATP:adenosine 5′-phosphotransferase, EC 2.7.1.20). Under these conditions, there was a decrease in the concentration of ATP in the cell and a 2- to 3-fold increase in the rate of formation of allantoin. This formation of adenosine was only slightly decreased by inhibition of the membranous 5′-nucleotidase; it led to the accumulation of S-adenosylhomocysteine in the presence of coformycin and an excess of L-homocysteine. It was concluded that, under basal conditions, the cytoplasmic 5′-nucleotidase present in the liver cell continuously produces adenosine, which is immediately reconverted into AMP by adenosine kinase, without giving rise to allantoin. This futile cycle between AMP and adenosine amounts to at least 20 nmol/min per g of liver and, thus, exceeds the basic rate of allantoin formation.**

An increase in hepatic ATP and total adenine nucleotides has been documented after the administration of adenosine to intact rats (1) and after the addition of the nucleoside to the perfusion medium of the isolated rat liver (2) or to suspensions of isolated rat hepatocytes (3, 4). The present work was undertaken in order to study the influence of this enlargement of the adenine nucleotide pool on the rate of its degradation. Indeed, no mechanism as yet is known that would adapt the rate of adenine nucleotide catabolism to an increased rate of synthesis. We came previously to the conclusion that AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) constitutes the limiting enzyme of hepatic adenine nucleotide catabolism under basal conditions and that deinhibition of this enzyme accounts for the increased formation of purine catabolites observed after the administration of fructose or in anoxia (see ref. 5 for a review). This conclusion was based on work with partially purified enzymes (6, 7) and was confirmed by studies in isolated hepatocytes (8, 9). The adenine nucleotides were labeled with [^{14}C]adenine, and their catabolism was investigated by following the appearance of the radioactivity in their breakdown products. By using the same technique, we now have reached the conclusion that the activity of AMP deaminase is stimulated in the conditions prevailing in the liver cell after the administration of adenosine. Furthermore, we report that, under normal conditions, there is a continuous production of adenosine by the cytoplasmic 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) which, however, does not contribute to the formation of allantoin but is part of a substrate cycle operating between AMP and adenosine. Part of this work has been presented at a symposium (10).

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MATERIALS AND METHODS

Chemicals and Enzymes. Coformycin {(*R*)-3-(β-D-erythropentofuranosyl)-3, 6, 7, 8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol} was a gift of H. Umezawa (Institute of Microbial Chemistry, Tokyo, Japan). 5-Iodotubercidin {4-amino-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine; ITu} was provided by L. B. Townsend, (University of Michigan College of Pharmacy, Ann Arbor, MI). GTP-agarose, 5′-amino-5′-deoxyadenosine, α,β-methyleneadenosine 5′-diphosphate, L-homocysteine thiolactone, and *S*-adenosyl-L-homocysteine (AdoHcy), were purchased from Sigma. The source of all other chemicals has been given (8).

AMP deaminase was partially purified from rat liver by the following procedure: a 20% (wt/vol) liver homogenate in 50 mM Tris buffer (pH 7.4) containing 0.1% 2-mercaptoethanol was centrifuged at 100,000 × *g* for 60 min at 2°C; 2 ml of the supernatant was subjected to affinity chromatography on a column (0.7 × 4 cm) of GTP-agarose equilibrated with 50 mM KH_2PO_4 buffer (pH 6.8). After the column was washed with 10 ml of the equilibration buffer, AMP deaminase was eluted as a single peak with the same buffer containing 10 mM ATP. The enzyme was purified 30- to 100-fold (to a specific activity of 400–600 nmol/min per mg of protein when assayed at 0.2 mM AMP and 5 mM ATP) and was free of myokinase and 5′-nucleotidase activity. Cytoplasmic 5′-nucleotidase from rat liver was prepared as described by Itoh (11) except that the enzyme was precipitated at 45% $(NH_4)_2SO_4$ and that the last step (precipitation at low ionic strength) was omitted. The specific activity of the enzyme measured at 0.2 mM IMP was 470 nmol/min per mg of protein. The origin of the auxilliary enzymes used in the analytical methods has been given (8).

