Separation of Effects of Adenosine on Energy Metabolism from Those on Cyclic AMP in Rat Thymic Lymphocytes*

(Received for publication, November 22, 1976, and in revised form, April 5, 1977)

STEVEN K. NORDEEN AND DONALD A. YOUNG‡
From the E. Henry Keutmann Laboratory, Endocrine-Metabolism Unit, Department of Medicine, and the Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14624

In rat thymic lymphocytes incubated for 2 h without exogenous energy-providing substrate, adenosine may be substituted for glucose as a means of maximally restoring energy metabolism and those cellular functions whose rates are sensitive to small changes in the energy balance, such as protein synthesis and uridine utilization for RNA synthesis (Nordeen, S. K., and Young, D. A. (1976) J. Biol. Chem. 251, 7295-7303). Since effects of adenosine in thymocytes and other cells have frequently been attributed to changes in cyclic AMP, this report investigates its possible involvement in these glucose-like restorative actions of adenosine.

Although the same range of doses of adenosine effective at raising cyclic AMP also elicit roughly parallel stimulation of protein synthesis and uridine utilization, further results dissociate the restorative actions from those on cyclic AMP. (a) Other purine nucleosides mimic the glucose-like actions of adenosine without increasing cyclic AMP; (b) conversely, prostaglandin E, mimics the cyclic AMP response without restoring energy metabolism or energy-dependent functions; and (c) potentiation of the cyclic AMP response, either by inhibiting phosphodiesterase or adenosine deaminase, does not enhance the restorative response to a range of doses of adenosine. Finally, cyclic AMP-mediated glycogenolysis cannot account for the glucose-like effects since addition of adenosine increases, not decreases, levels of glycogen.

Several of the results suggest that metabolism of adenosine itself as an energy-providing substrate might account for its glucose-like effects. Further experiments which reveal that carbon from the ribose moiety is metabolized to CO₂ in quantities comparable to carbon from glucose support this proposal. Although cyclic AMP does not appear to be involved in the restorative actions of adenosine on cellular energy metabolism, an enhanced sensitivity of the cyclic AMP response in cells where adenosine deaminase has been inhibited is consistent with proposals that cyclic AMP may mediate the immunosuppression associated with adenosine deaminase deficiency in man.

Investigations of the mechanisms by which adenosine produces its biological effects in a number of different types of cells, including effects on carbohydrate and lipid metabolism, have frequently focused on actions of adenosine on cyclic AMP (1-12). In rat thymic lymphocytes, our previous studies of interrelationships between energy production and rates of energy-dependent biosynthetic and transport processes have revealed that (a) these cells are dependent upon added glucose, or in its absence upon endogenous glycogen, to sustain ATP production at rates sufficient to support optimal energy balance (calculated as energy charge); and (b) that added adenosine can replace glucose as a means of maximally maintaining or (when added later) restoring glucose-6-P, energy charge, and such cellular functions as protein synthesis and uridine uptake (13) whose rates are sensitive to small changes in the energy balance (13-16). These observations suggest that glycogenolysis triggered by the previously observed (12) adenosine-induced increases in cyclic AMP may account for the glucose-like actions of adenosine. This suggestion is further supported by an observation that adenosine can enhance glycogenolysis in the liver (17). Since other readily metabolized, energy-providing substrates (pyruvate, lactate, or β-hydroxybutyrate) are only about one-half as effective as glucose at supporting thymus cell metabolism (perhaps due to the ability of glucose to furnish glucose-6-P for nuclear energy production) (18), the ability of adenosine to rival the efficacy of glucose is of interest.

The present study investigates the mechanism by which adenosine achieves its supportive role. We conclude that its glucose-like actions on thymocyte metabolism are not mediated by the increases in levels of cyclic AMP, nor by glycogenolysis; they may instead be attributed to metabolism of the ribose moiety of adenosine via energy-yielding pathways.

MATERIALS AND METHODS

Cell Preparation and Incubation—Juvenile male Sprague-Dawley rats (Charles River Breeding Laboratories) were decapitated and their thymuses rapidly removed and prepared as in previous experiments (14, 15). Briefly, thymuses pooled from 3 to 18 rats were
minced in Krebs-Ringer bicarbonate buffer (pH 7.4 in equilibrium with 95% O₂, 5% CO₂), and individual thymocytes dispensed by a hand-held glass homogenizer with a loose fitting pestle. The cells were then filtered, washed twice in 40 to 50 ml of buffer, resuspended, then filtered again before aliquots, either 0.5 or 1 ml, were placed into 10-ml glass Erlenmeyer flasks in a Dubnoff shaking metabolic incubator at 37 to 38°C. The time that elapsed between killing the first rat and the start of the incubations was 20 to 40 min. The preparation of cells was performed at room temperature. Incubation flasks were gassed periodically with 95% O₂, 5% CO₂ to ensure constant pH and O₂ content.

The results were qualitatively quite consistent from experiment to experiment; however, there is some degree of variation in absolute quantities day to day due in part to the inaccuracies introduced when standardizing the data by means of packed cell volume measurements. Hence, we frequently present results from a single typical experiment, representative of results observed repeatedly in other experiments, so that absolute quantities may be shown, as opposed to a percentage of the control response.

