Pathways of Purine Metabolism in Human Adipocytes

FURTHER EVIDENCE AGAINST A ROLE OF ADENOSINE AS AN ENDOGENOUS REGULATOR OF HUMAN FAT CELL FUNCTION

Horst Kather
From the Klinisches Institut für Herzinfarktforschung an der Medizinischen Universitätshôpital Heidelberg, Bergheimerstraße 58, D-6900 Heidelberg, West Germany

Previous results demonstrated that the adenosine that accumulates in human fat cell suspensions is derived from extracellular sources (Kather, H. (1988) J. Biol. Chem. 263, 8803–8809). To get insight into the mechanisms responsible for the lack of adenosine release, extracellular adenine nucleotide catabolism was minimized by 10 mmol/liter β-glycerophosphate and 10 μmol/liter α,β-methyleneadenosine 5’-diphosphate.

Intracellular adenine nucleotide catabolism resulted in a release of inosine and hypoxanthine under these conditions that was increased markedly by isoproterenol. Experiments with inhibitors of adenosine deaminase and adenosine kinase indicated that the production of inosine and hypoxanthine proceeded via AMP deamination. Consistently, IMP levels were increased transiently in the presence of isoproterenol. In addition, the cells possessed a nucleotide phosphomonoesterase that was resistant to the inhibitory actions of ATP and α,β-methyleneadenosine 5’-diphosphate and showed preference for IMP over AMP.

Adenosine (~1 nmol/10^6 cells/h) was also produced inside the cells. However, adenosine production was unrelated to ATP turnover via adenylate cyclase, and any adenosine formed was immediately reconverted to adenine nucleotides in the absence and presence of isoproterenol.

It was concluded that adenosine is not released by intact human adipocytes, because the alternative routes of intracellular AMP catabolism are compartmentalized (at least in functional terms), and adenosine kinase is not saturated with substrate in the absence and presence of isoproterenol.

Adenosine is a degradation product of adenine nucleotides postulated to act as an endogenous (feedback)-inhibitor of hormone-activated lipolysis (1–3). Its potent antilipolytic and cyclic AMP-lowering effects are well known and the processes mediating these events have been defined (1–7). The nucleoside is inevitably present in fat cell suspensions and is released by intact canine adipose tissue during prolonged sympathetic stimulation (1–7). However, the sources of this adenosine and the factors controlling its availability have remained unclear.

It has been proposed that the nucleoside is produced inside the adipocytes from cyclic AMP via the sequence cyclic AMP→AMP→adenosine (1–3). Once formed, adenosine may be deaminated, released into the pericellular space, or reconverted to adenine nucleotides by adenosine kinase (Fig. 1). Adenosine kinase has a low K₅₅ for adenosine (1–5 μmol/liter), whereas that for the deaminase is perhaps 50-fold higher (1). If adenosine kinase was saturated with substrate under basal conditions, a hormone-induced increase in cyclic AMP turnover could therefore result in intracellular adenosine accumulation and release.

A major problem in testing this hypothesis resides in the fact that adenosine can be produced via intra- and extracellular pathways (Fig. 1). Extracellular ATP catabolism proceeds exclusively via AMP dephosphorylation resulting in adenosine as the end product (8–14). In contrast, intracellular ATP catabolism appears to be mainly via deamination at the nucleotide level (AMP→IMP→inosine). Under physiological conditions, any adenosine formed inside the cells is immediately reconverted to AMP in virtually all cell types that have been studied in detail, e.g., hepatocytes (9), leukocytes (10), lymphoblasts (11), erythrocytes (12), cardiomyocytes (13), and probably also vascular endothelia (14). Consistently, human adipocytes released inosine and hypoxanthine, and their export was markedly increased by isoproterenol (8). By contrast, adenosine appeared to be derived from extracellular sources, and its concentration was not influenced by β-adrenergic catecholamines (8).

The current studies are detailed investigations into the pathways of purine metabolism in human adipocytes. As purine metabolism can proceed via intracellular and extracellular pathways and involves cyclic routes, analytical data alone are not sufficient to indicate the sources of individual purines (Fig. 1). Therefore, a pharmacological approach was used. The properties of the inhibitors used are listed in Table I.