Experimental Procedures. The activities of partially purified AMP deaminase and cytoplasmic 5′-nucleotidase were measured as described (6, 7) except that the separation of radioactive substrates and products was performed by thin-layer chromatography by the method of Reibel and Rovetto (12). The activity of uricase (urate:oxygen oxidoreductase, EC 1.7.3.3) was measured by the method of Scheibe *et al.*, (13) and AdoHcy hydrolase (EC 3.3.1.2) was assayed as described by Hershfield (14). In the experiments with L-homocysteine thiolactone, adenosine and AdoHcy were measured as described by Newby and Holmquist (15). The preparation of the isolated rat hepatocytes and the other methods used for the measurement of

Abbreviations: ITu, 5-iodotubercidin; AdoHcy, *S*-adenosyl-L-homocysteine.

enzymic activities and for the determination of metabolites have been given in detail (8, 9).

RESULTS

Influence of Adenosine on the Metabolism of the Adenine Nucleotides. Fig. 1 depicts the influence of 0.5 mM adenosine on the concentration and on the radioactivity of the adenine nucleotides in isolated hepatocytes and of their catabolites in the incubation medium. In control conditions, the removal of adenosine proceeded linearly at a rate of 0.3 μmol/min per g of cells and was complete in 25 min (Fig. 1*A*). In the presence of 50 μM coformycin, which inhibits completely hepatic adenosine deaminase and causes also a >90% reduction of the activity of AMP deaminase (8), this removal was slower by ≈75%. The total adenine nucleotide content of the hepatocytes increased continuously as long as adenosine was present (Fig. 1*C*); this increase reached 50% after 30 min in the absence of coformycin and 120% at 60 min in the presence of coformycin. Under the latter condition, all the adenosine removed was recovered in the adenine nucleotides at the end of the experiment. The increase in adenine nucleotides was almost exclusively due to an increase of the concentration of ATP (results not shown). The concentrations of GTP, IMP, and P_i were not modified (results not shown).

The addition of adenosine provoked also transient increases in the concentrations of inosine (Fig. 1*E*) and of hypoxanthine and xanthine (not shown), which were completely suppressed in the presence of coformycin. All three purine catabolites were undetectable in control hepatocytes. The production of allantoin expressed as nmol/min per g of cells (Fig. 1*G*) was 17 in control conditions, 106 in the presence of adenosine, and only 3 in the presence of both adenosine and 50 μM coformycin.

In control cells, the radioactivity in the adenine nucleotides decreased linearly with time (Fig. 1*D*). The more rapid loss of radioactivity, observed upon addition of adenosine, was partly restrained in the presence of coformycin. The addition of adenosine induced a prompt appearance of radioactivity in this nucleoside (Fig. 1*B*), which was transient in the absence of coformycin but increased steadily to a plateau in its presence. The time course of the formation of radioactive inosine (Fig. 1*F*) and allantoin (Fig. 1*H*) in the three experimental conditions was comparable to the rate of accumulation of the unlabeled catabolites.

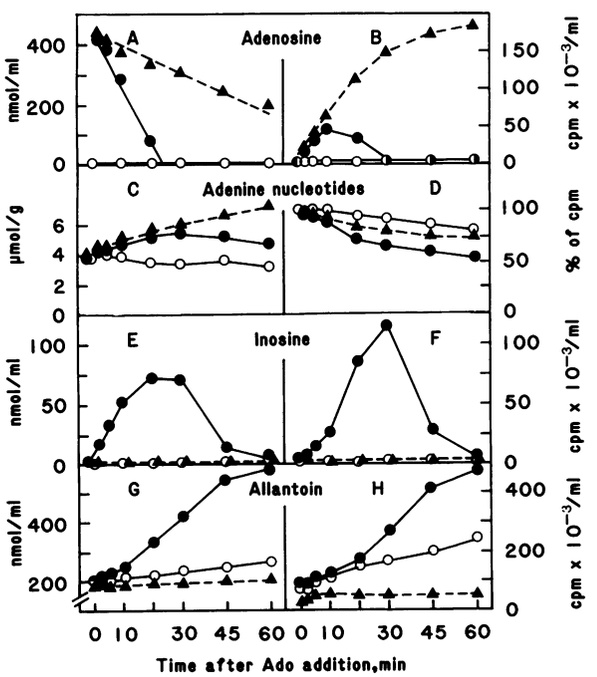

FIG. 1. Influence of adenosine on the metabolism of adenine nucleotides. (*Left*) Concentrations in nmol/ml of cell suspension (*A*, *E*, and *G*) or in μmol/g of cells (*C*). (*Right*) Radioactivity measurements in cpm × 10^{-3}/ml of cell suspension (*B*, *F*, and *H*) or in percentage of cpm at time 0 (*D*). Hepatocytes were preincubated for 15 min before time 0 in the presence of 1 μM [^{14}C]adenine without (○,●) or with (▲) 50 μM coformycin. ○, Control without adenosine.

