Adenosine, Cyclic AMP Metabolism, and Glycogenolysis in Rat Liver Cells*

(Received for publication, January 31, 1977, and in revised form, August 2, 1977)

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Rat liver cells rapidly metabolized adenosine added to the medium by incorporating the adenosine into nucleotides or oxidizing it to uric acid. In the presence of allopurinol (4-hydroxyprazololo[3,4-d]pyrimidine) the formation of uric acid was blocked and hypoxanthine accumulated as the end product of adenosine catabolism. Adenosine added to the medium at concentrations up to 20 μM was almost completely removed by liver cells during the first 5 min of incubation. In the presence of 200 μM adenosine about half of the adenosine was present after a 20-min incubation of rat liver cells in the presence of allopurinol and erythro-9-(2-hydroxy-3-nonyl) adenine which is an inhibitor of adenosine deaminase.

There was no increase in adenosine release by rat liver cells incubated with 270 nM glucagon. Adenosine release could be accelerated by valinomycin which markedly elevated labeled AMP and phosphorylase a. Valinomycin was only effective as an activator of phosphorylase a in the presence of calcium in the medium. In contrast, cyanide and dinitrophenol worked equally well as activators of phosphorylase a in regular or calcium-free buffer.

Adenosine (200 μM) inhibited the stimulation of glycogenolysis by glucagon or epinephrine in isolated rat liver cells. Basal glycogenolysis and the increases due to valinomycin were unaffected by 200 μM adenosine. In contrast, 100 μM 2',5'-dideoxyadenosine was ineffective as an inhibitor of glycogenolysis while 10 μM dideoxyadenosine was much more potent than 200 μM adenosine as an inhibitor of cyclic AMP accumulation by intact rat liver cells. Similarly, 5 to 10 μM 2',5'-dideoxyadenosine reduced the activation of adenylyl cyclase by glucagon to about the extent of 50 to 100 μM adenosine. Neither 200 μM adenosine nor 50 μM 2',5'-dideoxyadenosine affected glycogen phosphorylase a activity.

These data suggest that the inhibition of hormone-activated glycogenolysis seen in the presence of 200 μM adenosine is not secondary to inhibition of cyclic AMP accumulation in rat liver cells. Furthermore, the results with 2',5'-dideoxyadenosine suggest that modulation of phosphorylase activity and glucose release by rat liver cells can be dissociated from alterations in cyclic AMP.

Sattin and Rall (1) originally reported that adenosine stimulated the accumulation of cyclic AMP by brain slices and this effect was blocked by theophylline. However, Moriwaki and Foa (2) reported that rat liver adenylate cyclase was inhibited by high concentrations of adenosine which was confirmed by McKenzie and Bär (3). Fain et al. (4) found that fat cell adenylate cyclase was also inhibited by adenosine. Fain (5) and Ebert and Schwabe (6) reported that micromolar concentrations of adenosine (0.1 to 1 μM) inhibited the rise in cyclic AMP accumulation by intact rat fat cells incubated with lipolytic hormones. Furthermore, Schwabe et al. (7) found that incubated fat cells released adenosine to the medium.

Lund et al. (8) reported that millimolar concentrations of adenosine elevated ATP in rat liver cells and inhibited glucoseogenesis from lactate. Londos and Preston (9) found that under appropriate conditions adenosine and 2',5'-dideoxyadenosine were potent inhibitors of hepatic adenylate cyclase activity. The present study examines the possible physiological role of adenosine in regulating cyclic AMP metabolism, phosphorylase a, and glycogenolysis by rat liver cells.

EXPERIMENTAL PROCEDURES AND RESULTS
The procedures and results are described in the supplement. 1

DISCUSSION
Our data indicate that the inhibitory effect of adenosine on cyclic AMP accumulation by intact liver cells is far less than that on isolated rat fat cells (4-7). Liver cells metabolize adenosine so rapidly, even in the presence of an adenosine deaminase inhibitor, that little free adenosine is present. This is supported by our inability to see any increase in cyclic AMP accumulation by rat liver cells due to the addition of adenosine deaminase. These data do not negate the idea that adenosine is tightly bound to a regulatory site on hepatic

* This work was supported by United States Public Health Service Research Grants AM-01049 and AM 14648 from the National Institute of Arthritis, Metabolism, and Digestive Diseases. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
adenylate cyclase which restrains the enzyme. However, adenosine would have to be bound to this site so tightly that it is not available to hepatic adenosine kinase or deaminase. Changes in free adenosine content of liver cells probably occur only under severe hypoxia or other conditions where there is a large conversion of ATP to AMP as in the presence of valinomycin.