**Measurement of Metabolites—Assays for ATP, ADP, and AMP**

were done in neutralized perchloric acid extracts by modifications of standard enzymatic procedures (19). The modifications primarily involve a scaling down and, most importantly, a careful titration of the samples to within 0.05 pH unit of pH 7.5 with a microtitrator. Glucose was measured with amyloglucosidase, a-1,6-glucosidase, and glucose-6-P dehydrogenase by adaptation of the method of Passonneau and Lauderdale (20) following alkali degradation of pre-existing glucose and glucose-6-P (21).

**Cyclic AMP** was determined by a radioimmunoassay procedure adapted from a standard method (22, 23). The assay is a precipitate-reaction by adenosine was determined to be negligible. Levels of intermediates are reported as micromoles (or nanomoles) per ml of packed cell volume (micromoles/ml of PCC); 1 ml of packed cells equals approximately 1 g (wet weight) or 5 x 10⁶ cells. Packed cell volume was determined by a standard microhematocrit procedure, and depending on the experiment, ranged from 4.7 to 13%. To assay the metabolism of exogenous adenosine, a 50-μl aliquot of cell suspension was pipetted into an equal volume of 6% trichloroacetic acid, the precipitate pelleted by centrifugation, and a 50-μl aliquot of supernatant added to 950 μl of potassium phosphate buffer (0.15 M, pH 7.5). Adenosine was then determined by loss of absorbance at 265 nm following addition of adenosine deaminase. This procedure was adapted from a standard method for determination of adenosine (19).

**Radiolabeling of Protein, RNA, and CO₂** Incorporation of [5-³H]uridine into RNA or [14C]valine into protein was determined as previously reported (14, 15). In all cases, a concentration of 0.01 mg/ml of [5-³H]uridine (1.25 to 2.5 μCi/flask) and 0.1 mg/ml of [14C]valine (0.25 to 0.5 μCi/flask) was used. We have previously demonstrated that the experimental setting used in this study, changes in rates of incorporation reflect changes in rates of protein synthesis per se, as opposed to changes in amino acid uptake or in the specific radioactivity of intracellular amino acids (14, 16). In contrast to these conclusions about protein labeling, previous studies have indicated that the uptake of uridine is the rate-limiting process in the incorporation of uridine into RNA; substrate-induced stimulation of rates of labeling of RNA largely reflect changes in the specific activity of intracellular UTP (16).

Incorporation of radioactivity into CO₂ from glucose, adenosine, or inosine was determined as previously reported with phenethylamine as the CO₂-trapping agent (19).

**Materials—Enzymes, cofactors, nucleosides, and nucleotides** were purchased from Boehringer Mannheim and Sigma, phenethylamine and purchased adenosine, [*4-¹⁴C]adenosine, [*U-¹⁴C]adenosine, [*U-¹⁴C]inosine, and [*U-¹⁴C]glucose from Fisher, [*¹⁴C]valine, [*¹⁴C]uridine, (8-¹⁴C)-adenosine, (U-¹⁴C)adenosine, (U-¹⁴C)inosine, and (U-¹⁴C)glucose from Amersham/Searle. EHNA and Ro20-1724 were gifts from Dr. II. J. Schaefter and Hoffman-La Roche Inc., respectively.

1 Recent experiments by W. A. Guyette and D. A. Young (manuscript in preparation) demonstrate that the stimulatory effects of energy-providing substances and the inhibitory effects of cortisol (which inhibits ATP production) on the incorporation of amino acids into protein are paralleled exactly by the changes in activity of cell-free protein-synthesizing systems; (b) that these changes occur independently of changes in the size of amino acid pools; and (c) that the effects of substrates (or cortisol) are accompanied by increases (or decreases) in the fraction of ribosomes in polysomes.

2 The abbreviations used are: EHNA, erythro-9-(2-hydroxy-3-

**RESULTS**

**Relationships between Small Shifts in Levels or Ratios of Adenine Nucleotides and Changes in Rates of Protein Synthesis and Uridine Utilization—** As detailed in previous publications from this laboratory (13-16), isolated thymus cells provided with glucose maintain rather constant levels of ATP, ADP, and AMP, and rates of protein synthesis and uridine utilization. In the absence of glucose (or other energy-providing substrate), ADP and AMP begin to rise, and ATP declines slowly. The energy charge falls by about 7% by 2 h. There is also an associated but much larger decline (roughly by 75 to 80%) in some energy-dependent processes, such as protein synthesis and uridine utilization. As can be seen in Fig. 1, addition of glucose (or alternatively adenosine) at 2 h rapidly restores cellular energy charge to the level observed in cells that had been provided glucose (or adenosine) all along (compare open versus closed symbols). With adenosine, the total adenine nucleotide pool expands for about 10 min before reaching a new steady state level as can best be seen in Fig. 1 by comparing levels of ATP in the presence of glucose versus those with adenosine. Yet despite the higher levels of ATP and the expansion of the total adenine nucleotide pool with adenosine compared to glucose, the energy charge is restored to nearly identical values with equal rapidity (within 5 min).

