"Cell-type specificity of inhibition of glycolysis by 5-amino-4-imidazolecarboxamide riboside. Lack of effect in rabbit cardiomyocytes and human erythrocytes, and inhibition in FTO-2B rat hepatoma cells."

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Abstract
The nucleoside AICAriboside (5-amino-4-imidazolecarboxamide riboside) has been shown to inhibit glycolysis in isolated rat hepatocytes [Vincent, Bontemps and Van den Berghe (1992) Biochem. J. 281, 267-272]. The effect is mediated by AICA-ribotide (ZMP), the product of the phosphorylation of AICA-riboside by adenosine kinase. To assess the cell-type specificity of the effect, studies were conducted in rabbit cardiomyocytes, human erythrocytes and rat hepatoma FTO-2B cells. AICA-riboside had no effect on glycolysis in cardiomyocytes, human erythrocytes and rat hepatoma FTO-2B cells. AICA-riboside had no effect on glycolysis in cardiomyocytes, and a slight stimulatory effect in erythrocytes, but inhibited glycolysis by 65% at 250 microM concentration in FTO-2B cells, although only when tissue-culture medium was replaced by Krebs-Ringer bicarbonate buffer. At 500 microM AICAriboside, ZMP remained undetectable in cardiomyocytes, but reached 0.65 mM in erythrocytes and 5 mM in FTO-2B cells. In the latter, AICAriboside provoked up to 2-fold elevations of glucose 6-phosphate and fructose 6-phosphate,...

Référence bibliographique
Cell-type specificity of inhibition of glycolysis by 5-amino-4-imidazolecarboxamide riboside

Lack of effect in rabbit cardiomycocytes and human erythrocytes, and inhibition in FTO-2B rat hepatoma cells

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The nucleoside AICAriboside (5-amino-4-imidazolecarboxamide riboside) has been shown to inhibit glycolysis in isolated rat hepatocytes [Vincent, Bontemps and Van den Berge (1992) Biochem. J. 281, 267–272]. The effect is mediated by AICA-ribotide (ZMP), the product of the phosphorylation of AICAriboside by adenosine kinase. To assess the cell-type specificity of the effect, studies were conducted in rabbit cardiomycocytes, human erythrocytes and rat hepatoma FTO-2B cells. AICAriboside had no effect on glycolysis in cardiomycocytes, and a slight stimulatory effect in erythrocytes, but inhibited glycolysis by 65% at 250 μM concentration in FTO-2B cells, although only when tissue-culture medium was replaced by Krebs–Ringer bicarbonate buffer. At 500 μM AICAriboside, ZMP remained undetectable in cardiomycocytes, but reached 0.65 mM in erythrocytes and 5 mM in FTO-2B cells. In the latter, AICAriboside provoked up to 2-fold elevations of glucose 6-phosphate and fructose 6-phosphate, accompanied by a decrease in fructose 1,6-bisphosphate. This indicated inhibition of 6-phosphofructo-1-kinase (PFK-1). Accordingly, in FTO-2B cell-free extracts, the activity of PFK-1, measured under physiological conditions, was inhibited by approx. 70% by 5 mM ZMP. ZMP had a less pronounced effect on the activity of PFK-1 in normal rat liver; it did not influence the activity of PFK-1 in rat muscle, rabbit heart and human erythrocytes. It is concluded that the inhibitory effect of AICAriboside on glycolysis is dependent on both (1) the capacity of the cells to accumulate ZMP and (2) the presence of target enzymes which are sensitive to ZMP.

INTRODUCTION

AICAriboside (5-amino-4-imidazolecarboxamide ribofuranoside) is the nucleoside corresponding to AICAriboside 5′-monophosphate (AICAR or ZMP), an intermediate of the ‘de novo’ pathway of purine biosynthesis. AICAriboside is taken up and metabolized by various cell types, including erythrocytes and lymphoma cells [1], fibroblasts [2], isolated kidney tubules [3] and isolated hepatocytes [4]. The first step of the metabolism of AICAriboside is its phosphorylation to ZMP by adenosine kinase [1]. Thereafter, ZMP can be metabolized in two directions: (i) conversion into IMP by the sequential action of AICAR formyltransferase, which requires 10-formyl-tetrahydrofolate, and IMP cyclohydrolase; (ii) phosphorylation to the corresponding di- and tri-phosphates, ZDP and ZTP.