The specific radioactivity of the adenine nucleotides remained constant in control cells but, as expected, decreased progressively when their concentration was elevated by adenosine. From these values and from the radioactivity in their breakdown products, the amount of catabolites originating from the adenine nucleotides could be calculated. This allowed an estimate of the participation of AMP deaminase and the cytosolic 5′-nucleotidase under the three experimental conditions (Table 1). Under control conditions, the degradation of the adenine nucleotides leading to allantoin is known to proceed only through AMP deaminase (8). Upon addition of 0.5 mM adenosine, the rate of catabolism of the adenine nucleotides was increased ≈5-fold. This could be due to a stimulation of AMP deaminase and of 5′-nucleotidase, as evidenced by the formation of radioactive adenosine under this condition. In the presence of adenosine and 50 μM coformycin, the rate of degradation of the adenine nucleotides was increased only ≈2.5-fold. Because AMP deaminase is maximally inhibited at this concentration of coformycin, the observed rate reflects the participation of 5′-nucleotidase in the adenosine-induced catabolism. Thus, it could be calculated that the participation of AMP deaminase in the presence of adenosine accounted for 26–29 nmol/min per g of hepatocytes, which represents a 2-fold stimulation as compared to the basal condition.

Influence of Adenosine on the AMP-Degrading Enzymes. The stimulatory effect of adenosine on the catabolism of AMP prompted a study of the activities of both AMP-degrading enzymes in the conditions prevailing in the cells after the addition of the nucleoside. Hepatic AMP deaminase is stimulated by ATP and submitted to a synergistic inhibition by GTP and P_i. At physiological concentrations of AMP (0.1–0.2 mM) and of its effectors (3 mM ATP, 0.5 mM GTP, and 5 mM P_i) the enzymic activity is inhibited by 95% (6). The activity of purified AMP deaminase from rat liver measured in physiological conditions was not modified by the addition of adenosine but increased 2-fold, from 26 to 52 nmol/min per mg of protein, when ATP was raised to 5 mM, the concentration prevailing after the

Table 1. Influence of adenosine on the catabolism of the adenine nucleotides

	Conditions	Rate of catabolism, nmol/min per g of hepatocytes				Enzyme(s) involved
1	Control	12	12	12	15	AMP deaminase
2	With 0.5 mM adenosine	55	67	44	78	AMP deaminase and 5′-nucleotidase
3	With 0.5 mM adenosine/50 μM coformycin	29	38	ND	ND	5′-nucleotidase
	Difference: 2 − 3	26	29	—	—	AMP deaminase

The rates of catabolism were determined in four individual experiments by dividing for each time interval the radioactivity in the [^{14}C]-adenine nucleotide catabolites by the specific activity of the adenine nucleotides. Major catabolites in conditions 1–3: 1, allantoin; 2, adenosine, inosine, hypoxanthine, xanthine, and allantoin; 3, adenosine. ND, not done.

administration of adenosine (Fig. 2).

As already discussed (7), the cytoplasmic 5′-nucleotidase constitutes the only 5′-nucleotidase that qualifies for a role in the breakdown of intracellular 5′-nucleotides. This enzyme displays a markedly higher affinity for IMP and GMP than for AMP, is stimulated by ATP and GTP, and is inhibited by P_i (7, 16). In our previous study, we did not detect any activity of the cytoplasmic 5′-nucleotidase on AMP when measured in the presence of physiological concentrations of substrate (0.2 mM) and effectors. However, the use of a more active preparation now has allowed us to measure accurately, under these conditions, an activity equal to a few percent of that on the same concentration of IMP. This activity was not influenced by the addition of adenosine but was increased from 8 to 14 nmol/min per mg of protein by increasing the concentration of ATP to 5 mM (not shown).