EXPERIMENTAL PROCEDURES

Subjects
Subcutaneous adipose tissue was from surgical subjects undergoing elective abdominal or cosmetic breast surgery. Operations were performed after an overnight fast. Anesthesia was initiated with a short-acting barbiturate and maintained with oxygen, nitrous oxide, and halothane. The tissue specimens were obtained at the start of the operations.

Preparation of Fat Cells and Incubations
Tissue specimens were cut into small pieces and fat cells were isolated by the method of Rodbell, as described (6, 15). To achieve a complete inhibition of adenosine deaminase, concentrated cells were preincubated in the presence of 1 μmol/liter 2’-deoxycoformycin for 30 min at 37 °C (see below). Excess 2’-deoxycoformycin was removed by extensive washing (four times). Controls were handled in the same manner, except that 2’-deoxycoformycin was omitted. After washing,
catalyzes the conversion of ATP to AMP. The abbreviations used are: A.C., adenylate cyclase; 5'-Nucl., ecto-5'-nucleotidase; CAMP, cyclic AMP; Ado, adenosine; Ino, inosine; Hx, hypoxanthine.

the cells were suspended at densities of 20,000-60,000 cells/ml in Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 5 mmol/liter glucose, 20 g/liter human serum albumin, 10 μmol/liter α,β-methyleneadenosine 5'-diphosphate, and 10 mmol/liter β-glycerophosphate. All other inhibitors and hormones were added at the start of the final incubations. Performed at 37 °C under an atmosphere of 5% CO2 and 95% O2, as described (8). During standard incubations, the cells were maintained in suspension by gentle mechanical shaking and brief swirling by hand (approximately every 15 min). For rapid time courses a magnetic stirrer operating at the lowest possible speed was used to maintain cells in suspension, because swirling by hand resulted in lysis of 60-80% of the cells within 1 h under these latter conditions. Even with gentle mechanical stirring 30% of total lactate dehydrogenase activity was released per hour of incubation. At the time indicated, 0.2-0.3 ml of suspensions were removed, extracted by 1 mol/liter perchloric acid, and assayed for adenine nucleotide contents and cyclic AMP. Another aliquot of suspensions (1 ml) was centrifuged through silicon oil (6000 g, 10 s). 0.2 ml of the media was transferred to perchloric acid for determination of cyclic AMP contents and adenosine nucleotide concentrations. The remaining part of the media was inactivated by heating (95 °C, 5 min) and assayed for glycerol and purine contents.

Assays Purines and IMP—Adenosine, inosine, and hypoxanthine were determined by a chemiluminescent method which is based on the determination of H2O2 formed by sequential catabolism of purines to uric acid (16, 17).

For determination of IMP, the reaction sequence was extended by inclusion of 5'-nucleotidase. 50 μl of neutralized perchloric acid extracts were made up with H2O or standard (usually 100 μmol/liter) Krebs-Henseleit bicarbonate buffer, pH 8.2, containing 1 μmol/liter MgCl2, 25 μmol/liter luminol, 1 μl/ml peroxidase, 0.1 μl/ml xanthine oxidase, and 0.3 μl/ml nucleoside phosphorylase. After 1 min, the determination of IMP was initiated by injecting another 0.1 μl of bicarbonate buffer containing 25 μmol/liter luminol, 1 μl/ml peroxidase, 0.1 μl/ml xanthine oxidase, and 0.8 μl/ml Atroz venom 5'-nucleotidase. Light emission between 57 and 60 s was taken as a measure of IMP concentrations. The assay was specific for IMP; no interference was observed with AMP, IDP, or IT as up to a concentration of 1 μmol/liter. In neutralized perchloric acid extracts the detection limit for IMP was 20 nmol/liter.

Among the agents used, dipyridamole (10 μmol/liter) caused a marked suppression of light emission (8). When present, the inhibitor of nucleoside transport was therefore removed by repeated partition into chloroform (1 ml/ml of sample).