We have reported previously that addition of AICAriboside to suspensions of isolated rat hepatocytes causes a ZMP-mediated inhibition of glycolysis [5]. The inhibition was shown to result from a diminution of the phosphorylation of glucose to glucose 6-phosphate, accompanied by an inactivation of 6-phosphofructo-2-kinase (PFK-2). This inactivation provokes a decrease in fructose 2,6-bisphosphate (Fru-2,6-P₂), the main physiological stimulator of liver 6-phosphofructo-1-kinase (PFK-1).

Hepatic phosphorylation of glucose is mainly accomplished by the high- Kₘ isozyme of hexokinase, glucokinase. Liver also contains a PFK-2 isozyme specific for this tissue [6]. We therefore decided to investigate the effect of AICAriboside on glycolysis in other cell types, which possess different isozymes of these two enzymes. Isolated rabbit cardiomycocytes, human erythrocytes and FTO-2B cells, a rat hepatoma cell line, were chosen for this purpose.

MATERIALS AND METHODS

Materials

Collagenase was from Wako (Osaka, Japan), BSA from Serva (Heidelberg, Germany), and fatty-acid-free BSA from Sigma (St. Louis, MO, U.S.A.). Cell-culture components were purchased from Gibco (Paisley, Scotland, U.K.). AICAriboside and 6-phosphofructokinase (pyrophosphate-dependent) were from Sigma. [2-3H]Glucose (17.5 Ci/mmol) was from Amersham International (Amersham, Bucks., U.K.) and was re-purified by paper chromatography before use, to free it from a metabolizable contaminant [7]. 5-Iodotubercidin was from RBI (Natick, MA, U.S.A.). Auxiliary enzymes used in the spectrophotometric assays were from Boehringer (Mannheim, Germany). Sephadex G-25 (fine grade) was from Pharmacia (Uppsala, Sweden).

Isolation of cardiomycocytes

Cardiac ventricular myocytes were isolated from male New Zealand White rabbits weighing 1.7–2.3 kg, by a modification of the procedure of Altschuld et al. [8]. Hearts were perfused by the Langendorff method and myocytes isolated by addition of collagenase. After isolation, the preparations were enriched in

Abbreviations used: AICAriboside, 5-amino-4-imidazolecarboxamide riboside; ZMP (AICARbotide), AICAriboside 5′-monophosphate; PFK-1, 6-phosphofructo-1-kinase; PFK-2, 6-phosphofructo-2-kinase; Fru-1,6-P₂, fructose 1,6-bisphosphate; Fru-2,6-P₂, fructose 2,6-bisphosphate; KRB, Krebs–Ringer bicarbonate buffer; FBPase-1, fructose-1,6-bisphosphatase; FBPase-2, fructose-2,6-bisphosphatase.

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viable myocytes by three sequential sedimentations in 4% BSA. Before incubations, the myocytes were counted in a Nageotte cell chamber and their viability was assessed by Trypan Blue exclusion. Myocytes were adjusted to approx. 400,000 cells/ml. The protein content of cell suspensions was determined as described by Lowry et al. [9]. Myocytes were incubated in Krebs–Henseleit solution (pH 7.4) containing 118 mM NaCl, 4.8 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1 mM CaCl₂ and 25 mM Hepes, supplemented with 11 mM glucose and 1% BSA (fatty-acid-free), and gassed with 100% O₂. Incubations were performed at 37°C in a gently shaking water bath. Cells were preincubated for 20 min before starting the experiments.

Incubation of human erythrocytes

Fresh blood taken from a cubital vein of healthy human volunteers was collected on heparin. Isolation and washing of erythrocytes were performed in Krebs–Ringer bicarbonate buffer (KRB), pH 7.4, containing 118 mM NaCl, 4.8 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.5 mM CaCl₂ and 25 mM NaHCO₃, supplemented with 5 mM glucose and gassed with O₂/CO₂ (19:1) as described in [10]. The cells were resuspended in the same medium at a 25% haematocrit. Cells were incubated in a shaking water bath. To avoid modifications of pH of the incubation medium by loss of CO₂, vials were carefully resealed after removal of each sample.