Effect of Inhibitors of Adenosine Kinase on the Production of Purine Catabolites by Isolated Hepatocytes. The formation of radioactive adenosine from labeled adenine nucleotides, revealed by the addition of the unlabeled nucleoside to the hepatocyte suspension, and the reassessment of the activity of the cytoplasmic 5′-nucleotidase in physiological conditions raised the possibility that adenosine may be continuously formed from adenine nucleotides in basal conditions but not contribute to the production of allantoin because of its reutilization by adenosine kinase (ATP:adenosine 5′-phosphotransferase, EC 2.7.1.20). In order to check this hypothesis, experiments were performed with the adenosine kinase inhibitors 5-iodotubercidin (17) and 5′-deoxy-5′-aminoadenosine (18). It was first established that these compounds, when added to the hepatocyte suspension, inhibited adenosine kinase inside the cells. This was done by measuring the incorporation of [^{14}C]adenosine into the intracellular adenine nucleotides in the presence of 0.1 μM coformycin, a concentration that inhibits completely adenosine deaminase (8). A dose-dependent inhibition of this incorporation was observed, reaching 75% at 0.2 μM and about 98% at 0.2 mM ITu. It was verified that the inhibitors did not influence the activity of other purine degrading enzymes, namely AMP deaminase, cytoplasmic 5′-nucleotidase, adenosine deaminase, nucleoside phosphorylase, xanthine oxidase, and uricase. AdoHcy hydrolase also was neither inhibited nor inactivated by 100 μM ITu (results not shown). In isolated hepatocytes, addition of 0.5 μM ITu had no effect, but 10 and 100 μM ITu provoked a progressive loss of intracellular ATP, which decreased from 2.5 μmol/g of cells at time 0 to 1.2 μmol/g after 60 min (Fig. 3*A*). The inhibitor did not modify the concentrations of AMP and IMP but caused an accumulation of adenosine from prelabeled adenine nucleotides (Fig. 3*B*) and, after a short latency, a marked enhancement of the production of allantoin (Fig. 3*C*). The latter increased from 12.6 ± 1.0 nmol/min per g of hepatocytes in control conditions to 31.7 ± 2.7 nmol/min per g in the presence of 100 μM ITu (means ± SEM of five experiments each). Similar results were obtained with 5′-deoxy-5′-aminoadenosine, although higher concentrations of this adenosine kinase inhibitor were required (not shown).

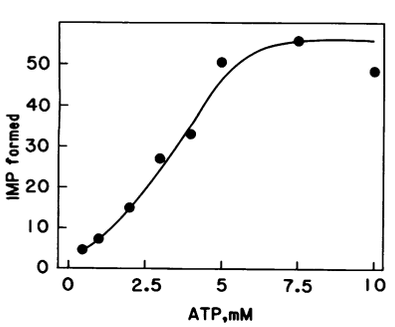

FIG. 2. Influence of the concentration of ATP on the activity of AMP deaminase from rat liver. The activity of the enzyme (nmol of IMP formed per min/mg of protein) was measured at 0.2 mM AMP in the presence of 0.5 mM GTP/5 mM P_i.

Influence of Inhibitors of the Membranous 5′-Nucleotidase and of Aspecific Phosphatases on the Production of Adenosine by Isolated Hepatocytes. In view of the short-lived functional and morphological integrity of isolated hepatocytes, the possibility also was considered that adenosine may be formed from adenine nucleotides released in the incubation medium by ruptured cells. This formation of adenosine may be catalyzed by various phosphatases either liberated from broken cells or present as ectoenzymes on the hepatocyte, among them being the membranous 5′-nucleotidase. In order to assess this possibility, experiments were performed in the presence of β-glycerophosphate (a competitive inhibitor of aspecific phosphatases) and of α,β-methyleneadenosine 5′-diphosphate [a potent inhibitor of the membranous 5′-nucleotidase in various tissues (19)], which, however, did not affect the activity of the cytoplasmic 5′-nucleotidase from rat liver (results not shown). When 10 mM β-glycerophosphate/2.5 mM α,β-methyleneadenosine 5′-diphosphate was added to an hepatocyte suspension prior to the addition of 20 μM AMP, the rate of degradation of the nucleotide was reduced to 2 nmol/min per g of cells, as compared to ≈2 μmol/min per g of cells in control conditions. Preincubation of isolated hepatocytes with the inhibitory mixture decreased the accumulation of adenosine and allantoin induced by 100 μM ITu by <15%.

