Stimulation of rat liver glycogen synthesis by the adenosine kinase inhibitor 5-iodotubercidin

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The adenosine kinase inhibitor 5-iodotubercidin (Itu) was found to have the following effects on glycogen metabolism in hepatocytes of fasted rats. (1) Itu strongly stimulated glycogen synthesis from different substrates (glucose, lactate plus pyruvate, dihydroxyacetone, glycerol and fructose). In cells incubated with these substrates, the well-known stimulating effect of amino acids and that of Itu was more than additive. (2) In parallel with the increase in glycogen deposition, there was an increase in synthase *a* and a decrease in phosphorylase *a* concentrations after administration of Itu. Synthase *a* was increased by Itu and amino acids in an additive manner, whereas the observed activation of phosphorylase after addition of amino acids was antagonized by Itu. (3) In contrast with amino acids, Itu increased neither the cell volume nor the aspartate and glutamate concentrations. (4) Itu enhanced the levels of cyclic AMP. The stimulation of glycogen deposition in the presence of Itu persisted when the cyclic AMP concentration was further increased by adenosine or 2-chloroadenosine. (5) Itu decreased the concentration of ATP, but its effects on glycogen synthesis, synthase *a* and phosphorylase *a* concentrations persisted when the ATP catabolism was prevented by adenosine. (6) The effect of Itu on glycogen synthesis was not the result of inhibition of adenosine kinase, since 5′-amino-5′-deoxyadenosine, another inhibitor of this enzyme, had no effect on glycogen deposition.

INTRODUCTION

5-Iodotubercidin (Itu) is a potent and selective inhibitor of adenosine kinase [1–3]. This enzyme is responsible for the phosphorylation of adenosine to AMP within the cells. Itu has been used to distinguish between the effects of adenosine and its phosphorylated products [4]. Adenosine interacts with P_1-receptors that activate adenylate cyclase, whereas ATP interacts with P_{2y}-receptors and evokes a transient Ca^{2+}-response [4–6]. Itu alone has no effects on P_1- or P_2-receptors [3,4]. It has been reported that addition of Itu leads to an accumulation of adenosine in rat hepatocytes [1] and potentiates the action of exogenously added adenosine on heart rate, blood pressure and adenosine-stimulated cyclic AMP generation [3]. Itu provokes ATP catabolism without modification of AMP or IMP concentrations [1].

Initially we studied the effect of adenosine on glycogen metabolism. We had evidence that the known stimulating effect of amino acids on glycogen synthesis [7–11] may be due to a purine compound, since the increase in glycogen production by amino acids is strongly blocked by inhibitors of purine synthesis [8] and because this inhibition is correlated with a decrease of the formation of adenine nucleotides [12]. Adenosine has been reported to stimulate glycogen synthesis *in vivo* [13] and glycogen synthase activity in isolated liver preparations [11,14]. However, in our incubations adenosine failed to affect glycogen synthesis at low and was inhibitory at high concentrations (100–500 μM) [12]. We used Itu in order to inhibit phosphorylation of adenosine, which is known to be rapidly metabolized [15]. To our surprise, we observed that Itu causes a strong stimulation of glycogen formation which is independent of adenosine addition [12].

To our knowledge, Itu has never been used in studies on glycogen metabolism in the absence of added adenosine. Therefore we focused our interest on the direct effects of Itu on glycogen synthesis and on the concentrations of glycogen synthase *a* and phosphorylase *a*. Furthermore, we investigated whether the stimulation of glycogen synthesis by Itu and amino acids is based on a common mechanism of action. We also tested whether the effects of Itu are due to changes in the concentrations of cyclic AMP and ATP.

EXPERIMENTAL

Materials

The chemicals used are listed with their sources: enzymes, coenzymes, 5′-amino-5′-deoxyadenosine, 2-chloroadenosine (Sigma, St. Louis, MO, U.S.A.); lactate, adenosine (Serva, Heidelberg, Germany); alanine, glutamine (Fluka, Buchs, Switzerland); glucose, glycine (Merck, Basel, Switzerland); pyruvate (Calbiochem, La Jolla, CA, U.S.A.); Itu (generously given by L. B. Townsend, University of Michigan College of Pharmacy, Ann Arbor, MI, U.S.A., and of Research Biochemicals, MA, U.S.A.); BSA fraction V (Miles, Zurich, Switzerland); UDP-D-[U-^{14}C]glucose. All other chemicals were of analytical grade.

Methods

Preparation of hepatocytes and incubation procedure

Hepatocytes from 18–24 h-fasted male Wistar rats were prepared and incubated as described previously [16]. Then $(8–15)\times10^6$ cells were shaken under O_2/CO_2 (19:1) in stoppered 25 ml flasks at 37 °C in Krebs bicarbonate buffer containing 0.5% (w/v) BSA, defatted and dialysed by the method of Chen [17]. The cells were preincubated in the presence or absence of inhibitor or effector for 20 min before substrates were added. The flasks were regassed and the incubation was continued for the time periods indicated.