Cell cultures

Monolayer cultures of rat hepatoma FTO-2B cells were grown in Dulbecco's Modified Eagle's Medium/Ham's F12 (v/v) supplemented with 10% fetal-calf serum, 1% penicillin (5000 i.u./ml)/streptomycin (5000 µg/ml) and 2 mM glutamine, at 37°C, in an atmosphere of air containing 5% CO₂.

Incubation of FTO-2B cells

The day before the experiment, plastic Petri dishes (60 mm diameter) were seeded with 1×10⁶ cells. The day of the experiment, the medium was removed and the cells were washed with 2 ml of KRB. Experiments were started by the addition of 5 or 10 mM glucose, immediately followed by AICAriboside. When used, 10 µM 5-iodotubercidin was added immediately before AICAriboside. For measurement of the release of ³H₂O from [²H]glucose, 0.02 µCi of labelled glucose/ml of KRB was added simultaneously with unlabelled glucose. When Fru-2,6-𝑃₂ was measured, the cells were preincubated with glucose for 1 h before the addition of AICAriboside.

Measurements of metabolites

Lactate and ³H₂O were measured in samples of the incubation medium deproteinized in 2% HClO₄ and neutralized with 3 M K₂CO₃. For nucleotide measurements, the incubation medium was removed and the cells were treated with 1 ml of 2% HClO₄, scraped with a rubber spatula, and the extracts neutralized with 3 M K₂CO₃. For measurement of glycolytic intermediates, the cells were treated with 0.5 ml of 2% trichloroacetic acid, scraped, and centrifuged. The supernatants were neutralized by extraction with 3×5 vol. of ether. For the determination of Fru-2,6-𝑃₂, the cells were extracted with 1 ml of 50 mM NaOH containing 1% (v/v) Triton X-100, scraped, heated for 15 min at 80°C and frozen in acetone/solid CO₂.

Lactate [11], triose phosphates and Fru-1,6-𝑃₂ [12] and hexose 6-phosphates [13] were determined spectrophotometrically, and nucleotides and nucleosides were quantified by h.p.l.c. as described in [4]. ³H₂O was separated from [²H]glucose on Dowex AG-1 (borate form) columns [7]. Fru-2,6-𝑃₂ was measured by its stimulatory effect on potato 6-phosphofructokinase (pyrophosphate-dependent), as described by Van Schaftingen [14], except that commercial enzyme was used.

Enzyme assays

For measurement of enzyme activities in FTO-2B cells, approx. 400×10⁶ cells were trypsin-treated, washed twice in PBS (150 mM NaCl/2.5 mM KCl/8 mM Na₂HPO₄/1.5 mM KH₂PO₄, pH 7.4) and resuspended in 6 ml of buffer A (50 mM Hepes/100 mM KF/15 mM EGTA, pH 7.1). The suspension was sonicated and centrifuged for 15 min at 60,000 g. The supernatant was filtered on a Sephadex G-25 (fine grade) column (20 cm×1 cm), using buffer A for equilibration and elution. For the measurement of PFK-1 activity, 1 µM ATP and 1 µM Fru-2,6-𝑃₂ were added in buffer A in order to stabilize the enzyme (buffer B). Hexokinase, PFK-1 and PFK-2 activities were measured as described in [7], [15] and [16] respectively. Fructose-1,6-bisphosphatase (FBPase-1) was assayed as given in [17]. Protein concentration was measured with the Bio-Rad assay [18].

For the assay of PFK-1 in rat liver and muscle and in rabbit heart, tissues were homogenized in 4 vol. of buffer B. The homogenate was centrifuged for 15 min at 11,000 g. The supernatant was filtered on glass wool, followed by centrifugation for 30 min at 60,000 g. The supernatant was incubated on Sephadex G-25 (fine), using buffer A for equilibration and buffer B for elution. PFK-1 activity was measured as given in [15].