Adenine Nucleotides—Adenine nucleotides were assayed using the firefly luciferase as indicator reaction (6, 18). α,β-Methyleneadenosine 5'-diphosphate and β-glycerophosphate were free of contaminating adenosine nucleotides. At the concentrations used (10 μmol/liter and 10 mmol/liter, respectively), the inhibitors of ectophosphatase activities had neither an influence on ATP-induced light output, nor did these compounds produce a measurable signal on their own. In addition, the phosphatase inhibitors did not interfere with the conversion of AMP and ADP to ATP.

Other Determinations—Glycerol was determined by a lumimetric method (19). Cyclic AMP was measured by a commercially available radioimmunoassay after acetylation (Amersham, Bucks, United Kingdom). Cell number was determined by counting all cells in appropriately diluted aliquots of cell suspensions. Lactate dehydrogenase activity was measured in aliquots of the media that were removed prior to heat inactivation and kept on ice until measurement. To assess the activity of the enzyme in whole suspensions, 3 volumes of H2O were added to suspensions and cell lysis was completed by a brief sonication (1 s). Total lactate dehydrogenase activity did not change in the course of incubations.

Assessment of Inhibitor Requirements

The effects of 2'-deoxycoformycin were determined by measuring the degradation of added adenosine (10 μmol/liter) in hypotonic lysates of cells that had been preincubated in the presence of various concentrations of the inhibitor for 30 min at 37 °C and were washed extensively (four times) prior to cell lysis. The reaction mixture contained 50 mmol/liter Na2PO4, pH 7.4, 10 μmol/liter adenosine, and homogentisic acid at a final dilution of 1:4 relative to suspensions containing 40,000-80,000 cells/ml. The rate of inosine and hypoxanthine formation was linear for 1 h under these conditions. At 1 μmol/liter 2'-deoxycoformycin, adenosine deaminase was inhibited by 95%. Half-maximal effects occurred at approximately 10 μmol/liter. When 2'-deoxycoformycin-treated cells were washed and incubated a further 3 h in the absence of the inhibitor, adenosine deaminase was still inhibited by more than 90%.

The concentration of α,β-methyleneadenosine 5'-diphosphate required to suppress the activity of the ecto-5'-nucleotidase in intact cells was determined by its ability to prevent the dephosphorylation of exogenously supplied AMP in suspensions containing 10 μmol/liter β-glycerophosphate. The specificity of ecto-5'-nucleotidase inhibition was assessed by determining the effects of the ADP analogue on the hydrolysis of AMP and IMP by soluble and particulate (30,000 × g) fractions of fat cell lysates that had been dialyzed (two times) against 100 volumes of phosphate-free Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 1 g/liter charcoal to remove endogenous nucleotides. Reactions were carried out for 30 min at 37 °C in 0.1 ml of phosphate-free Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 5 μmol/liter 5'-deoxy-5-iodotubercidin, 60 μmol/liter β-glycerophosphate, 20 g/liter human serum albumin, and various concentrations of AMP or IMP (1 μmol/liter-30 μmol/liter) in the absence and presence of 10 μmol/liter α,β-methyleneadenosine 5'-diphosphate and/or 3 mmol/liter ATP. Protein concentrations corresponded to a density of 30,000 cells/ml. The rates of product formation were linear with time; ATP degradation was negligible, and more than 90% of the AMP that was dephosphorylated was recovered as adenosine under the conditions used.

The ability of 5'-deoxy-5-iodotubercidin to inhibit adenosine kinase in situ was determined by assessing its capacity to prevent a net uptake of exogenously supplied adenosine (1 μmol/liter) in suspensions containing 10 μmol/liter α,β-methyleneadenosine 5'-diphosphate and 10 mmol/liter β-glycerophosphate.

Chemicals

2'-Deoxycoformycin and 5'-deoxy-5-iodotubercidin (Ro 88-0414 701) were gifts of Dr. M. Sufficient, Naughton Products Branch, Division of Cancer Treatment, National Institutes of Health, Bethesda, MD, and Dr. Berlepsch, Hoffmann La Roche Company, Basel, Switzerland, respectively. 5'-Nucleotidase from Crotalus atrox was from Sigma, Deisenhofer, West Germany. Luminol was obtained from Fluka AG, Basel, Switzerland. The sources of all other reagents and chemicals were the same as reported (8).