Many experiments, such as that in Fig. 2, have also shown that in conjunction with these rather subtle increases in energy charge there is a rapid re-establishment of maximal rates of protein synthesis¹ and uridine uptake (3- to 6-fold increase). Thus, measurements of protein synthesis and uridine utilization provide the most sensitive indices of small changes in cellular energy metabolism. Data in Fig. 2 also show that adenosine approaches the efficacy of glucose at re-establishing maximal rates of protein synthesis and uridine utilization, but addition of adenosine together with glucose does not yield an additive stimulation of either. In fact, adenosine exerts a transient suppression of glucose-stimulated uridine utilization. This is the result of a short lived, dose-dependent, inhibitory effect of adenosine on uridine incorporation into RNA as previously noted (13). After this initial inhibition (5 to 10 min), rates of uridine utilization do tend to reflect the changes in energy balance.

**Effects of Adenosine and Analogues on Cyclic AMP and on Rates of Energy-dependent Functions—** Experiments such as that in Fig. 3 show that levels of adenosine that restore rapid protein synthesis and uridine utilization also increase levels of cyclic AMP. However, a closer comparison of dose responses reveals only a rough correlation between levels of cyclic AMP and observed rates of protein synthesis. For example, levels of adenosine between 0.25 and 2.5 mM are about equally effective at restoring protein synthesis (left) and initially (at 5 min) increasing cyclic AMP accumulation (right). At later times, however, the higher adenosine levels (e.g. 2.5 mM) further increase cyclic AMP without concurrent increases in protein synthesis. As can also be seen from Fig. 3 (center) these same relationships probably also hold for uridine utilization, providing that one takes into account the transient inhibition by high levels of adenosine as mentioned earlier. Here this inhibition is superimposed on the simultaneous stimulation of uridine utilization that results from the restoration of the energy balance. It is also important to note that substrate deprivation is itself
Adenosine's Actions on cAMP and Lymphocyte Energy Metabolism

Fig. 1. Rapid restoration of adenine nucleotide levels and energy charge by adenosine and glucose. At either the start of the incubation (closed symbols) or at 120 min (open symbols), thymus cells were given glucose (○, 5.5 mM) or adenosine (□, 2.5 mM). Controls (△) received buffer alone. Aliquots of 1 ml were removed at 125 min and 180 min and were pipetted into 1.5-ml polypropylene tubes (Eppendorf) containing 200 μl of cold 0.5 N perchloric acid. Levels of adenine nucleotides were determined on the neutralized perchloric acid extracts as described. Levels of adenine nucleotides, reported as micromoles per ml of packed cells, represent the mean of values obtained in four separate incubation flasks. Standard errors are within or slightly greater than the symbol in all cases. Energy charge is calculated according to Atkinson (22) as ([ATP] + 1/2 [ADP])/(2ATP) + [ADP] + [AMP]).

without effect on basal levels of cyclic AMP; starved cells exhibit cyclic AMP levels identical with those seen in cells provided glucose from the start, or at later times.3

To further assess a possible role of cyclic AMP, the ability of adenosine to restore biosynthetic processes was compared with that of added cyclic AMP, or various adenosine analogues (Fig. 4). Cyclic AMP itself (at concentrations up to 1 mM) fails to increase protein synthesis or uridine utilization; however, this failure may possibly be attributed to the inability of added cyclic AMP to penetrate thymus cells (23). Although dibutyryl cyclic AMP (at 1 mM) apparently stimulates these energy-dependent functions somewhat, this may not be due to its cyclic AMP-like effects, but instead to the energy-yielding metabolism of butyrate derived from decylation of the dibutyryl derivative.4 In Fig. 4, inosine, guanosine, AMP, and ATP (also at concentrations of 1 mM) all are effective at restoring rates of energy-dependent processes. AMP and ATP, like adenosine, also rapidly raise levels of cyclic AMP. Yet with ATP, the restoration of protein synthesis and uridine uptake is substantially delayed, suggesting that the increase in cyclic AMP is itself either insufficient or incidental to the restoration; instead further metabolism of the ATP (perhaps to adenosine) may be required.5 The concept that the rise in cyclic AMP is incidental to the restorative actions of adenosine is supported by further data which indicate that other purine nucleosides, inosine and guanosine, are equally as effective at supporting protein synthesis and uridine utilization without altering intracellular levels of cyclic AMP. The purine nucleoside configuration appears to be integral to the ability to support thymocyte metabolism because neither the pyrimidine nucleoside, uridine, nor the purine base, adenine, stimulates protein synthesis or uridine incorporation. In addition, neither uridine nor adenine increases cyclic AMP. Other data (not shown) indicate that neither prostaglandin E1 (1 μM), which elicits a cyclic AMP response similar to that of 1 mM adenosine (12), nor ribose, nor a combination of adenine and ribose can mimic the restorative actions of adenosine.

Experiments that Rule Out Involvement of Glycogenolysis in Glucose-like Effects of Adenosine—As discussed earlier (see the introduction), glycogenolysis seems a likely mechanism by which adenosine, through its stimulation of cyclic AMP, could rapidly increase levels of glucose-6-P and support energy metabolism and energy-dependent functions. However, experiments like those in Fig. 5 demonstrate that net breakdown of glycogen cannot explain these actions, since addition of adenosine (squares) at the start of the incubation, like glucose (triangles), largely prevents the decline in levels of glycogen seen in substrate-deprived cells (circles). Furthermore, addition of adenosine at 2 h leads to a net gain of glycogen.