In order to have any appreciable amount of free adenosine present in the medium a very high concentration (200 μM) must be added to incubated liver cells. Lund et al. (8) using isolated rat liver cells and Wilkening et al. (10) using perfused rat livers demonstrated that 100 to 1000 μM adenosine increased total hepatic ATP. In the presence of 200 μM adenosine they saw about a doubling of total hepatic AMP after incubating for 1 h. This complicates the interpretation of adenosine effects on hepatic metabolism because it is difficult to tell whether the effects of adenosine are due to increases in ATP or inhibition of adenylyl cyclase. In view of the increases in total ATP which would be anticipated in the presence of 200 μM adenosine it was surprising to find no change in basal glucose release or of glycogen phosphorylase α by liver cells. Adenosine (200 μM) did inhibit the increases in glucose release due to epinephrine and glucagon but this was not mediated through any change in glycogen phosphorylase α activity which was present in liver cell homogenates.

It was possible to separate effects of adenosine on ATP from those on cyclic AMP by the use of 2',5'-dideoxyadenosine which cannot be phosphorylated (due to lack of a 5'-hydroxyl group) or deaminated. Furthermore, this adenosine analog is a more effective inhibitor of hepatic adenylyl cyclase than is adenosine (9). We found similar results with respect to cyclic AMP accumulation by rat liver cells. However, despite the fact that 2',5'-dideoxyadenosine gave a much greater inhibition of cyclic AMP accumulation due to hormones it was ineffective in blocking hormone-induced increases in hepatic glucose release. At first glance these data provide another instance in which cyclic AMP accumulation can be dissociated from a metabolic response (glycogenolysis). However, it should be noted that epinephrine alone did not measurably elevate cyclic AMP while it elevated glycogenolysis to the same extent as glucagon (Fig. 2). These data can be interpreted as supporting the hypothesis of Bornbaum and Fain (11) that there are other factors besides cyclic AMP involved in hepatic glycogenolysis. Alternatively one could postulate that 2',5'-dideoxyadenosine inhibits supermial increases in cyclic AMP but not the small elevations which are required for activation of glycogenolysis.

The increase in labeled AMP due to valinomycin probably reflects on uncoupling action of this drug as noted in Ehrlich ascites tumor cells (12, 13). Two minutes after the addition of valinomycin there was an increase not only in labeled AMP but also of labeled adenosine, IMP, inosine, and hypoxanthine. The addition of 200 μM adenosine also markedly increased labeled hypoxanthine formation over 20 min. However, the mechanisms involved were quite different since adenosine did not produce any increase in labeled AMP formation and markedly increased labeled inosine accumulation over 20 min. Adenosine addition at concentrations between 50 and 100 μM has been shown to elevate total ATP in rat liver cells (8) while valinomycin should have the opposite effect. Possibly the explanation for their similar effects on the formation of labeled hypoxanthine from ATP is as follows. Adenosine increases the turnover of label by elevating total ATP which has been shown to activate adenylyl deaminase in rat heart and lung (14) and this accounts for the much greater accumulation of labeled inosine in the presence of 200 μM adenosine. In contrast valinomycin increases the accumulation of labeled AMP which is then cleaved by adenylyl phosphatase to give labeled adenosine. Interestingly, in the presence of both valinomycin and 200 μM adenosine there was a marked increase in labeled adenosine accumulation and drop in labeled ATP.

In rat liver cells incubated with allopurinol, hypoxanthine accumulates as the end product of nucleoside catabolism. If adenosine deaminase is blocked as in the present experiments inosine is derived from the cleavage of IMP and its major metabolic fate is as a precursor of hypoxanthine. Adenosine, inosine, and hypoxanthine can all be converted to nucleotides by liver. Furthermore, Pritchard et al. (15) have shown that perfused rat livers can take up hypoxanthine and release adenosine.

It is difficult to relate our results to those of Chagoya de Sánchez and associates who found that the intraperitoneal injection of 200 mg/kg of adenosine markedly increased liver glycogen turnover (16) and liver ATP (17). They found an 8-fold increase in the active form of glycogen synthetase 1 h after the in vivo administration of adenosine which was blocked by cycloheximide (16). Since the phosphatase which activates glycogen synthase is the same enzyme which inactivates glycogen phosphorylase, one would have expected a marked drop in glycogenolysis due to adenosine. However, our studies were short term (10 min or less) and any effect of adenosine which was mediated via protein synthesis would likely be minimal in this period. Our failure to see an effect of adenosine on glycogen phosphorylase activity, except for a slight increase, argue against short term regulation by adenosine of glycogen phosphorylase phosphatase.