RESULTS

Specificity of Inhibitors—Human fat cells possess ectophosphatases capable of sequentially degrading ATP to adenosine (8). The cells are fragile (Table II). Their short-lived integrity resulted in an unacceptable background of adenine nucleotide catabolism from broken cells. Therefore, the current experiments were carried out in the presence of 10 mmol/liter β-glycerophosphate, an inhibitor of nonspecific phosphatases, and 10 μmol/liter α,β-methyleneadenosine 5'-diphosphate, a
Selective inhibitor of ecto-5′-nucleotidase\(^1\) (Table I).

None of the inhibitors used had a detectable influence on cellular adenine nucleotide contents or lactate dehydrogenase release (not shown). ATP levels dropped by approximately one-third in the course of incubations (Table II). In contrast to conventional incubations, the decrease in ATP was counterbalanced by a corresponding accumulation of ADP and AMP. The concentration of AMP dropped by about one-third in the course of incubations (Table II).

\(^1\) Despite its specificity as an inhibitor of ecto-5′-nucleotidase, α,β-methyleneadenosine 5′-diphosphate should not be used uncritically, as at higher concentrations than those required to inhibit the ecto-enzyme (≥30 μmol/liter), it can also suppress cyclic AMP levels in the presence of isoproterenol. Unlike the cyclic AMP-lowering action of 5′-deoxy-5-iodotubercidin, the latter effect was not reversed by adenosine deaminase. The issue whether this inhibitory action is mediated via A\(_1\) adenosine receptors (7), P\(_2\)-purinergic receptors (38), or other mechanisms remains to be clarified.

Inhibition of ectophosphatase activities was specific, in as much, as α,β-methyleneadenosine 5′-diphosphate and β-glycerophosphate failed to suppress the activity of a distinct nucleotide phosphomonoesterase recovered in fat cell lysates (Fig. 2). Similar to the soluble 5′-nucleotidases from chicken and rat liver (29, 30), the latter enzyme displayed marked preference for IMP over AMP. With half-saturation occurring at -0.3 mmol/liter (IMP) and -3 mmol/liter (AMP), its substrate affinity was low, however, in comparison to the ectoenzyme exhibiting an apparent \(K_m\) value for AMP of -5 nmol/liter. Further investigation of ecto-5′-nucleotidase inhibitors revealed that both ≥300 times higher than the \(K_m\) value for AMP and the \(K_m\) value for AMP were always in the same range (22-26).

### Table I

**Cellular targets and side effects of various inhibitors of purine metabolism**

| Compound | Principle effect | Refs. | Other relevant properties | Refs. |
|----------|-----------------|-------|--------------------------|-------|
| α,β-Methyleneadenosine 5′-diphosphate | Inhibition of ecto-5′-nucleotidase, \(K_m\) in intact cells: 0.1-7 μmol/liter (endothelium), 18 μmol/liter (perfused rat heart). | 14, 20, 21 | Presumably nonpenetrating. No inhibition of other phosphatases up to 1 nmol/liter. No hydrolysis. Inhibition of cyclic AMP accumulation (>30 μmol/liter). | 20, 24, 25 |
| 2′-Deoxycoformycin | Tight-binding inhibitor of adenosine deaminase (\(K_m\) 2–7 mmol/liter). Slowly penetrating. | 22, 25 | Inhibition of AMP deaminase (\(K_m\) depending on cell type, but usually higher (~100 times) than for inhibition of adenosine deaminase). | 22–26 |
| 5′-Deoxy-5-iodotubercidin | Most potent inhibitor of adenosine kinase. \(K_m\) in guinea pig and rat brain: 0.1–0.5 μmol/liter. | 27, 28 | Weak agonist activity (if any) at A\(_1\)/A\(_2\)-adenosine receptors. | 28 |