Abbreviation used: Itu, 5-iodotubercidin.

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Analytical methods

Glycogen was measured as glucose after acid hydrolysis [18]. ATP [19], aspartate [20] and glutamate [21] were determined spectrophotometrically in neutralized $HClO_4$ extracts of the cell suspension as indicated in the references given. None of these metabolites was found in the medium after 60 min of incubation, and the values therefore represent cellular contents. Glycogen synthase *a* activity was measured at 25 °C in the presence of 5 mM UDP-[U-^{14}C]glucose, 1 % glycogen, 4 mM EDTA, 20 mM KF, 50 mM Hepes, pH 7.8, and 10 mM Na_2SO_4 [22]. Phosphorylase *a* activity was measured at 25 °C in the presence of 50 mM glucose 1-phosphate, 1 % glycogen, 150 mM NaF and 0.5 mM caffeine, pH 6.1 [23].

For determination of cyclic AMP, the cells were freeze-thawed in 50 mM Tris buffer, pH 7.5, containing 4 mM EDTA (Titriplex III) to prevent enzymic degradation of cyclic AMP [24], followed by heating for several minutes to coagulate protein. After centrifugation, cyclic AMP was determined in the supernatant by using the cyclic AMP [^{3}H] assay system from Amersham International.

The cell volume was measured by assessing the wet and dry wt. of cell suspensions [25]. In agreement with current literature, 1 g wet wt. corresponded to 0.216 ± 0.009 g dry wt. [25,26].

Expression of results

A value of 125×10^6 cells per g liver wet wt. was used for the calculations [27,28]. With the exceptions mentioned in the corresponding Figures, the results are given as means ± S.E.M. for the indicated numbers of cell preparations. Statistical differences ($n = 3$ or more cell preparations) were tested by Student's unpaired t test [29].

RESULTS

Concentration-dependent effects of Itu in the presence and absence of amino acids

The effects of Itu, an inhibitor of adenosine kinase [1–3], were studied in cells incubated for 60 min with substrates (Figure 1). A mixture containing 10 mM glucose, 4.5 mM lactate and 0.5 mM pyruvate was chosen to study effects of Itu at various concentrations in the absence and presence of the amino acids glutamine, alanine and glycine (5 mM each). The cells were preincubated for 20 min with Itu and the substrates were added at zero time.

Itu led to a dose-dependent stimulation of glycogen deposition, which reached maximal levels at about 20 μM (Figure 1a). At higher concentrations of Itu, glycogen levels fell in the absence of amino acids and continued to increase slightly in their presence. Glycogen synthase was activated by Itu in parallel with glycogen synthesis (Figure 1b). The increases in synthase *a* and in glycogenesis due to Itu correlated better in the presence of amino acids ($r = 0.981$) than in their absence ($r = 0.859$). Itu strongly decreased the concentration of phosphorylase *a* (Figure 1c), and the effect was more pronounced when the amino acids were included in the substrate mixture (phosphorylase *a* versus glycogen: $r = 0.992$ with and 0.861 without amino acids).

Unlike amino acids, Itu did not increase the cell weight (Figure 1d) and the concentration of aspartate plus glutamate (Figure 1e). Itu even led to a slight decrease in aspartate (results not shown). The ATP content of the cells decreased upon addition of Itu (Figure 1f), and this decrease was more pronounced when amino acids were absent. At 10 and 20 μM Itu, the ATP catabolism was significantly greater in the absence of amino acids ($P < 0.05$). The concentration of cyclic AMP increased in response to Itu (Figure 1g), and this increase was significant in the presence of 50 and 100 μM Itu ($P < 0.05$).

Itu time curve in the presence and absence of amino acids

We chose 10 μM Itu for study of the time-dependent effects. When Itu was absent, addition of amino acids led to a 4.3-fold increase in glycogen production (Figure 2a), a 3-fold increase in synthase *a* (Figure 2b) and also a 1.4-fold increase in phosphorylase *a* concentrations (Figure 2c) after 60 min of incubation. The