For the assay of PFK-1 in human erythrocytes, fresh blood was collected on heparin. After three washes in 0.9% NaCl, the cell pellet was frozen in aceton/solid CO₂, followed by thawing at room temperature, and addition of 4 vol. of 10 mM Hepes (pH 7.0)/10 mM KCl/1 µM Fru-2,6-𝑃₂/1 µM ATP. The activity of PFK-1 was measured as in [15], but with 50 mM Tris buffer (pH 7.4)/200 µM Mg-ATP/1 mM magnesium acetate/50 µM fructose 6-phosphate/150 µM glucose 6-phosphate/10 µM AMP.

Statistics

Unless stated otherwise, experiments shown are representative of at least three studies that gave similar results. Results of averaged experiments are given as means ± S.E.M. Significance was tested by Student’s t test.

RESULTS

Effects of AICAriboside in rabbit cardiomyocytes

In isolated rabbit cardiomyocytes incubated in Hepes-buffered Krebs–Henseleit solution, the production of lactate from 11 mM glucose was 90±10 µmol/h per g of cells (n = 3). Addition of 100–500 μM AICAriboside to the cell suspensions did not modify this rate. The rate of disappearance of 250 μM AICAriboside was 2.4±0.3 µmol/h per g of cells (n = 3). Both in the absence and in the presence of 100–500 μM AICAriboside, Z-nucleotides remained undetectable over the whole duration (60 min) of the incubations.

Effects of AICAriboside in human erythrocytes

In human erythrocytes incubated in KRB with 5 mM glucose, the production of lactate was 1.56±0.11 µmol/h per ml of packed cells (n = 3). Addition of 500 µM AICAriboside slightly stimulated this production, to 2.09±0.15 µmol/h per ml of packed cells (P = 0.046). In the absence of AICAriboside Z-
addition of AICAriboside. The insert shows the effect of AICAriboside on the rate of production of lactate. Production of lactate was calculated over the 2–6 h time interval after addition of the nucleoside.

Cells were incubated in KRB medium with 10 mM glucose, without (○) or with additions of AICAriboside (250 μM) and/or ITu (10 μM), as indicated.

(Figure 1). The dose-dependency of the effect (Figure 1, insert) shows that half-maximal inhibition was obtained with a AICAriboside concentration between 50 and 100 μM, and maximal (65%) inhibition with a concentration of 250 μM. In FTO-2B cells incubated in tissue-culture medium, AICAriboside had no effect on the production of lactate.

In FTO-2B cells incubated in tissue-culture medium or in KRB supplemented with 10 mM glucose, Z-nucleotides were undetectable in the absence of AICAriboside. In tissue-culture medium, incubation of the cells with 250 μM AICAriboside provoked accumulation of ZMP, up to 1.5 μmol/g of cells after 4 h; ZDP remained undetectable and ZTP accumulated to 0.09 μmol/g of cells. In cells incubated in KRB, AICAriboside induced much higher increases in Z-nucleotides. This is most likely due to the absence in KRB of folate compounds, which allow conversion of ZMP into IMP in tissue medium. Figure 2 depicts the effect of the addition of 25–500 μM AICAriboside on the level of Z-nucleotides after 6 h of incubation. At 500 μM AICAriboside, ZMP (Figure 2a), ZDP and ZTP (Figure 2b) reached approx. 5.5, 0.035 and 0.6 μmol/g of cells respectively. The accumulation of Z-nucleotides reached a plateau after 2 h of incubation and persisted for as long as 24 h without much decline, provided that AICAriboside was still present (results not shown). The rate of disappearance of 50 μM AICAriboside was 1.04 ± 0.05 μmol/h per g of cells (n = 3). Since inhibition of glycolysis by AICAriboside was only observed in FTO-2B cells incubated in KRB, subsequent experiments were performed in this cell type and incubation medium.