Potential of Cyclic AMP Response does not Enhance Restorative Effects of Adenosine—Experiments in which the actions of adenosine on cyclic AMP are independently enhanced further dissociate these actions from the restorative effects. As shown by the shaded bars in Fig. 6, Ro20-1724, an inhibitor of phosphodiesterase, potentiates the cyclic AMP response to doses of adenosine which range from otherwise ineffective to optimal; however, the rate of protein synthesis is not similarly enhanced (compare closed versus open (control) symbols).

A second means of augmenting the cyclic AMP response is to inhibit the degradation of adenosine with EHNA, an inhibitor of adenosine deaminase. Without EHNA, adenosine at a dose of 1 mM is undetectable in the medium 10 min after its addition (cf. Fig. 7). One might infer from this that adenosine
Effect of different doses of adenosine on levels of cyclic AMP and on restoration of rates of protein synthesis and uridine utilization. Thymus cells were incubated for 120 min without exogenous energy-providing substrate prior to addition of adenosine at the indicated concentrations. Ten minutes before this addition, a mixture of [14C]valine and [5-3H]uridine was added to each flask. Aliquots of cell suspension, 50 μl, were removed at 5, 10, and 30 min after addition of adenosine, pipetted into cold 6% trichloroacetic acid, and cyclic AMP determined as described. Aliquots of 50 μl were also removed 15, 35, and 60 min after addition of adenosine, plated onto filter paper discs which were then floated onto cold 10% trichloroacetic acid, and radioactivity in RNA and protein determined as described. Each point represents the mean of values obtained from three separate incubation flasks, except the control values which are from duplicate flasks. Standard errors, where larger than the symbol, are depicted by vertical lines; packed cell volume, PCV.

Comparison of the effect of adenosine and various analogues on cyclic AMP, protein synthesis, and uridine utilization. As before, thymus cells were incubated for 120 min without substrate at which time adenosine (ADO) or various analogues, inosine (INO), guanosine (GUA), AMP, ATP, dibutyryl cyclic AMP (dbcAMP), cyclic AMP (cAMP), uridine (URD), or adenine (ADE), were added to give a concentration of 1 mM. Controls (Con) received buffer alone. Levels of cyclic AMP and of radioactivity in protein and RNA were determined at the indicated times. Each point represents the mean of values obtained from three separate flasks. Standard errors, where larger than the symbol, are depicted by vertical lines. Results (not included) indicate that the rates of protein synthesis and uridine incorporation in cells which received cyclic AMP and dibutyryl cyclic AMP at 0.1 mM are not different from the rates in no-substrate controls. PCV, packed cell volume.

Adenosine's metabolic effects are no longer required after 10 min, having already generated some signal which leads to the restoration and maintenance of cellular metabolism. Alternatively, metabolites derived from adenosine may be responsible for the glucose-like effects. The latter mechanism predicts that if deamination of adenosine is prevented with EHNA then no metabolites could accumulate, and thus no restorative effects would be seen. Conversely, the first mechanism predicts that preservation of adenosine should augment (or at least not change) adenosine's restorative actions on thymocyte metabolism. EHNA at 1 μM par-
Adenosine's Actions on cAMP and Lymphocyte Energy Metabolism

Fig. 5. Effects of adenosine and glucose on levels of glycogen in cells deprived of substrate. Glycogen levels were measured in three separate experiments. Thymus cells received adenosine (□, □, □), glucose (△, △, △, ◯), or buffer alone (○, ○, ◯) at the beginning of the incubation or after 120 min of incubation without substrate. In each experiment, adenosine was added to give a concentration of 2.5 mM. In Experiments A (open symbols) and B (closed symbols), glucose was added to give 5.5 mM. In Experiment C (half-filled symbols), glucose was added to 2.5 mM (△) or 15 mM (◇). At the indicated times, 1 ml of cell suspension was pipetted into 1.5-ml polypropylene tubes containing 100 μl of 2 N NaOH, capped, mixed in a Vortex mixer, and placed in a boiling water bath. Glycogen was determined as described. The symbols represent the mean of values obtained from two or usually three separate incubation flasks. In all cases, the range of values is within or slightly greater than the size of the symbol. In each experiment, levels of glycogen are significantly (p < 0.005) increased in adenosine-treated cells at 180 min when compared to levels in substrate-deprived cells at the time adenosine was added (120 min). Glycogen levels in substrate-deprived cells from Experiments A and B have been replotted on a semilog scale in the inset to demonstrate the first order kinetics of the net utilization of glycogen under these conditions. PCV, packed cell volume.

Fig. 6. Effect of a phosphodiesterase inhibitor on the rise in cyclic AMP and on the restoration of protein synthesis due to adenosine. As in previous experiments, thymus cells were incubated for 120 min before addition of adenosine, 1 mM (◇, ◇), 100 μM (○, ○), or 10 μM (△, △). Controls received buffer (□, □). Earlier (at 90 min) an addition of either Ro20-1724 (0.5 mM, closed symbols and shaded bars) or buffer (open symbols and open bars) was made. An addition of [14C]valine followed at 112 min. Aliquots were withdrawn at 128 min for determination of cyclic AMP and at indicated times for radioactivity in protein. The data represents the mean of values obtained from four separate flasks. Standard errors, where larger than the symbols, are represented by vertical lines.