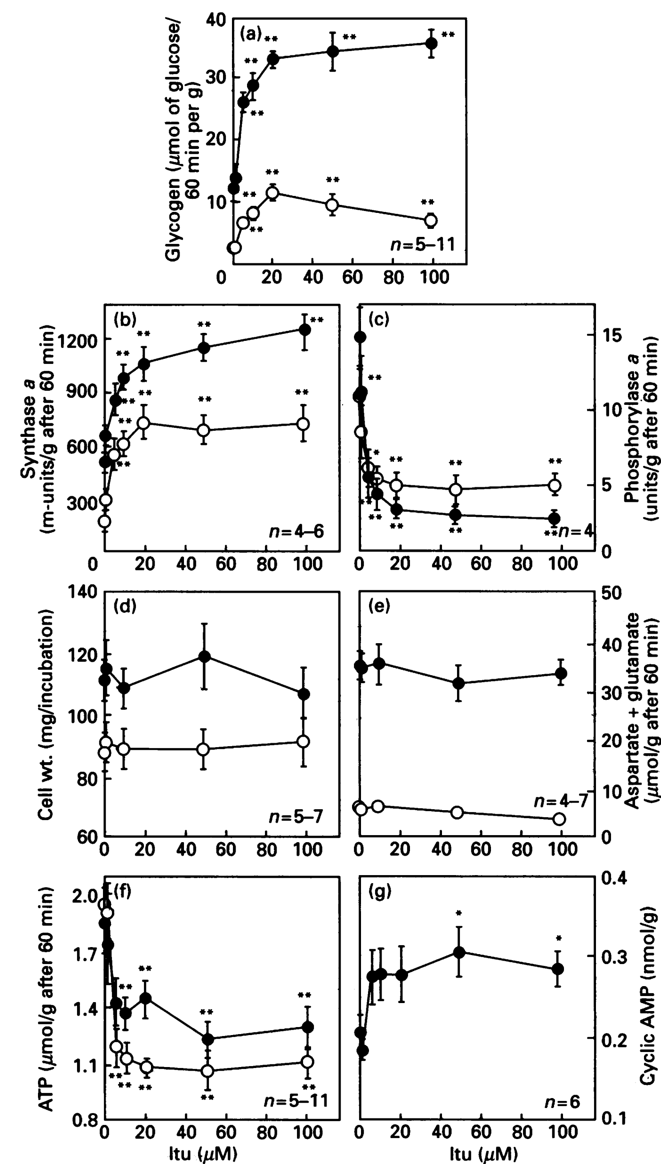

Figure 1 Concentration-dependent effects of Itu in the absence and presence of amino acids

Hepatocytes were incubated for 60 min with 10 mM glucose, 4.5 mM lactate and 0.5 mM pyruvate in the absence (○) or presence (●) of an amino acid mixture containing glutamine, alanine and glycine (5 mM each). Itu was preincubated with the cells for 20 min. The numbers of cell preparations are indicated by n in each panel: *$P < 0.05$ and **$P < 0.01$ versus control values without Itu.