Allopurinol is an analog of hypoxanthine which is converted to oxipurinol (4,6-dihydroxypyrazolo[3,4-d]pyrimidine) by xanthine oxidase (18). Allopurinol and oxipurinol both inhibit xanthine oxidase (18) and are converted in trace amounts to mononucleosides in rat liver (19). Allopurinol does not affect the utilization of labeled hypoxanthine or nucleic acid synthesis or interfere with the growth of cultured skin fibroblasts which lack xanthine oxidase (20). Abnormally high amounts of hypoxanthine accumulate in the presence of allopurinol and this may have resulted in greater recycling of label due to reutilization of hypoxanthine. Ordinarily hypoxanthine would further oxidize to uric acid and allantoin which are not recycled.

We conclude that there is no appreciable effect of micromolar concentrations of adenosine on cyclic AMP metabolism by isolated rat parenchymal cells even in the presence of erythro-9-(2-hydroxy-3-nonyl) adenine and allopurinol. In intact cells there is seldom enough adenosine around, even if its removal by deamination is blocked, to affect adenylyl cyclase. This is particularly true since fairly high concentrations of adenosine are required to inhibit cyclic AMP accumulation by rat liver cells.

Acknowledgments—The authors wish to acknowledge the technical assistance of Mrs. S. H. Li. We are grateful to Dr. C. Londos and M. S. Preston of the National Institutes of Health for providing manuscripts prior to publication.

REFERENCES
Adenosine, Cyclic AMP, and Hepatic Glycogenolysis


Additional references are found on p. 8070.
Adenosine, Cyclic AMP, and Hepatic Glycogenolysis

Supplementary Material

Adenosine, cyclic AMP Metabolism and Analogues in Rat Liver Cells

John R. Parks and Raymond R. Shepherd

Experimental Procedures

Male albino rats were isolated from the pregnant mothers and sacrificed by a rapid decapitation after a 4-hour fast. The livers were immediately removed and cut into two halves. Each half was placed in a separate 10-cm tissue culture dish containing 10 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5.5 mM glucose and 150 mM sodium lactate. The liver slices were incubated for 20 minutes at 37°C in a atmosphere of 95% O2/5% CO2. The buffer was then changed to one containing 2.5 mM 3-mercaptopropionic acid (MPA) and the incubation was continued for an additional 40 minutes.

RESULTS

Adenosine was relatively ineffective when added to isolated liver slices in concentrations up to 100 μM. However, when the slices were pretreated with 10 μM of theophylline, an adenosine receptor antagonist, the adenosine concentration required for half-maximal stimulation was reduced to 1 μM. The results are presented in Table I.

<table>
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<th>Concentration (μM)</th>
<th>Adenosine Activity (%)</th>
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<td>100</td>
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Table I. Effect of Adenosine on Glycogenolysis in Rat Liver Slices

Table II. Effect of Adenosine, MPA, and Allopurinol on Cyclic AMP Accumulation in Rat Liver Slices

<table>
<thead>
<tr>
<th>Adenosine</th>
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<th>Allopurinol</th>
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Table III. Effect of Adenosine and MPA on Glycogenolysis in Rat Liver Slices

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<th>Concentration (μM)</th>
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Supplemental Material

Adenosine, cyclic AMP Metabolism and Analogues in Rat Liver Cells

Table IV. Effect of Adenosine on Glycogenolysis in Rat Liver Slices

<table>
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<th>Adenosine</th>
<th>MPA</th>
<th>Allopurinol</th>
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<tr>
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Table V. Effect of Adenosine and MPA on Glycogenolysis in Rat Liver Slices

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Adenosine did not affect the increase in glycogenolysis due to either glucose or epinephrine. Moreover, it is significant that the glycogenolysis was not increased by epinephrine when the liver was perfused with a high adenosine concentration (0.1 nM). This indicates that the glycogenolysis is not affected via a mechanism that involves adenosine metabolism.

There was a clear correlation of concentration in the liver cells and the glycogenolysis due to epinephrine. The glycogenolysis was increased by a decrease in the concentration of adenosine, temperature, or ATP. ATP is a major metabolite of adenosine, temperature, and other factors that can influence glycogenolysis.

The effect of adenosine on glycogenolysis is shown in Figure 4. The glycogenolysis was increased by a decrease in the concentration of adenosine. The glycogenolysis was also increased by a decrease in the concentration of ATP. The glycogenolysis was not increased by a decrease in the concentration of temperature.

In conclusion, the results show that adenosine and ATP are important factors in the regulation of glycogenolysis. The decrease in the concentration of adenosine or ATP increases glycogenolysis, while the increase in the concentration of adenosine or ATP decreases glycogenolysis.
Adenosine, cyclic AMP metabolism, and glycogenolysis in rat liver cells.
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