### Table II

**Changes in lactate dehydrogenase activity and adenine nucleotide contents in media and suspensions**

| Compound | Principle effect | Refs. | Other relevant properties | Refs. |
|----------|-----------------|-------|--------------------------|-------|
| 2′-Deoxycoformycin | Tight-binding inhibitor of adenosine deaminase (\(K_m\) 2–7 mmol/liter). Slowly penetrating. | 22, 25 | Inhibition of AMP deaminase (\(K_m\) depending on cell type, but usually higher (~100 times) than for inhibition of adenosine deaminase). | 22–26 |
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Fig. 3 shows the effects of 2′-deoxycoformycin and/or 5′-deoxy-5-iodotubercidin on basal and hormone-activated rates of lipolysis and cyclic AMP levels in the presence of isoproterenol. A treatment of the cells with 2′-deoxycoformycin had no effect on lipolysis and cyclic AMP accumulation (Fig. 3, A and B). By contrast, 5′-deoxy-5-iodotubercidin caused a marked inhibition of nonstimulated glycerol release (Fig. 3A). The isoproterenol-induced increase in cyclic AMP was also
markedly reduced, e.g. from 6.2 ± 1.3 nmol/10⁶ cells (controls) and 5.1 ± 0.7 nmol/10⁶ cells (2'-deoxycoformycin-treated cells) to 2.2 ± 0.7 and 2.3 ± 0.7 nmol/10⁶ cells, respectively. However, the lipolytic effect of the β-adrenergic agonist was unimpaired, consistent with the frequent notion that the increases in cyclic AMP caused by activating hormones may be orders of magnitude higher than those required for a maximal stimulation of lipolysis (31). The inhibitory effects of 5'-deoxy-5-iodotubercidin could be overcome by adenosine deaminase (0.5 unit/ml), indicating that its antilipolytic and unimpaired, consistent with the frequent notion that the increase in adenine nucleotide synthesis that became predominant at the end of incubations (Fig. 4D).

2'-Deoxycoformycin had no perceptible effect on the conversion of exogenously supplied adenosine to adenine nucleotides, as expected (Fig. 4D). It was surprising, however, that the production of inosine and hypoxanthine was reduced by only 30% in 2'-deoxycoformycin-treated cells. Even at the highest concentration used (1 μmol/liter), two-thirds of the adenosine that had been removed from the media were incorporated into adenine nucleotides, whereas only one-third was converted to inosine and hypoxanthine (Fig. 4, A and C; Table III). In controls, adenine nucleotide concentrations displayed a steady increase up to 2.5 h of incubation in the presence of 1 μmol/liter of added adenosine and then declined, indicating that the increase in adenine nucleotide synthesis was in part counterbalanced by an enhancement of adenine nucleotide catabolism that became predominant at the end of incubations (Fig. 4D).

2'-Deoxycoformycin and/or 5'-deoxy-5-iodotubercidin on the removal and metabolic fate of exogenously supplied adenosine. A, rates of disappearance of added adenosine (1 μmol/liter). B, corresponding changes in cyclic AMP concentrations in the presence of isoproterenol (Iso). C, release of inosine (Ino.) and hypoxanthine (Hx.). D, changes in adenine nucleotide contents of suspensions. Initial adenine nucleotide concentrations were (nmol/10⁶ cells): 25 ± 3.8 (Control), 30 ± 3 (dCF), and 27 ± 4 (dITu), respectively. Values are means ± S.E. of 4 (dCF + dITu) or 5 (other conditions) paired experiments carried out with fat cells from different donors.

The metabolism of exogenously supplied adenosine was
Values are those obtained in the experiments shown in Fig. 4 after 2.5 h of incubation (when adenine nucleotide concentrations peaked). Adenosine uptake refers to the amount of adenosine that had disappeared from the media. The net synthesis of inosine and hypoxanthine was calculated by subtracting the spontaneous release of these latter purines from the total amount produced in the presence of exogenously supplied adenosine. The flux through adenosine deaminase was calculated from the difference in inosine and hypoxanthine release between untreated and 2'-deoxycoformycin-treated cells. The rate of phosphorylation was calculated from the difference between adenosine uptake and deamination.