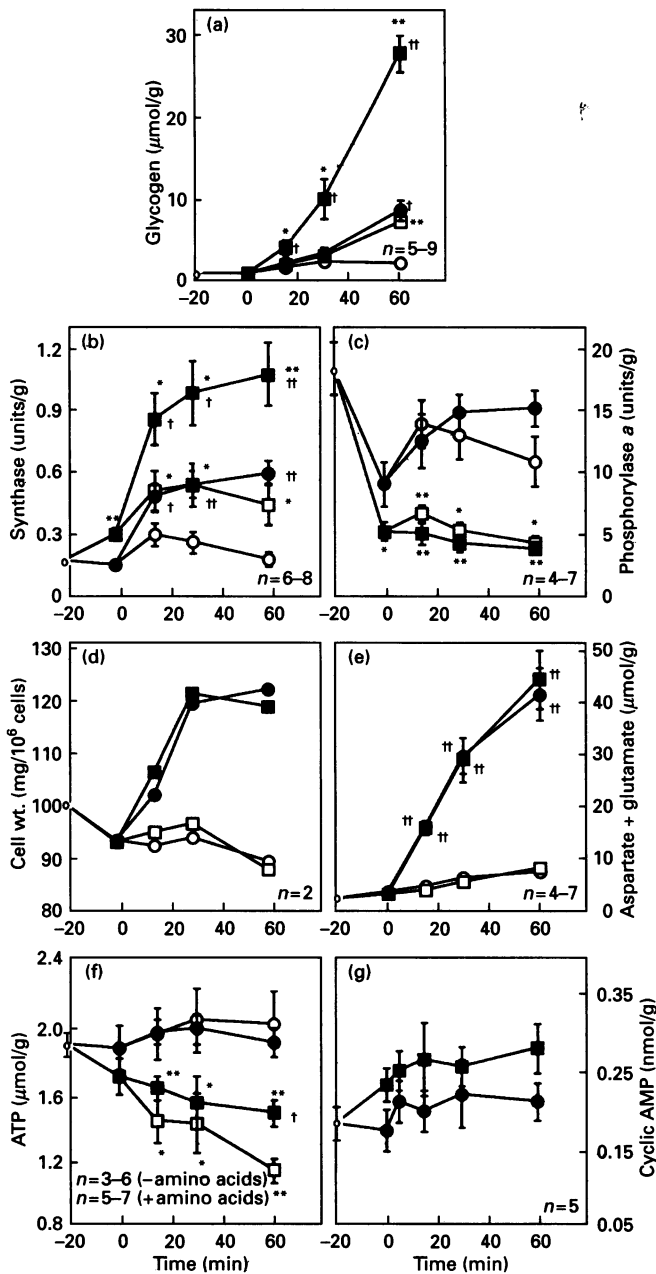

Figure 2 Itu time curve in the absence and presence of amino acids

Substrate conditions are given in the legend of Figure 1: ○, ●, without Itu; □, ■, preincubated with 10 µM Itu; in the presence (●, ■) or absence (○, □) of amino acids. The preincubation time is also shown in the Figure because Itu had effects already during this period. Values are the means ± S.E.M. for the indicated numbers of experiments, except for the cell weight, where the means of two experiments are given. The data at each time point were compared for statistical difference: *$P < 0.05$ and **$P < 0.01$ versus control values in the absence of Itu; †$P < 0.05$ and ††$P < 0.01$ for values with amino acids versus values without amino acids.

ATP concentration was not significantly altered by the presence of amino acids (Figure 2f).

The stimulation of glycogen synthesis due to Itu became significant already 15 min after substrate addition in the presence of amino acids. In their absence the stimulation by Itu was slower and became significant only after 60 min (Figure 2a). Already a preincubation of the cells with Itu for 20 min resulted in an activation of glycogen synthase and in a decrease in phosphorylase *a* (Figures 2b and 2c). A further increase in synthase *a* occurred rapidly after addition of substrates; Itu and amino acids acted additively. Phosphorylase *a* increased after substrate addition, and this increase was completely suppressed by Itu. In the presence of amino acids but in the absence of Itu, there was a simultaneous activation of synthase and phosphorylase.

Amino acids increased the cell volume (Figure 2d) and the concentration of aspartate plus glutamate in a time-dependent manner (Figure 2e), whereas Itu did not alter these parameters. The ATP content of the cells was lowered during preincubation with Itu and further decreased after substrate addition (Figure 2f). After 60 min, the decrease in ATP due to Itu was significantly greater in the absence of amino acids than in their presence. The concentration of cyclic AMP was almost constant during the whole 80 min of incubation in the absence of Itu and was slightly but insignificantly elevated in its presence (Figure 2g). The cyclic AMP levels after a 15 min incubation period were identical in the presence and absence of amino acids (0.244 ± 0.025 and 0.246 ± 0.014 nmol/g respectively; $n = 3$; results not shown).

Results with various gluconeogenic substrates

Itu stimulated glycogen deposition from gluconeogenic precursors such as lactate plus pyruvate, dihydroxyacetone, glycerol and fructose also in the absence of glucose (Table 1). The activation of glycogen production by Itu and amino acids was more than additive.

The concentration of synthase *a* was raised by Itu. This increase was similar for all gluconeogenic substrates. Itu and amino acids had an additive effect. Phosphorylase *a* concentration was decreased on addition of Itu. This effect was more pronounced in the presence of amino acids, which, except in the presence of fructose, increased phosphorylase *a*. Johnson and Miller [30] have reported that fructose, at concentrations of 5 mM and higher, causes an increase in cyclic AMP levels which results in an activation of glycogen phosphorylase. Neither Carabaza et al. [11] nor we could observe such an effect on cyclic AMP. In our experiments, phosphorylase *a* increased on addition of fructose (Table 1), whereas the concentration of cyclic AMP even slightly decreased from 0.20 ± 0.04 nmol/g (control value after 60 min of incubation) to 0.17 ± 0.01 with 5 mM and to 0.15 ± 0.02 nmol/g with 10 mM fructose respectively ($n = 3$).

Correlation between ATP content and glycogen formation

The results presented so far indicate that Itu and amino acids do not share a common mechanism of action and that neither Itu nor amino acids exert their effects via changes in cyclic AMP. However, the stimulating effect of Itu on glycogen production was reflected by a decrease in the ATP content (Figures 1f and 2f). The possibility of a correlation between variations of the ATP concentration and glycogen synthesis was further studied (Table 2).

It has been reported that fructose activates glycogen synthase and phosphorylase by lowering the concentration of ATP [11]. Addition of 10 mM fructose indeed caused a large ATP catabolism and a stimulation of glycogen synthesis (Table 2). However, Itu strongly further increased glycogen formation from fructose, and this 3.4-fold increase was not correlated with additional changes in the ATP content.