Fig. 7. Effect of an inhibitor of adenosine deaminase, EHNA, on the metabolism of added adenosine. Thymus cells were added to flasks already containing EHNA, and the incubation continued for 5 min until adenosine (1 mM) was added. Aliquots of cell suspension were removed immediately, at 5, and at 10 min after this addition for determination of adenosine as described. Each symbol represents the mean of values obtained from three separate incubation flasks.

Adenosine's Actions on cAMP and Lymphocyte Energy Metabolism

Fig. 8. Effect of an inhibitor of adenosine deaminase, EHNA, on the metabolism of added adenosine. Thymus cells were added to flasks already containing EHNA, and the incubation continued for 5 min until adenosine (1 mM) was added. Aliquots of cell suspension were removed immediately, at 5, and at 10 min after this addition for determination of adenosine as described. Each symbol represents the mean of values obtained from three separate incubation flasks.

Like actions, since glucose-restored (diamonds), inosine-restored (squares), and basal (triangles) rates of synthetic functions are either uninhibited or only slightly inhibited. Measurements of adenine nucleotide levels in Table I also indicate that EHNA also has some inhibitory effect in the presence of glucose; however, this effect is much smaller than when adenosine is the restoring agent. Also, since the results (in Fig. 9 and Table I) suggest deamination of adenosine to inosine is a prerequisite for the restorative effects, it seems likely that...
Adenosine's Actions on cAMP and Lymphocyte Energy Metabolism

**Fig. 8.** Effect of EHNA on the adenosine-induced increase in levels of cyclic AMP. EHNA (closed symbols) or water (open symbols) was added to substrate-deprived thymus cells at 115 min. Adenosine (0, +, 1 mM; 0, 0, 100 μM; 0, n, 10 μM) was added at 120 min; controls received buffer (a, A). Levels of cyclic AMP were determined 5, 10, and 30 min after this addition. Symbols represent the mean of values obtained from triplicate flasks. Standard errors, where larger than the symbol, are depicted by vertical lines. PCV, packed cell volume.

**Table I**

Inhibition by EHNA of restoration of energy charge by adenosine

At 120 min, substrate-deprived thymus cells received adenosine (1 mM), glucose (5.5 mM), or buffer alone. EHNA (100 μM) was added at the same time to one-half of the flasks which received either glucose or adenosine. At 130 min, cold 60% perchloric acid was added to stop the incubation, and the flasks were immediately placed in an ice water bath. Adenine nucleotides were determined on the neutralized perchloric acid extracts as described. Values represent the mean of determinations from four flasks ± S.E.

| Condition          | Adenine nucleotides at 130 min | Energy charge | AMP | ADP | ATP |
|--------------------|--------------------------------|---------------|-----|-----|-----|
| No substrate       | 0.1104 ± 0.0015                 | 0.565 ± 0.003 | 2.24 ± 0.01 | 0.865 |
| Adenosine          | 0.0678 ± 0.0062                 | 0.200 ± 0.008 | 2.76 ± 0.02 | 0.910 |
| Adenosine + EHNA   | 0.1273 ± 0.0027                 | 0.597 ± 0.015 | 2.42 ± 0.01 | 0.864 |
| Glucose            | 0.0425 ± 0.0022                 | 0.355 ± 0.015 | 2.53 ± 0.06 | 0.925 |
| Glucose + EHNA     | 0.0691 ± 0.0028                 | 0.447 ± 0.015 | 2.41 ± 0.01 | 0.900 |

* PCV, packed cell volume.

adenosine acts via the same cyclic AMP-independent means as does inosine (and perhaps guanosine, cf. Fig. 4).

**Utilization of Adenosine as Energy-providing Substrate** – These last findings, combined with the observations that adenosine restores energy-dependent functions (Figs. 2 and 3) while increasing glycogen (Fig. 5), suggest that the further metabolism of adenosine via energy-yielding pathways may account for its glucose like effect. Accordingly, experiments were done to test this alternative to a quasi-hormonal, cyclic AMP-mediated mechanism. As can be seen in Table II (Experiments A to D), the rate of metabolism of 14C from uniformly labeled adenosine to 14CO2 is similar to that of glucose. Because thymus cells lack uricase (24, 25), the only enzyme in the purine degradation pathways of the rat that catalyzes a

**Fig. 9.** Effect of EHNA on the restoration of protein synthesis and uridine utilization by adenosine. At 120 min, substrate-deprived thymus cells received either glucose (Gl, 5.5 mM, adenosine (A), 1 mM, inosine (I), 1 mM, or buffer (B). Prior to this (at 100 min), all flasks received [3H]valine and [14C]uridine and (at 110 min) either 100 μM EHNA (E, closed symbols), or water. Radioactivity in protein and RNA was determined at the indicated times. Symbols represent the mean of values obtained from three replicate flasks. Standard errors, where larger than the symbols, are depicted by vertical lines.