Effect of L-Homocysteine Thiolactone on the Production of Adenosine by Isolated Hepatocytes. The possibility also was considered that the accumulation of adenosine, observed upon inhibition of adenosine kinase, may arise from the hydrolysis of AdoHcy. In order to explore this hypothesis, the effect of L-homocysteine thiolactone on the ITu-induced accumulation of adenosine was investigated, and the formation of AdoHcy was measured under various conditions. Because the AdoHcy hydrolase reaction has its equilibrium far in the synthetic direction (20), the simultaneous addition of adenosine and of L-homocysteine results in the accumulation of large amounts of

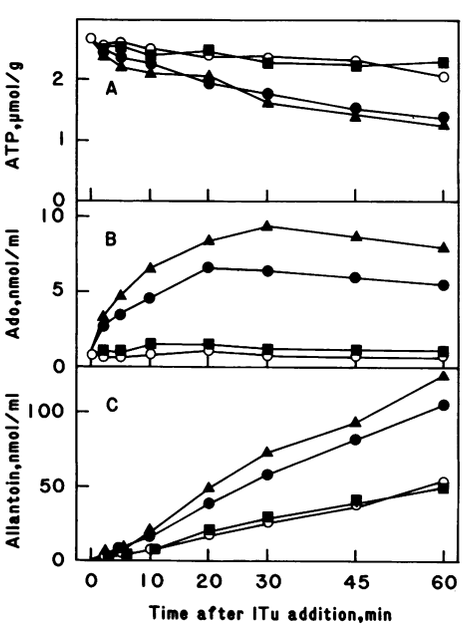

FIG. 3. Influence of ITu on the concentration (μmol/g of cells) of ATP (*A*) and on concentrations (nmol/ml of cell suspension) of adenosine (*B*) and allantoin (*C*). After 15 min of preincubation in the presence of 1 μM [^{14}C]adenine, the hepatocytes were incubated without (○) or with ITu at 0.5 (■), 10 (●), and 100 (▲) μM. The concentrations of adenosine and allantoin were calculated as given in Table 1.

AdoHcy in various tissues (21, 22). Thus, in the absence of exogenous adenosine and provided homocysteine is available, an ITu-induced increase in the concentration of AdoHcy would indicate that adenosine is produced by another enzyme than AdoHcy hydrolase. The experiment depicted in Fig. 4 was performed with hepatocytes that had been preincubated with 0.1 μM coformycin to inhibit adenosine deaminase. This resulted in an increased capacity of the cells to accumulate adenosine upon addition of ITu (Fig. 4*A*) as compared with the experiment described in Fig. 3. Concomitantly, the production of allantoin was not modified in comparison with the control condition (results not shown). The addition of ITu alone induced an accumulation of AdoHcy (Fig. 4*B*), which proceeded at the rate of about 5 nmol/min per g of cells. When L-homocysteine thiolactone was added together with ITu, the build-up of adenosine was delayed by 20 min. The accumulation of AdoHcy proceeded, however, ≈5-fold faster. The sum of both metabolites increased linearly for 60 min at a rate of 40 nmol/min per g of cells. In the absence of ITu, the addition of L-homocysteine induced only a small increase in the concentration of AdoHcy, which reached a plateau after 5 min.

DISCUSSION

This work (*i*) provided an insight into the mechanism whereby the rate of catabolism of the adenine nucleotides in liver cells adapts to an increased rate of synthesis and (*ii*) revealed the operation of a futile cycle between AMP and adenosine. These two aspects will be discussed separately.

Influence of an Increase in the Concentration of the Adenine Nucleotides on Their Rate of Breakdown. The increase in hepatic adenine nucleotides, induced by 0.5 mM adenosine (Fig. 1*C*), is explained by the phosphorylation of the nucleoside by adenosine kinase. In accordance with Lund *et al.* (3), adenosine incorporation into the adenine nucleotides accounted for only 25% of the adenosine removal in control conditions (Fig. 1*A*), the remainder being metabolized by way of adenosine deaminase towards allantoin (Fig. 1*G*). The enhancing effect of coformycin, a potent inhibitor of adenosine deaminase in various tissues (23, 24), on the adenosine-induced increase in concentration of ATP can be explained by the exclusive utilization of adenosine by adenosine kinase under this condition. The finding that the production of allantoin was nearly suppressed in the presence of 50 μM coformycin is in accordance with the previous demonstration of an additional inhibition of hepatic AMP deaminase at this concentration of the inhibitor (8).