Another inhibitor of adenosine kinase, 5′-amino-5′-deoxyadenosine [1,2], led, as already observed with Itu, to a

Table 1 Effect of Itu on glycogen synthesis and on glycogen synthase *a* and phosphorylase *a* activities in the presence of various gluconeogenic substrates

Hepatocytes were incubated for 60 min with the substrates indicated. Concentrations were: lactate, 4.5 mM; pyruvate, 0.5 mM; dihydroxyacetone, 10 mM; glycerol, 10 mM; fructose, 5 mM; amino acids (glutamine, alanine, glycine), 5 mM each. Itu was preincubated with the cells for 20 min. Initial synthase *a* and phosphorylase *a* activities were 0.19 ± 0.02 and 28.0 ± 1.0 units/g of liver respectively. Phosphorylase *a* activity decreased to 11.5 ± 1.0 units/g in the absence and 7.9 ± 0.4 units/g in the presence of Itu after preincubation. Synthase *a* activity was 0.22 ± 0.03 unit/g without and 0.41 ± 0.08 unit/g with Itu before substrates were added. The glycogen content (0.71 ± 0.02 μmol/g) remained constant during preincubation and was not altered by Itu. *$P < 0.05$ and **$P < 0.01$ versus control values in the absence of Itu; †$P < 0.05$ and ††$P < 0.01$ for values with amino acids versus values without amino acids.

		Glycogen production (μmol of glucose/g)		Synthase *a* activity (units/g)		Phosphorylase *a* activity (units/g)	
Gluconeogenic substrate	Itu	No amino acids	+amino acids	No amino acids	+amino acids	No amino acids	+amino acids
None	–	0.68 ± 0.09 (4)	2.26 ± 0.43 (5)††	0.15 ± 0.03 (4)	0.36 ± 0.06 (5)††	10.1 ± 1.9 (5)	27.4 ± 2.0 (5)††
	10 μM	0.95 ± 0.09 (4)**	10.06 ± 1.93 (5)**††	0.29 ± 0.09 (4)	0.77 ± 0.13 (5)**††	7.2 ± 1.6 (4)	9.0 ± 1.4 (5)**
Lactate plus pyruvate	–	1.08 ± 0.16 (6)	3.08 ± 0.87 (6)††	0.12 ± 0.03 (5)	0.29 ± 0.04 (6)††	7.7 ± 0.4 (5)	20.9 ± 1.5 (6)††
	10 μM	1.30 ± 0.16 (6)	10.28 ± 2.24 (6)**††	0.30 ± 0.05 (5)*	0.58 ± 0.12 (6)**††	5.8 ± 0.3 (5)**	9.0 ± 1.2 (6)**†
Dihydroxyacetone	–	1.36 ± 0.23 (5)	7.71 ± 2.04 (5)	0.27 ± 0.05 (5)	0.36 ± 0.07 (5)	13.0 ± 2.2 (5)	20.2 ± 3.0 (5)††
	10 μM	5.09 ± 1.28 (5)**	21.78 ± 2.89 (5)**	0.54 ± 0.12 (5)**	0.72 ± 0.16 (5)**	6.2 ± 1.4 (6)**	5.9 ± 1.1 (5)**
Glycerol	–	0.88 ± 0.15 (5)	2.07 ± 0.27 (5)†	0.12 ± 0.01 (4)	0.30 ± 0.04 (4)††	21.4 ± 3.3 (4)	30.5 ± 2.7 (4)†
	10 μM	1.32 ± 0.23 (5)**	10.54 ± 2.47 (4)**††	0.35 ± 0.07 (4)**	0.69 ± 0.17 (4)**†	13.8 ± 2.5 (4)**	9.1 ± 1.4 (4)**
Fructose	–	4.43 ± 0.55 (4)	16.42 ± 2.10 (4)††	0.31 ± 0.06 (4)	0.53 ± 0.04 (4)††	19.3 ± 4.2 (4)	18.8 ± 1.2 (4)
	10 μM	15.08 ± 0.77 (4)**	38.91 ± 4.32 (4)**††	0.73 ± 0.07 (4)**	1.18 ± 0.05 (5)**††	7.9 ± 0.9 (4)**	6.8 ± 0.9 (4)**

Table 2 Effect of decreasing the ATP concentration on glycogen formation

Cells were incubated for 60 min with the substrates indicated. The concentrations of amino acids, glucose, lactate and pyruvate are given in Figure 1. The effectors were preincubated with the cells for 20 min. Abbreviations: ADA, 5′-amino-5′-deoxyadenosine; CA, 2-chloroadenosine. $n = 5$ in all cases. *$P < 0.05$ and **$P < 0.01$ versus control values.