**Table II**

Incorporation of 14C from glucose or adenosine into 14CO2

In all experiments, thymus cells were incubated for 120 min without energy-providing substrate. At that time, (8-14C)glucose or uniformly labeled (14C)adenosine or (14C)inosine was added at the concentrations indicated. In Experiments A to C, the incubation was halted at 180 min by addition of 2 N HCl. In Experiments D to F, the incubation was ended at 150 min. In Experiment D, EHNA (100 μM) was added 15 min before adenosine or glucose; in Experiment F, EHNA was added concurrently with adenosine or inosine. The number of replicate flasks for each condition is given in parentheses; PCV refers to packed cell volume.

| Experiment | Substrate | CO2 Produced from added substrate | Inhibition |
|------------|-----------|----------------------------------|------------|
|            | Glucose, 5.5 mM | 5.49 ± 0.07 (10) |            |
|            | Adenosine, 2.5 mM | 4.93 ± 0.20 (10) |            |
|            | Glucose, 5.5 mM | 8.94 ± 0.24 (6) |            |
|            | Adenosine, 2.5 mM | 5.60 ± 0.60 (6) |            |
|            | Glucose, 5.5 mM | 5.81 ± 0.08 (4) |            |
|            | Glucose, 2.0 mM | 5.73 ± 0.06 (3) |            |
|            | Adenosine, 2.0 mM | 4.73 ± 0.14 (5) |            |
|            | Glucose, 1.0 mM | 5.87 ± 0.10 (8) |            |
|            | Glucose + EHNA | 4.77 ± 0.12 (7) | 18.7       |
|            | Adenosine, 1.0 mM | 7.15 ± 0.07 (7) |            |
|            | Adenosine + EHNA | 2.73 ± 0.08 (9) | 61.8       |
|            | Adenosine, 1.0 mM | 4.07 ± 0.05 (8) |            |
|            | Inosine, 1.0 mM | 3.90 ± 0.06 (8) |            |
|            | (8-14C)Adenosine | ≤0.001 (8) |            |
|            | Adenosine, 1.0 mM | 4.70 ± 0.06 (8) |            |
|            | Adenosine + EHNA | 1.70 ± 0.05 (7) | 63.8       |
|            | Inosine, 1.0 mM | 4.43 ± 0.14 (7) |            |
|            | Inosine + EHNA | 3.24 ± 0.03 (8) | 26.9       |
Effects of adenosine are mimicked by an analogue, N-phenylcyclic AMP metabolism (here decreasing levels) and not as in mine-induced lipolysis and, under some circumstances, in thymus cells through its own further metabolism. (a) The increases glucose oxidation, it appears to work via a different metabolism of ribose-5-P by enzymes associated with the hexokinase (carbon 8) is used. We interpret these results as demonstrating that the ribose provided by adenosine is metabolized by energy-yielding pathways about as effectively as glucose. The large inhibition of CO₂ production from adenosine by EHNA (Experiments E and F) confirms the implication of the results in Fig. 9 and Table I, that deamination to inosine is necessary for the utilization of the ribose moiety as a substrate. Also, as this conclusion predicts, uniformly labeled inosine supports ¹⁴CO₂ production as effectively as adenosine (Experiments E and F). In repeated experiments, EHNA at 100 μM also inhibits ¹⁴CO₂ production from glucose and from inosine somewhat, but always to a much smaller extent than is seen with adenosine.

**DISCUSSION**

This report investigates mechanisms which may account for the previously reported glucose-like supportive actions of adenosine on thymus cell metabolism (13). Cyclic AMP, which may play an essential role in some adenosine actions including certain actions in lymphoid tissues (10, 11, 26, 27), does not appear to be involved in the actions of adenosine on energy metabolism in thymic lymphocytes. Here adenosine is able to substitute for glucose by donating its ribose moiety for metabolism as an energy-providing substrate. Adenosine supports cellular metabolism, whereas ribose itself is ineffective, possibly because adenosine provides ribose in a utilisable form or serves as a means to carry ribose into the cell by a specific transport mechanism (or both).

Several observations indicate that adenosine may provide carbohydrate to energy-yielding pathways by the following route:

\[
\text{Adenosine} \rightarrow \text{inosine} \rightarrow \text{ribose-1-P} \rightarrow \text{ribose-5-P} + \text{hypoxanthine}
\]

where (1) adenosine deaminase, (2) purine nucleoside phosphorylase, and (3) phosphoribosyltransferase. First, adenosine is rapidly deaminated by thymus cells (Fig. 7). Second, enzymes catalyzing Reactions 2 and 3 are present in thymus (28). Third, both inosine and guanosine, substrates for purine nucleoside phosphorylase (29), mimic the supportive actions of adenosine (Fig. 4). Fourth, when an inhibitor of adenosine deaminase is present, then adenosine, a poor substrate itself for the nucleoside phosphorylase (29), is unable to restore either energy charge or macromolecular labeling (Fig. 9, Table I). Finally, metabolism of ribose-5-P by enzymes associated with the hexose monophosphate shunt, the glycolytic, and the glycogen synthetic pathways could account for the ability of adenosine to increase levels of glucose-6-P, lactate, and glycogen (Ref. 13 and Fig. 5).

Among the best studied actions of adenosine are its "insulin-like" effects on the metabolism of glucose and lipids in fat cells (5-7). Although adenosine, like insulin, inhibits catecholamine-induced lipolysis and, under some circumstances, increases glucose oxidation, it appears to work via a different mechanism than insulin (6, 7). Conclusive evidence indicates that in fat cells adenosine is acting through modulation of cyclic AMP metabolism (here decreasing levels) and not as in thymus cells through its own further metabolism. (a) The effects of adenosine are mimicked by an analogue, N-phenylpropyl adenosine (which is not deaminated) and are not reproduced by inosine, the deamination product of adenosine (5); (b) inclusion of adenosine deaminase in the medium abolishes the effects of adenosine (6, 7); and (c) such structurally diverse agents as prostaglandin E, and nicotinic acid which affect fat cell cyclic AMP-like adenosine, reproduce the effects of adenosine on the metabolism of glucose and lipids (5).