### TABLE III

| Condition | Adenosine uptake | Net production of inosine and hypoxanthine | Adenine nucleotide synthesis | Adenosine kinase | Adenosine deaminase | Recovery (nmol/10⁶ cells/2.5 h) |
|-----------|-----------------|------------------------------------------|-------------------------------|------------------|---------------------|---------------------------------|
| Control   | 24.0 ± 3.5      | 9.2 ± 1.3                                | +12.0 ± 1.3                   | 18.7             | 3.1                 | 87 (Fig. 5, A)                  |
| dCF³      | 22.7 ± 3.9      | 6.1 ± 1.3                                | +12.4 ± 1.3                   | 18.5             | 0.8                 | 80 (Fig. 5, B)                  |
| dITu      | 14.4 ± 2.6      | 12.0 ± 1.4                               | -1.1 ± 0.3                    | 12.0             | 3.1                 | 83 (Fig. 5, C)                  |

*a dCF, 2'-deoxycoformycin; dITu, 5'-deoxy-5-iodotubercidin.

TABLE IV

| Condition | Adenosine release (nmol/10⁶ cells/3 h) |
|-----------|---------------------------------------|
| dCF + dITu| 3.1 ± 0.8                             |
| dCF + dITu + DPD | 1.3 ± 0.6                          |

### Purine Release—Summarized in Fig. 5 are the effects of 2'-deoxycoformycin and/or 5'-deoxy-5-iodotubercidin (Fig. 4, A and D). The latter finding demonstrated that adenosine deaminase and adenosine kinase are the sole routes of intracellular metabolism of exogenously supplied adenosine and implied that both enzymes were virtually completely blocked in situ at the inhibitor concentrations used. As a blockade of adenosine deaminase alone resulted in only 30% inhibition of inosine and hypoxanthine formation, the latter observation also indicated that two-thirds of the inosine and hypoxanthine accumulations in the media in the presence of 1 μmol/liter of exogenously supplied adenosine had been produced via a pathway involving adenosine kinase (adenosine→AMP→IMP→inosine), consistent with the notion that not only the synthesis, but also the breakdown of adenine nucleotides was increased under these latter conditions (Fig. 4D, Table III).

### Purine Release—Summarized in Fig. 5 are the effects of 2'-deoxycoformycin and/or 5'-deoxy-5-iodotubercidin on purine release in the absence and presence of isoproterenol. As reported previously (8), adenosine concentrations were beyond detectable levels (≤5 nmol/liter) in the presence of ecto phosphatase inhibitors, regardless of whether ATP turnover via adenylyl cyclase was increased by isoproterenol or not (Fig. 5A). By contrast, the cells released inosine and hypoxanthine (Fig. 5, B and C). In controls, their concentrations were 2.3 ± 0.8 nmol/10⁶ cells (inosine) and 0.75 ± 0.2 nmol/10⁶ cells (hypoxanthine) after 3 h of incubation. Isoproterenol caused a 3.5-fold increase in inosine output that was paralleled by a corresponding enhancement of hypoxanthine release (Fig. 5, B and C). 2'-Deoxycoformycin had no effect on purine output or the distribution of individual purines in the absence and presence of isoproterenol, indicating that the purines accumulating in the media had been produced via deamination at the nucleotide level (AMP→IMP→inosine).

By contrast, an inhibition of adenosine kinase resulted in the appearance of measurable amounts of adenosine in the media. The rates of adenosine accumulation were linear with time and not influenced by isoproterenol (Fig. 5A). On the average, 1 nmol of adenosine was released by 10⁶ cells within 3 h in the presence of 5'-deoxy-5-iodotubercidin (Fig. 5A). In the absence of isoproterenol, the release of inosine and hypoxanthine was also increased (by 1.6 nmol/10⁶ cells; Fig. 5, B and C). The latter effect could be reversed by 2'-deoxycoformycin, indicating that a major fraction of the adenosine that accumulated upon inhibition of adenosine kinase was deaminated. Consistently, adenosine release was increased to 3 nmol/10⁶ cells/3 h when its further metabolism was completely blocked by the combined action of 2'-deoxycoformycin and 5'-deoxy-5-iodotubercidin (Fig. 5A). The release of adenosine was reduced 60% by dipyridamole, an inhibitor of nucleoside transport, under these latter conditions, indicating that it was mainly derived from intracellular sources (Table IV).