Substrate(s)	Effector	Glycogen (μmol of glucose/ g of liver)	ATP (μmol/g of liver)
10 mM fructose	None	6.03 ± 1.24	0.74 ± 0.12
	10 μM Itu	20.75 ± 3.16**	0.68 ± 0.03
Amino acids, glucose, lactate and pyruvate	None	14.62 ± 0.59	1.80 ± 0.04
	400 μM ADA	14.07 ± 0.70	1.51 ± 0.09*
	None	10.56 ± 1.69	1.90 ± 0.10
	100 μM CA	0.61 ± 0.05**	1.09 ± 0.15**

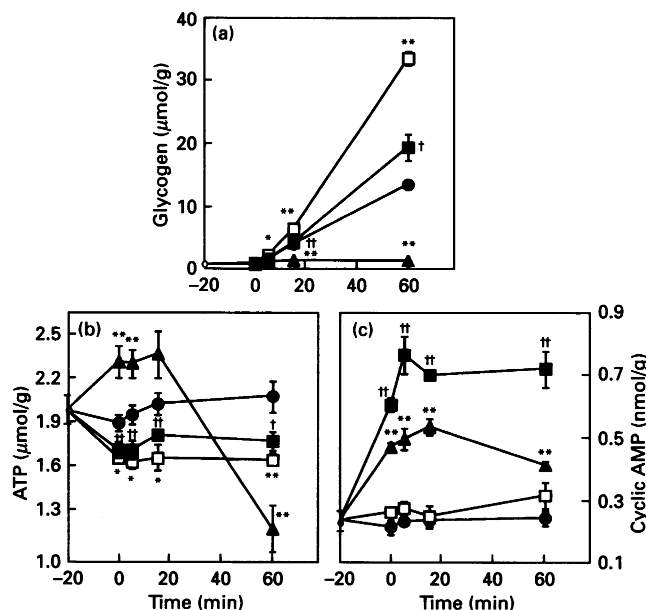

Figure 3 Time curve with 100 μM 2-chloroadenosine in the presence and absence of 10 μM Itu

Hepatocytes were preincubated with 2-chloroadenosine and/or Itu for 20 min. Substrates (glucose, lactate, pyruvate and amino acids; concentrations as in Figure 1) were added at zero time. ●, Control without effector; ▲, 100 μM 2-chloroadenosine; □, 10 μM Itu; ■, 2-chloroadenosine plus Itu. Values are given as means ± S.E.M. for 3 experiments, except for the 5 min value of cyclic AMP ($n = 5$): *$P < 0.05$ and **$P < 0.01$ when Itu or 2-chloroadenosine was compared with control values; †$P < 0.05$ and ††$P < 0.01$ when Itu plus 2-chloroadenosine was compared with 2-chloroadenosine.

decrease in ATP, whereas the formation of glycogen was unaffected. In this case, neither an inhibition of adenosine kinase nor a lower ATP concentration had an effect on glycogen synthesis (Table 2).

In the course of our investigations, we also studied the effects of the adenosine analogue 2-chloroadenosine. Upon addition of 100 μM 2-chloroadenosine, we observed a marked decrease in ATP after 60 min of incubation with substrates and amino acids (Table 2). Despite the low ATP content, there was no glycogen formation in the presence of 2-chloroadenosine. Since this ATP-depleting effect of 2-chloroadenosine was new, we performed additional experiments.

Itu and 2-chloroadenosine

Some groups have reported that 2-chloroadenosine is not metabolised by the cells and does not affect the concentration of ATP [4,11], whereas Claeyssens et al. [31] have observed an increase in cellular ATP on addition of 2-chloroadenosine which has been ascribed to its phosphorylation. ATP catabolism in the presence of 2-chloroadenosine as shown in Table 2 was thus unexpected, and we conducted experiments with shorter incubation times. Figure 3 shows the time-dependent effects of