The increased rate of degradation of the adenine nucleotides induced by adenosine (Table 1), indicates that mechanisms exist that counteract the enhancement of the rate of synthesis of ATP. Its reduction by 50 μM coformycin denotes that it involves AMP deaminase. The fact that the degradation of the adenine nucleotides still proceeds in the presence of 50 μM coformycin and is accompanied by a sustained increase in the concentration of labeled adenosine (Fig. 1*B*) indicates that there is also a dephosphorylation of AMP. This unexpected observation is discussed in detail in the next section. The adaptation of the rate of degradation of the adenine nucleotides to an increased rate of synthesis can be explained by the fact that the increased concentration of ATP in the cell causes an at least proportional increase in the activity of both AMP deaminase (Fig. 2) and the cytoplasmic 5′-nucleotidase.

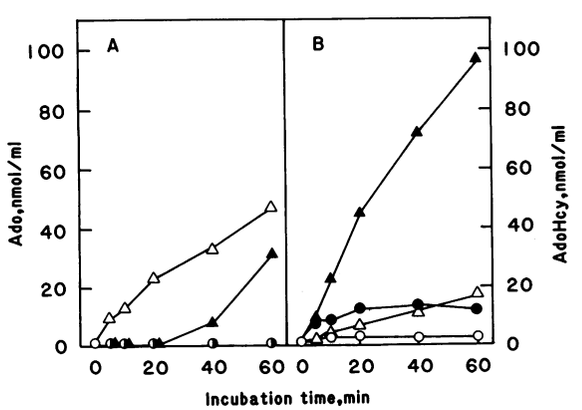

FIG. 4. Influence of ITu and homocysteine thiolactone on the concentrations (nmol/ml of cell suspension) of adenosine (*A*) and AdoHcy (*B*). After 15 min of preincubation with 0.1 μM coformycin, the hepatocytes were incubated without additions (○) or with 0.5 mM L-homocysteine thiolactone (●), 100 μM ITu (△), or both (▲).

The AMP-Adenosine Cycle. The presence of 5′-nucleotidase and adenosine kinase in various tissues of a large range of animals (25) has led Arch and Newsholme to postulate that a substrate cycle operates between AMP and adenosine as a means to regulate the concentration of adenosine (26). Although these authors state that adenosine is formed by the membranous 5′-nucleotidase acting primarily on intracellular AMP, it also could be produced by the cytoplasmic 5′-nucleotidase or by AdoHcy hydrolase, an enzyme of the *S*-adenosylmethionine-dependent transmethylation pathway. In the present work, we bring unequivocal evidence for the continuous operation of the AMP–adenosine cycle in the liver and show that it involves the cytoplasmic 5′-nucleotidase. This evidence relies on several facts.

The formation of adenosine from ^{14}C-labeled nucleotides immediately after the addition of 0.5 mM unlabeled adenosine. This observation (Fig. 1*B*) can be explained by a trapping effect of the unlabeled nucleoside and suggests that adenosine is already formed in the hepatocytes in basal conditions. Indeed, adenosine did not increase the concentration of hepatic AMP, had no action by itself on the activity of the cytoplasmic 5′-nucleotidase, and inhibits rather than stimulates the membranous 5′-nucleotidase (27). It is of interest to mention that similar results have been obtained with perfused rat livers (unpublished observations).

The accumulation of [^{14}C]adenosine in the presence of ITu at concentrations that block nearly completely adenosine kinase. Observed without addition of exogenous adenosine, this accumulation was seen in the absence (Fig. 3*B*) and presence (Fig. 4*A*) of coformycin and was markedly enhanced in the latter condition. It was followed by a decrease in the concentration of ATP (Fig. 3*A*), confirming that the formation of adenosine proceeds continuously in the hepatocytes at the expense of the adenine nucleotide pool. In the absence of ITu, this catabolism does not go beyond adenosine: the nucleoside is not converted to allantoin by way of adenosine deaminase because it is immediately rephosphorylated. Indeed, it appears that adenosine kinase is far from saturated under normal conditions, as indicated by the elevation of ATP concentration upon addition of adenosine (Fig. 1*C*) and by the fact that its partial inhibition by 0.5 μM ITu remained without effect on the catabolism of the adenine nucleotides (Fig. 3). This conclusion is also in agreement with our previous observation that the production of allantoin by isolated hepatocytes is not decreased by the concentration of coformycin that inhibits selectively adenosine deaminase (8).