The stimulatory effect of isoproterenol on inosine and hypoxanthine release was reduced by 5'-deoxy-5-iodotubercidin, as expected because cyclic AMP levels were markedly suppressed by the adenosine that accumulated in the media under these conditions (Figs. 3 and 5, A–D). The decrease in
inosine and hypoxanthine release was moderate, however, and became only apparent in 2'-deoxycoformycin treated cells. In untreated cells the 5'-deoxy-5-lodotubercidin-induced inhibition was probably masked, because inosine and hypoxanthine were not only produced from IMP, but also from adenosine under these latter conditions.

Isoproterenol-activated rates of inosine and hypoxanthine release were markedly nonlinear with time (Fig. 6). Approximately one-third of the cyclic AMP present in human fat cell suspensions was released into the media in the course of incubations, a figure that closely corresponded to lactate dehydrogenase release, indicating that not only adenine nucleotides, but also the majority of cyclic AMP was released via nonspecific mechanisms (Fig. 6B, Table II). A comparison of the time courses of cyclic AMP accumulation inside the cells with purine production during each of the 30-min intervals revealed that the rates of net production of inosine and hypoxanthine paralleled the changes with time of intracellular cyclic AMP, suggesting that the stimulatory effect of isoproterenol on the release of these latter purines was related to its cyclic AMP-elevating property (Fig. 6, B and C), which is in fact the case (32). Accordingly, the net production of inosine and hypoxanthine displayed only a small initial rise in the absence of the β-adrenergic agonist.

The linear phase of purine release was accompanied by a transient 2- to 5-fold increase in cellular IMP contents that was maximal within 6–10 min and could be detected in 8 out of 10 experiments (Fig. 7). Purine release became progressively slower within 20–30 min in the presence of isoproterenol. Concomitantly, IMP concentrations returned to control levels, indicating that the flux through adenylate deaminase in fact transiently increased by the β-adrenergic agonist, consistent with the conclusions drawn from the inhibitor studies. Isoproterenol had no detectable influence on intracellular AMP. However the amount of AMP accumulating in the media was large in relation to the intracellular concentrations of the nucleotide. Therefore, minor increases in intracellular AMP cannot be excluded.

**DISCUSSION**

In the current studies, the pathways of adenine nucleotide catabolism and the metabolic fate of adenosine were analyzed in human adipocytes by means of specific inhibitors of purine production and metabolism. The potential pitfalls inherent in this approach were considered carefully, and the results of various control experiments are consistent with the view that the known side effects of these latter compounds played a minor role, if any, under the conditions used (Fig. 3, Table II).

The observation that the extracellular concentrations of adenine nucleotides (and cyclic AMP) were related to lactate dehydrogenase release in the presence of ectophosphate inhibitors, whereas adenosine concentrations dropped beyond detectable levels (<5 nmol/liter), provides direct evidence in support of the previous conclusion that the production of adenosine, occurring extracellularly, reflects the catabolism of adenine nucleotides from broken cells, and confirms that inosine and hypoxanthine are the sole products of adenine nucleotide breakdown that are released by intact human adipocytes in absence and presence of isoproterenol (8).

2'-Deoxycoformycin had no influence on basal or isoproterenol-stimulated rates of inosine and hypoxanthine accumulation, even though adenosine deaminase was effectively blocked in situ (Fig. 4). The latter finding is consistent with the results obtained in other cell types under physiological conditions (9–14) and indicates that the formation of inosine and hypoxanthine proceeded via deamination at the nucleotide level (AMP → IMP → inosine). IMP levels were consistently increased transiently in the presence of isoproterenol (Fig. 7).

A release of adenosine could only be observed upon inhibition of adenosine kinase, indicating that some adenosine was produced and was immediately recycled into adenine nucleotides (Fig. 5). Along with the observation that exogenously supplied adenosine was mostly phosphorylated, that latter finding indicated that adenosine kinase constituted the primary route of adenosine metabolism, and was not saturated with substrate irrespective of whether ATP turnover via adenylate cyclase was increased by isoproterenol or not. Indeed, phosphorylation was so dominant over deamination that the conversion of exogenously supplied adenosine to inosine and hypoxanthine also took place mainly via a circuitous route bypassing adenosine deaminase, e.g. adenosine → AMP → IMP → inosine, consistent with the conclusions drawn from similar observation in rat hepatocytes (9).