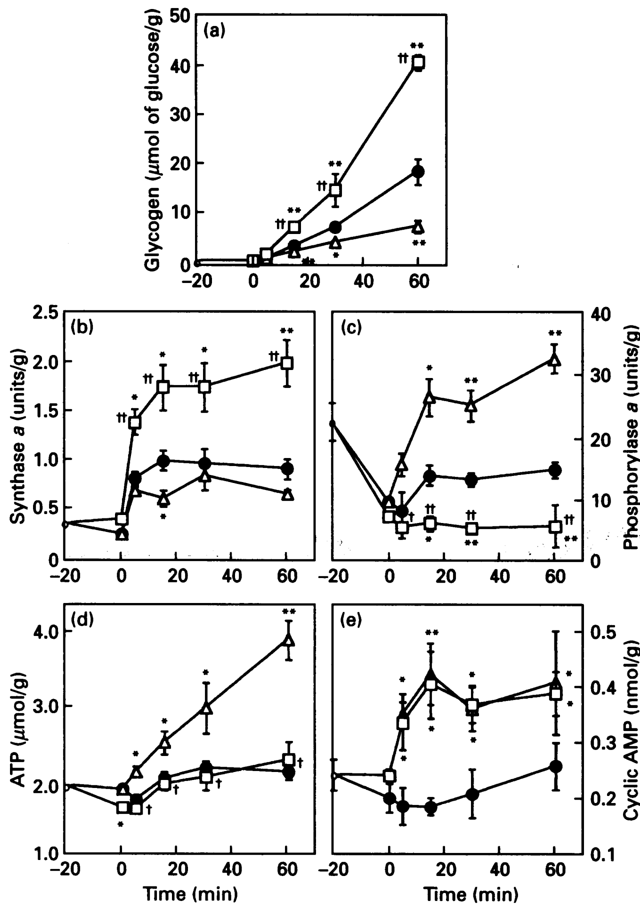

Figure 4 Time curve with 0.5 mM adenosine in the presence and absence of Itu

Hepatocytes were preincubated for 20 min in the presence (□) or absence (●, △) of 10 μM Itu. The cells were further incubated with glucose, lactate, pyruvate and amino acids (see Figure 1) in the presence (△, □) or absence (●) of 0.5 mM adenosine. Values are given as means ± S.E.M. for 3 different cell preparations: $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control values; $\dagger P < 0.05$ and $\dagger\dagger P < 0.01$ when Itu plus adenosine was compared with adenosine.

100 μM 2-chloroadenosine in the absence and presence of 10 μM Itu. Glycogen formation was completely inhibited in the presence of 2-chloroadenosine, and this inhibition was relieved by Itu (Figure 3a). The concentration of ATP (which may represent ATP + 2-chloroATP [31]) was increased by 2-chloroadenosine during preincubation and the first 15 min of incubation with substrates, and then fell sharply. Both the increase and the fall in ATP were antagonized by Itu (Figure 3b). The observed initial increase of ATP in the presence of 2-chloroadenosine does not exclude the possibility that the agent may be phosphorylated as stated in [31]. However, we did not observe a constant increase of ATP in the presence of 2-chloroadenosine, as did Claeyssens et al. [31].

2-Chloroadenosine is known to be a P_1-agonist [31] and to increase cyclic AMP in hepatocytes [31,32]. An increase in cyclic AMP would therefore explain the inhibiting effect of 2-chloroadenosine on glycogen formation. As shown in Figures 3(a) and 3(c), glycogen formation was totally suppressed by 2-chloroadenosine, and this inhibition was accompanied by an increase in cyclic AMP. However, the inhibition of glycogen synthesis caused by 2-chloroadenosine was relieved by Itu, whereas the increase in cyclic AMP was paradoxically even potentiated by Itu.

Itu and adenosine

Adenosine has also been reported to increase the concentration of cyclic AMP [32,33] and, after phosphorylation, the concentration of ATP in the cells. When rat hepatocytes were incubated with glucose, lactate, pyruvate and amino acids, addition of 0.5 mM adenosine caused a time-dependent inhibition of glycogen production, which reached 59% at 60 min (Figure 4a). Synthase *a* concentration decreased slightly, whereas phosphorylase *a* was increased 2-fold in the presence of adenosine (Figures 4b and 4c). Under the same conditions, adenosine led to a significant increase in ATP and cyclic AMP concentrations (Figures 4d and 4e).

Preincubation of the cells with 10 μM Itu for 20 min prevented the effects of adenosine on glycogen, synthase *a* and phosphorylase *a* concentrations. On the other hand, the ATP concentration decreased only to control values in the presence of adenosine plus Itu, and the cyclic AMP levels raised by adenosine were not affected by Itu.

DISCUSSION

Comparison of the effects of Itu and amino acids

It is well documented that amino acids stimulate glycogen synthesis from glucose and gluconeogenic precursors [7–11], and we determined whether Itu acts in a similar manner. Promotion of glycogen synthesis by added amino acids appears to be due to hepatocyte swelling after their uptake and the accumulation of intracellular catabolites such as glutamate and aspartate [34–37]. Furthermore, amino acids activate glycogen synthase with either no effect on [10] or activation of [11] glycogen phosphorylase.

Itu affected neither the cell volume nor the contents of aspartate and glutamate in the cells (Figures 1 and 2). The mechanism of Itu action thus seems not to be related to cell volume changes. Furthermore, the stimulation of glycogen synthesis and the increase in synthase *a* in response to Itu and amino acids was additive (Figure 1, Table 1). In agreement with Carabaza et al. [11], we observed an increase in phosphorylase *a* concentration in the presence of amino acids (Figure 1, Table 1). Only with 10 mM fructose as substrate was the already high level of phosphorylase *a* not further increased by amino acids (Table 1). Itu significantly decreased phosphorylase *a*, and this effect was not dependent on the presence of amino acids (Table 1). It is therefore concluded that the stimulatory action of Itu and amino acids is not based on a common mechanism.