However, some actions of adenosine in other tissues may be attributable to the energy-yielding metabolism of the ribose moiety. Degradation of cyclic AMP to adenosine or the subsequent metabolism of adenosine (or both) may be involved in the ability of millimolar levels of either agent to increase levels of glycogen in HeLa cells, an effect opposite to that elicited by dibutyryl cyclic AMP (30), and to increase several biosynthetic parameters (including levels of glycogen) in substrate-deprived uterine horn (31). In the latter studies, other purine nucleotides or nucleosides (or both) also produced adenosine-like effects. The metabolism of adenosine as an energy source probably also accounts for the increase in lactate output in ascites tumor cells given adenosine (32) and contributes to the preservative effects of adenosine (and inosine) on the storage of red blood cells (33) (see also Ref. 34 for recent discussion).

Recently, adenosine, a stimulator of adenyl cyclase in cultured astrocytoma cells (3), was used in conjunction with a phosphodiesterase inhibitor to study the effects of cyclic AMP on glycogen metabolism (35). Somewhat surprisingly, instead of a glycogenolytic response, adenosine partially reversed the glycogen-lowering effect of the phosphodiesterase inhibitor. While an accurate assessment is difficult, it is conceivable that the metabolism of adenosine as an energy source may have favored glycogen synthesis, especially since glycogen metabolism was shown to be regulated by the availability of energy-providing substrate.

In this same study, Passonneau and Crites (35) further showed glycogen metabolism to be additionally and independently regulated by cyclic AMP. Little is known about the regulation of glycogen metabolism in thymic lymphocytes, possibly because low levels are difficult to measure. However, in our experiments, adenosine can cause net deposition of glycogen in spite of substantial increases in cyclic AMP, suggesting that here cyclic AMP is not the controlling factor. Furthermore, experiments such as those in Fig. 7 suggest that, under conditions of stringent energy supply, there may be yet another factor involved in the control of glycogen metabolism; the rate of glycogen breakdown appears to be dependent upon the amount of glycogen itself. First order kinetics of glycogen catabolism are indicated by the linear fit obtained when levels of intracellular glycogen in substrate-deprived cells are plotted as a function of incubation time on a semilog scale (inset, Fig. 5). We have found that as breakdown slows sufficiently in substrate-deprived cells levels of ADP and AMP rise as ATP drops, and concurrently those biosynthetic and transport functions that are sensitive to small changes in adenosine phosphate ratios, e.g., protein synthesis and uridine utilization, first slow, and then are virtually shut down (cf. 13, 14). The low levels of glycogen in thymus cells probably explain why rates of these processes begin to fall within 30 to 45 min unless the cells are maintained with exogenous energy-providing substrate.

In lymphoid tissues, the relationship between the metabolism of adenosine and possible physiological effects is of particular interest in light of the association of adenosine deaminase deficiency in man with a form of severe combined immunodeficiency.
Adenosine's Actions on cAMP and Lymphocyte Energy Metabolism

Adenosine has several actions that are relevant to the immune system. In the presence of cyclic AMP (cAMP), adenosine can modulate cyclic nucleotide responsiveness, as well as interfere with the pyrimidine metabolism of lymphocytes. These effects are of particular importance in the context of immunodeficiency (36-38).

Cytoxicity (36-38). Afflicted individuals, who usually experience chronic bacterial and viral infections early in infancy, have involuted thymuses and lymphocytes that respond poorly to mitogenic stimulation (39). The observation that the sensitivity of the cyclic AMP response to adenosine is markedly increased by EHNA supports a suggestion of Wolberg et al. (10) that adenosine deaminase deficiency may leave lymphocytes unprotected from cyclic AMP-elevating actions, which in turn have been implicated experimentally in adenosine's immunosuppressive effects (10, 11, 26, 27). In addition, our findings together with those of another study (40), which imply that human lymphocytes undergoing blastogenesis are particularly sensitive to EHNA, further suggest that the increase in adenosine uptake (44). We previously have observed an inhibition of the deaminase deficiency may increase cellular susceptibility to pyrimidine starvation (43). Adenosine also inhibits pyrimidine deaminase deficiency, which normally is rapidly relieved as the adenosine is metabolized (13). However, as shown here, when EHNA is used to prevent deamination, the suppression of uridine incorporation is prolonged. This finding suggests that effects on pyrimidine metabolism as well as those on cyclic AMP might also be involved in the pathogenesis of immunodeficiency.