Unexpectedly, isoproterenol had no effect on adenosine production, although the output of inosine and hypoxanthine was increased markedly by the β-adrenergic agonist. The molecular mechanisms responsible for the apparent compartmentation of the alternative routes of AMP catabolism are presently unclear. The simplest solution to the problem would be that the accumulation of adenosine observed in the absence of its further metabolism reflected a residual breakdown of
adenine nucleotides from broken cells, due to an incomplete inhibition of ctc5'-nucleotidase or involvement of the newly discovered nucleotide phosphomonoesterase which is resistant to the inhibitory actions of ATP and 5'-methyleneadenosine 5'-diphosphate. Because of its low affinity for AMP, the latter enzyme did not measurably contribute to extracellular adenine nucleotide catabolism, however, even though one-third of the cells were damaged in the course of incubations (Table II). Adenosine accumulation was reduced 60% consistently by dipyridamole in the absence of its further metabolism, implying that at least two-thirds of the nucleoside had been produced inside the cells (Table IV).

The adenosine formed inside the cells could be derived from sources other than AMP, e.g. S-adenosylhomocysteine (30-35). However, the equilibrium of S-adenosyl-1-homocysteine hydrolase is in the direction of S-adenosyl-1-homocysteine synthesis (36). Production of adenosine via the S-adenosyl-1-homocysteine hydrolase pathway, therefore, requires a rapid removal of adenosine, which was blocked by the combined action of 2'-deoxycoformycin and 5'-deoxy-5-iodotubercidin, suggesting that the intracellular formation of adenosine proceeded from AMP (at least in the absence of its further metabolism).

Popular procedures of accelerating ATP catabolism, e.g. substrate deprivation, metabolic poisoning, and/or uncoupling of mitochondrial respiration, alter the equilibrium among the three adenine nucleotides in the direction of AMP (9-14, 36). By contrast, isoproterenol primarily reduced the size of the adenine nucleotide pool without substantially affecting the concentrations of ADP and AMP (32). Considering that the nucleotide phosphomonoesterase activity identified in human fat cell lysates displayed preference for IMP over AMP and that AMP levels were not increased substantially in the presence of isoproterenol, it is not surprising that adenosine production was not increased by the β-adrenergic agonist. Rather, the crucial question is why the release of inosine and hypoxanthine was increased selectively by isoproterenol.

The observation that the isoproterenol-induced increase in inosine and hypoxanthine release was related to cyclic AMP accumulation in kinetic terms supports the view that the stimulatory effects of the β-adrenergic catecholamine on adenine nucleotide catabolism are related to increased substrate turnover via the adenylyl cyclase-phosphodiesterase cycle (Fig. 6). This conclusion is substantiated by a forthcoming report (32), and implies that AMP turnover is largely determined by the velocity of cyclic AMP hydrolysis in the presence of isoproterenol. The hormone-induced increase in AMP-metabolic flux occurred with little change in its concentration. Similar observations made during induced ATP catabolism in Ehrlich ascites tumor cells led to the proposal that the activity of adenylate deaminase in situ is related primarily to AMP-metabolic flux and to a lesser extent to AMP levels or the concentrations of known regulators of the enzyme (37). A more complete understanding of the mechanisms responsible for the selective increase in AMP deamination seen in the presence of β-adrenergic catecholamines will, therefore, not only depend on a further characterization of the enzymes involved in AMP metabolism, but will also require quantitative estimates of cyclic AMP turnover as well as an identification of the factors controlling the partitioning of substrate between adenylate kinase and adenylate deaminase reactions in situ.

In conclusion, the present findings demonstrate that intact human adipocytes fail to release adenosine in the absence and presence of isoproterenol under the conditions used, because the alternative routes of intracellular adenine nucleotide catabolism are compartmentalized (at least in functional terms), and adenosine kinase is not saturated with substrate irrespective of whether ATP turnover via adenylyl cyclase is increased by the β-adrenergic agonist or not. Along with previous observations (8), the current findings therefore strongly suggest that the adenosine that ineffectually accumulates in fat cell suspensions is almost exclusively derived from adenine nucleotides that are extruded by broken or damaged cells.

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