The persistence of the formation of adenosine upon inhibition of the membranous 5′-nucleotidase or after the addition of L-homocysteine thiolactone. The observation that the accumulation of adenosine provoked by ITu was only slightly modified when the membranous 5′-nucleotidase of the hepatocytes was >99% inhibited confirms our (7) as well as others' (28–30) belief that this enzyme does not intervene in the formation of the nucleoside from intracellular 5′-nucleotides.

One explanation for the accumulation of AdoHcy induced by

ITu and L-homocysteine thiolactone (Fig. 4*B*) could be an inhibition or an inactivation (14) of the AdoHcy hydrolase because of a continuous generation of AdoHcy by the S-adenosylmethionine-dependent transmethylation pathway. However, this possibility may be ruled out because ITu had no effect on AdoHcy hydrolase. Furthermore, although inhibition of the hydrolysis of AdoHcy by L-homocysteine has been demonstrated (20), the limited, nonprogressive accumulation of AdoHcy recorded upon addition of 0.5 mM of the amino acid, indicates that no significant inhibition occurred at this concentration. Another interpretation for the accumulation of AdoHcy induced by ITu is a reversal of the AdoHcy hydrolase reaction, caused by the build-up of adenosine (Fig. 4*A*) and the availability of L-homocysteine. This conclusion implies the existence inside the hepatocytes of a source of adenosine different from the transmethylation pathway, which is most likely the hydrolysis of AMP by the cytoplasmic 5′-nucleotidase. The ITu-induced accumulation of AdoHcy recorded in the absence of exogenous L-homocysteine (Fig. 4*B*), shows that there is a continuous albeit limited production of this amino acid in basal conditions. The progressive increase in the concentration of adenosine, observed after 20 min in the presence of ITu and L-homocysteine thiolactone (Fig. 4*A*), might be explained by a slowdown of the rate of synthesis of AdoHcy because of a gradual metabolization of the amino acid or an inhibition of the reaction by its product.

The increases in adenosine and AdoHcy, measured in the presence of ITu and coformycin (Fig. 4), allow an estimation of the rate of dephosphorylation of AMP by the cytoplasmic 5′-nucleotidase. Under basal conditions, this rate approximated 20 nmol/min per g of cells and was similar to the increase in production of allantoin caused by ITu alone (calculated from Fig. 3*C*); it was raised to 40 nmol/min per g in the presence of 0.5 mM homocysteine, most likely because of the known inhibitory action of AdoHcy on adenosine kinase (31). These rates markedly exceed the formation of adenosine by the transmethylation pathway, which can be estimated at ≈4 nmol/min per g from the rate of accumulation of AdoHcy in hepatocytes in which AdoHcy hydrolase had been inactivated by adenine arabinoside (32). Preliminary studies with this compound have shown that it reduced only slightly the ITu-induced accumulation of adenosine (unpublished results). Thus, the activity of the cytoplasmic 5′-nucleotidase and, consequently, the rate of recycling represent at least twice the normal rate of production of allantoin by the hepatocytes. Therefore, it is not surprising that interruption of the cycle by inhibition of adenosine kinase results in an ATP depletion. This indicates that the rephosphorylation of adenosine plays a vital role in the maintenance of the adenine nucleotide pool in the liver cells.

As discussed by Arch and Newsholme (26), few data are presently available that demonstrate wittingly the operation of the AMP–adenosine cycle in tissues other than the liver. Lomax and Henderson (33) have briefly alluded to the possibility that simultaneous breakdown and resynthesis of AMP may be a normal occurrence in Ehrlich ascites tumor cells, and this recycling rate has been estimated at 6 nmol/hr per g of cells (34). The apparently normal viability of the several adenosine kinase-deficient cell lines that have been described (35–37) may be explained either by the absence of a quantitatively important AMP–adenosine cycle in the parental cell lines or by a compensation of the enzyme defect by increased purine synthesis in the mutant ones. The role of the strikingly active AMP–adenosine cycle discovered in hepatocytes remains now to be further investigated. It may intervene in the regulation of the various physiological effects of adenosine as well as in the transfer of purines to other tissues (see ref. 38 for a review).

F.B. was research fellow of the International Institute of Cellular and Molecular Pathology and G.V.D.B. is Maître de Recherches of the Belgian Fonds National de la Recherche Scientifique. This work was supported by the Fonds de la Recherche Scientifique Médicale.

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