**Effect of inhibition of adenosine kinase in FTO-2B cells**

To study if AICAriboside by itself or, as in isolated hepatocytes [5], its metabolite ZMP, was responsible for the inhibition of the glycolytic pathway, FTO-2B cells were incubated with 250 μM AICAriboside, without or with 10 μM 5-iodotubercidin, a powerful inhibitor of adenosine kinase [19]. As expected, incubation with 5-iodotubercidin completely prevented the formation of ZMP from AICAriboside (results not shown). 5-Iodotubercidin by itself had a slight inhibitory effect on the production of lactate (Figure 3). When the cells were incubated with 250 μM AICA-riboside and 10 μM 5-iodotubercidin, the inhibition of glycolysis from glucose was suppressed. This result indicates that the inhibitory effect of AICAriboside on glycolysis requires its phosphorylation.
In a 75% manner, up-dose-dependent concentrations removal of tissue-culture cells, Fru-2,6-P2, a no significant 1 Fru-2,6-P2 dependently of the concentration of In isolated hepatocytes, these cells. The usual influence on the concentration of lactate production from 1 h in KRB medium with 10 mM glucose, before the addition of various concentrations of AICAriboside. In FTO-2B cell extracts, the release of 3H2O from [2-3H]glucose was measured 6 h after addition of 50 μM AICAriboside. Cross-over plots revealed that after 2 h of incubation (Figure 6a) glucose 6-phosphate and fructose 6-phosphate increased by 246% and 174% respectively, whereas Fru-1,6-P2 decreased by 32.5 ± 1.2 μmol/min g of protein (n = 5). Figure 7 shows that ZMP inhibited the activity of PFK-1 in a dose-dependent manner, reaching 70% with 5 mM ZMP.

The finding that ZMP had an inhibitory effect on PFK-1 in FTO-2B cells prompted a study of its influence on the activity of PFK-1 in other cell types, namely normal rat liver and muscle, rabbit heart, and human erythrocytes. In cell-free extracts of rat liver, ZMP inhibited the activity of PFK-1 to a lesser extent than in FTO-2B cells: 5 mM ZMP provoked only 30% inhibition.

Figure 6 Effect of AICArriboside on the concentrations of glycolytic intermediates

FTO-2B cells were incubated in KRB medium with 10 mM glucose. Metabolite concentrations in cells incubated for (a) 2 h or (b) 6 h with 500 μM AICArriboside are plotted as percentages of concentrations measured in the absence of the nucleoside. Control values (expressed as nmol/m of medium for lactate, and as nmol of cells for other metabolites) were: at 2 h: glucose 6-phosphate (G6P) 34, fructose 6-phosphate (F6P) 15, Fru-1,6-P2(F1,6P2) 65, fructose phosphates (TP) 108, and lactate (LAC) 239; at 6 h: G6P 90, F6P 11, F1,6P 172, TP 242, and LAC 465. Values are means ± S.E.M. (n = 3) (*P < 0.05; **P < 0.01; ***P < 0.001).

Figure 7 Effect of ZMP on the activity of PFK-1

The enzyme activity was measured at pH 7.4 in a Sephadex-G-25-filtered high-speed supernatant of FTO-2B cells in the presence of 50 μM fructose 6-phosphate, 2.5 mM ATP, 100 μM AMP, 2.5 mM Fru-2,6-P2, and various concentrations of ZMP (*P < 0.05).

The activities of PFK-1 in extracts of rat muscle, rabbit heart and human erythrocytes were not influenced by up to 5 mM ZMP.

Influence of ZMP on other glycolytic and gluconeogenic enzymes

To explain the inhibitory effect of AICArriboside on the release of 3H2O from [2-3H]glucose, the influence of ZMP on the phosphorylation of glucose was measured in FTO-2B cell extracts. That the Km for glucose was 40–50 μM, and phosphorylation was feedback-inhibited by glucose 6-phosphate, confirmed that it was accomplished not by glucokinase but by hexokinase. Its activity, measured in the presence of a physiological concentration of glucose 6-phosphate, was approx. 1 μmol/min per g of protein. No effect of AICArriboside, ZMP or ZTP on hexokinase activity could be detected under various conditions (results not shown).

The activity of phosphoglucone isomerase, measured with 20 μM glucose 6-phosphate as substrate, was 82.4 ± 1.7 μmol/min per g of protein (n = 3) in FTO-2B cell extracts. It was inhibited by 70% by 5 mM ZMP (results not shown).

As evidenced by the absence of production of glucose from dihydroxyacetone, FTO-2B cells lack glucose-6-phosphatase and/or FBPase-1. The activity of FBPase-1, measured at 50 μM Fru-1,6-P2, was 0.6 μmol/min per g of protein in FTO-2B cell extracts, which is approx. 100-fold lower than in normal hepatocytes. This confirms the known predominance of the glycolytic over the gluconeogenic pathway in hepatoma cells. As in isolated rat hepatocytes [4], 5 mM ZMP inhibited FBPase-1 by 95% (results not shown).