REFERENCES

1. Sattin, A., and Rall, T. W. (1970) Mol. Pharmacol. 6, 13-23
2. Schultz, J., and Daly, J. W. (1973) J. Biol. Chem. 248, 860-866
3. Clark, R. B., Gross, R., Su, Y.-F., and Perkins, J. P. (1974) J. Biol. Chem. 249, 5296-5303
4. Blume, A. J., and Foster, C. J. (1975) J. Biol. Chem. 250, 5005-5008
5. Wieser, P. B., and Fain, J. N. (1975) Endocrinology 96, 1221-1225
6. Fain, J. N., and Wieser, P. B. (1975) J Biol Chem 250, 1037-1040
7. Schwabe, U., Ebert, R., and Erbcher, H. C. (1975) Adv. Cyclic Nucleotide Res. 5, 505-584
8. Peck, W. A., Carpenter, J., and Mesinger, K. (1974) Endocrinology 92, 148-154
9. Mills, D. C. R., and Smith, J. R. (1971) Biochem. J. 121, 185-196
10. Wolberg, G., Zimmerman, T. P., Hiemstra, K., Winston, M., and Chu, L.-C. (1975) Science 187, 957-959
11. Zenser, T. V. (1975) Biochim. Biophys. Acta 404, 202-213
12. Nordeen, S. K., and Young, D. A. (1976) Fed. Proc. 35, 1712
13. Nordeen, S. K., and Young, D. A. (1976) J. Biol. Chem. 251, 7295-7303
14. Young, D. A. (1980) J. Biol. Chem. 244, 2219-2221
15. Young, D. A. (1970) J. Biol. Chem. 245, 2747-2752
16. Young, D. A., Giddings, S., Swonger, A., Kluftner, G., and Miller, M. (1970) in Proceedings of the 3rd International Congress on Hormonal Steroids (James, V. H. T., and Martini, L., eds) pp. 624-635, International Congress Series 219, Excerpta Medica, Amsterdam
17. Chagoya de Sanchez, V., Briones, R., and Pina, E. (1971) Biochem. Pharmacol. 20, 2455-2541
18. Giddings, S. J., and Young, D. A. (1974) J. Cell. Physiol. 93, 409-418
19. Bergmeyer, H. U., ed (1969) Methods of Enzymatic Analysis, pp. 491, 543, and 574, Academic Press, New York
20. Passonneau, J. V., and Lauderdale, V. R. (1974) Anal. Biochem. 60, 405-412
21. Lust, W. D., Passonneau, J. V., and Crites, S. K. (1975) Anal. Biochem. 68, 328-331
22. Atkinson, D. E. (1968) Biochemistry 7, 4030-4034
23. MacManus, J. P., Whittfield, J. F., and Braceland, B. (1971) Biochem. Biophys. Res. Commun. 42, 569-569
24. Eyers, S. O., Friedman, M., and Garfield, M. M. (1947) Am. J. Physiol. 150, 677-681
25. Lang, F., Greger, R., and Deetjen, P. (1974) Pfluegers Arch. Gesamte Physiol. Menschen Tiere 351, 323-330
26. Hirschhorn, R., Grossman, J., and Weissman, G. (1970) Proc. Soc. Exp. Biol. Med. 133, 1361-1365
27. Smith, J. W., Steiner, A. L., and Parker, C. W. (1971) J. Clin. Invest. 50, 442-448
28. MclLwain, L. A., and Lampen, J. O. (1951) J. Biol. Chem. 191, 95-104
29. Parks, R. E., and Agarwal, R. P. (1972) in The Enzymes (Boyer, P. D., ed) 3rd Ed, Vol. 7, p. 500, Academic Press, New York
30. Hitz, H., and Kaukel, E. (1973) Mol. Cell. Biochem. 1, 229-239
31. Hechter, O., Yoshinaga, K., Harkerston, I. D. K., and Birchall, K. (1967) Arch. Biochem. Biophys. 122, 449-465
32. Vusako, W. D. (1971) J. Biol. Chem. 246, 1607-1617
33. Gabrio, B. W., Finch, C. A., and Huennekens, F. M. (1956) Blood 11, 103-113
34. Brewer, G. J. (1974) in The Red Rilled Cell (Surgenor, D. M., ed) 2nd Ed. Vol. 1, pp. 410-413, Academic Press, New York
35. Passonneau, J. V., and Crites, S. K. (1976) J. Biol. Chem. 251, 9615-9620
36. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B., and Meuwissen, H. J. (1972) Lancet 2, 1067-1069
37. Knudsen, B. B., and Dissing, J. (1973) Clin. Genet. 4, 343-347
38. Yount, J., Nichols, P., Ochs, H. D., Hammar, S. P., Scott, C. R., Chen, S.-H., Giblett, E. R., and Wedgwood, R. J. (1974) J. Pediat. 84, 173-177
39. Meuwissen, H. J., Pollara, B., and Pickering, R. J. (1975) J. Pediat. 86, 169-181
40. Carson, D. A., and Seegmiller, J. E. (1976) J. Clin. Invest. 57, 274-282
41. Hall, J. G. (1963) Aust. J. Exp. Biol. Med. Sci. 41, 93-98
42. Koehler, L. H., and Benz, E. J. (1962) Clin. Pharmacol. 20, 5003-5008
43. Green, S. D., and Chan, T.-S. (1973) Science 182, 836-837
44. Plagemann, P. G. W. (1971) Biochim. Biophys. Acta 233, 688-701
Separation of effects of adenosine on energy metabolism from those on cyclic AMP in rat thymic lymphocytes. S K Nordeen and D A Young

*J. Biol. Chem.* 1977, 252:5324-5331.

Access the most updated version of this article at [http://www.jbc.org/content/252/15/5324.citation](http://www.jbc.org/content/252/15/5324.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/252/15/5324.citation.full.html#ref-list-1](http://www.jbc.org/content/252/15/5324.citation.full.html#ref-list-1)