DISCUSSION

This study shows that the inhibitory effect of AICArriboside on glycolysis, initially observed in normal rat hepatocytes [5], is not seen in isolated rabbit cardiomyocytes and human erythrocytes, but can be evidenced in FTO-2B cells, a rat hepatoma cell line, although only under certain conditions. From the data presented it appears that the inhibitory effect of AICArriboside depends on: (1) the capacity of the cells to accumulate ZMP; (2) the presence of target enzymes that are sensitive to inhibition and/or inactivation by ZMP. These points will be discussed separately.

Accumulation of ZMP

We have shown previously that in normal rat hepatocytes AICArriboside induces a dose- and time-dependent build-up of ZMP, reaching approx. 3 μmol/g of cells after 20 min in the presence of 500 μM nucleoside [4]. In the present study, incubation with 500 μM AICArriboside resulted in undetectable ZMP in isolated rabbit cardiomyocytes after 60 min, and in 0.6 μmol of ZMP/g of cells after 3 h in human erythrocytes. In FTO-2B cells, 250 μM AICArriboside induced accumulation of ZMP to 1.5 μmol/g of cells after 4 h of incubation in tissue-culture medium, and to 5 μmol/g of cells after 1 h in KRB (Figure 2a). The variability of the capacity of the different cell types to accumulate ZMP is most likely explained by differences in the activity of adenosine kinase, the enzyme that phosphorylates AICArriboside to ZMP [1]. Indeed, its activity is several-fold lower in heart and erythrocytes than in liver [23]. Accordingly, the rate of utilization of 500 μM AICArriboside which we measured in rabbit cardiomyocytes and human erythrocytes was approx. 20- and 60-fold lower than in isolated hepatocytes [4]. The absence of detectable accumulation of ZMP in rabbit cardiomyocytes accords with the observation that, in dog hearts in vivo, ZMP concentration reached only approx. 0.07 μmol/g of cells after a 24 h perfusion with AICArriboside [24].

The rate of conversion of ZMP into IMP also plays a role in the accumulation of ZMP: that in FTO-2B cells ZMP build-up was several-fold higher in KRB than in tissue-culture medium can be explained by the presence of folate compounds in the latter, which allow ZMP to be formylated and subsequently converted into IMP and other purine compounds. The lower build-up of ZMP in the presence of folates in FTO-2B cells raised the possibility that the accumulation of ZMP in isolated rat hepatocytes, recorded upon incubation in KRB [4,5], might also be decreased in the presence of folates. However, the observation that, on administration of AICArriboside to rats in vivo, hepatic ZMP accumulates rapidly to concentrations which are comparable with those observed in isolated hepatocytes (M. F. Vincent and G. van den Bergh, unpublished work) indicates
that even in the presence of physiological levels of folates there is little conversion of ZMP into IMP in liver. This may be due to the 2–4-fold lower activity of AICAr transformylase in liver compared with other tissues [4]. In cancerous liver cells, with higher rates of synthesis de novo, the accumulation of ZMP could be enhanced by inhibition of AICAr transformylase by anti-folates such as methotrexate.

The inhibitory effect of AICArbose on glycolysis is dependent on the capacity of the cells to accumulate ZMP, as demonstrated by suppression of the effect when adenosine kinase is inhibited by 5-iodotubercidin (Figure 3). The absence of effect of AICArbose on glycolysis in rabbit cardiomyocytes can thus be explained by their inability to accumulate measurable amounts of ZMP. However, even in cells that are able to accumulate ZMP, there is no clear correlation between ZMP concentration and inhibition of glycolysis: in isolated rat hepatocytes, half-maximal inhibition occurred at a ZMP concentration of 0.25 μmol/g of cells [4,5], whereas in FTO-2B cells incubated in KRb it required 2 μmol/g of cells (compare Figures 1 and 2a); in erythrocytes and in FTO-2B cells incubated in tissue-culture medium, which accumulated ZMP to 0.6 and 1.5 μmol/g of cells respectively, glycolysis was not inhibited. This indicates that the target enzymes for ZMP, and/or their sensitivity, vary from one cell type to another.

**Target enzymes for ZMP**

In normal rat hepatocytes, AICArbose inhibits glycolysis [5] by both decreasing the phosphorylation of glucose, which is predominantly effected by glucokinase in this cell type, and inactivating PFK-2, the enzyme that catalyses the synthesis of Fru-2,6-P₂, the principal stimulator of hepatic glycolysis. That in FTO-2B cells the rate of production of lactate remained constant when the concentration of glucose was decreased from 15 to 1 mM, and that in FTO-2B cell extracts glucose was phosphorylated with a K₅₀ of 40–50 μM, shows that these cells contain only hexokinase. Similarly to other hepatoma cells, FTO-2B cells, although displaying a highly differentiated hepatic phenotype [23], thus lack glucokinase. That AICArbose did not decrease glucose 6-phosphate and fructose 6-phosphate in FTO-2B cells, as in normal hepatocytes [5], indicates that it does not act primarily on hexokinase. The observation that ZMP accumulation did not inhibit glycolysis in erythrocytes, which lack glucokinase, also accords with the proposal that the latter is a target for ZMP, whereas hexokinase is not.

The decrease in the release of ³H₂O from [³H]glucose after addition of AICArbose to FTO-2B cells could, in theory, be explained by inhibition of hexokinase and/or phosphoglucomutase. Implication of the 70% inhibition of phosphoglucomutase by 5 mM ZMP in the inhibition of detrition and glycolysis can be ruled out, because residual isomerase activity remained 25-fold higher than that of hexokinase. The inhibition of the release of ³H₂O is thus probably a consequence of feedback inhibition of hexokinase by the increased concentration of glucose 6-phosphate resulting from the inhibition of PFK-1.

FTO-2B cells also differ from hepatocytes with respect to the bifunctional PFK-2/fructose-2,6-bisphosphatase (FBPase-2), the enzyme that synthesizes and degrades Fru-2,6-P₂. Compared with the normal liver enzyme, the FTO-2B enzyme has a 4-fold higher ratio of kinase to phosphatase activity [26], no inactivating cyclic-AMP-dependent phosphorylation site [27], and a different cloned cDNA, resulting from alternative splicing of the gene encoding the liver enzyme [27]. The small and delayed decrease in Fru-2,6-P₂ which AICArbose induced in FTO-2B cells (Figure 4) indicates that, in contrast with normal hepatocytes, their PFK-2/FBPase-2 is not a target for ZMP. The mechanism of the retarded decrease in Fru-2,6-P₂ is not immediately apparent, since the concentration of its precursor, fructose 6-phosphate, was still twice as high in control conditions after 6 h of incubation with AICArbose (Figure 6).

The metabolite cross-over at the level of PFK-1, combined with the absence of effect of AICArbose on the production of lactate from dihydroxyacetone, indicated that AICArbose inhibits glycolysis at the level of PFK-1 in FTO-2B cells. Accordingly, the activity of PFK-1 in FTO-2B cell extracts was inhibited by 70% by concentrations of ZMP reached in cells incubated with 250 μM AICArbose. The observation that ZMP inhibited the activity of PFK-1 in FTO-2B cells and in normal rat hepatocytes, but not in rat muscle, rabbit heart and human erythrocytes, suggests that only the liver isoenzyme of PFK-1 is ZMP-sensitive. That the inhibitory effect was more pronounced in FTO-2B cell extracts than in hepatocyte extracts accords with the finding of modified PFK-1 isoenzymes in hepatomas (reviewed in [28]).

Taken together, our previous study in isolated rat hepatocytes [5] and the present work indicate that glucokinase and PFK-2 of normal liver are most sensitive to ZMP, whereas hexokinase is insensitive. In FTO-2B cells, which lack glucokinase and have a different PFK-2 which is not sensitive to ZMP, glycolysis can nevertheless also be inhibited by AICArbose via ZMP. This is due to the inhibitory effect of ZMP on PFK-1. Why liver PFK-1 is sensitive to ZMP, whereas the skeletal-muscle, heart and erythrocyte enzymes are not, is unknown.

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