Role of ATP and cyclic AMP

Itu is an inhibitor of adenosine kinase and consequently interferes with adenosine and ATP metabolism. ATP and adenosine are known to affect the activity state of synthase and phosphorylase [4–6,11,15,32,38–40]. Adenosine has been reported to activate adenylate cyclase in the livers from fed rats [4–6,32,33] whereas other groups failed to observe an increase in cyclic AMP in the presence of adenosine in livers of fasted rats [11,15]. We noticed a concentration-dependent increase in cyclic AMP after addition of Itu (Figure 1). The effects of Itu on glycogen formation can therefore not be attributed to an inhibition of the adenylate cyclase system. In contrast with Carabaza et al. [11] and Fain and Shepherd [15], we observed an increase in cyclic AMP after adenosine addition. Itu did not inhibit accumulation of cyclic AMP in the presence of adenosine (Figure 4) and even further increased cyclic AMP in the presence of 2-chloroadenosine

(Figure 3), whereas its effects of glycogen synthesis, synthase *a* and phosphorylase *a* persisted. These results suggest that Itu acts in a manner independent of adenosine and cyclic AMP, and even cancels the effects of cyclic AMP on glycogen metabolism.

Itu caused a decrease in ATP concentration. ATP is known to inhibit glycogen synthase phosphatase in a glycogen-particle system from rat liver [38–40]. A decrease in ATP in the presence of Itu could therefore explain the observed increase in synthase *a* concentrations (Figures 1b and 2b). Effects of ATP on phosphorylase phosphatase have also been described in sub-cellular liver preparations, and stimulatory [39] as well as inhibitory [41,42] actions have been noticed. On the one hand, if ATP inhibits the enzyme, then a decrease in the nucleotide in the presence of Itu should result in an inhibition of phosphorylase *a*, as observed in Figures 1(c) and 2(c). Nevertheless, the decrease in phosphorylase *a* due to Itu should have been less marked in the presence of amino acids, since the fall in ATP was also smaller in their presence. However, the decrease in phosphorylase *a* due to Itu was much more marked when amino acids were present (Figures 1c and 1f, 2c and 2f). On the other hand, a stimulation of phosphorylase phosphatase by ATP [39] would not explain the large decrease in phosphorylase *a* levels observed in the presence of Itu. It is difficult to draw definite conclusions from these experiments, especially since the physiological relevance of the ATP effects on phosphorylase phosphatase has been questioned by the authors [39,41]. However, the following findings strongly argue against an action of Itu via ATP catabolism.

(i) The effects of Itu on glycogen production, synthase *a* and phosphorylase *a* persisted in the presence of adenosine, although the ATP concentration did not decrease below the control values, as with Itu alone (compare Figures 1 and 4). (ii) The increase in glycogenesis observed with the ATP-depleting substrate fructose was more than tripled in the presence of Itu, whereas the ATP content was not additionally influenced by Itu (Table 2). (iii) 2-Chloroadenosine depressed glycogen production irrespective of an increased (−20 to +15 min of incubation) or a decreased ATP content (60 min of incubation with substrates). Itu reversed the biphasic effects of 2-chloroadenosine on the ATP concentration and abolished the inhibition of glycogen synthesis during the whole incubation period. After 60 min, the ATP as well as the glycogen content was higher in the presence of Itu and 2-chloroadenosine than with 2-chloroadenosine alone (Figure 3). (iiii) 5′-amino-5′-deoxyadenosine, another inhibitor of adenosine kinase [1,2], had no effect on the formation of glycogen, although the ATP concentration was decreased by the agent (Table 2). From these results it can be concluded that the effect of Itu on glycogen synthesis cannot be explained by a decrease of the ATP concentration. Furthermore, the results with 5′-amino-5′-deoxyadenosine show that the observed effects of Itu on glycogen metabolism are not due to an inhibition of adenosine kinase, but to another as yet unknown action of Itu.

Itu and proglycosyn

When similar experiments are compared, the effects obtained with Itu are almost identical with those of the phenacyl imidazolium compound proglycosyn (LY 177507) described by Harris et al. [43], Yamanouchi et al. [44] and Guo et al. [45]. Proglycosyn stimulates glycogen synthesis from glucose and gluconeogenic substrates, and this stimulation is markedly increased further in the presence of glutamine and asparagine. Both proglycosyn and Itu activate glycogen synthase, inhibit glycogen phosphorylase and do not increase the cell volume. The only difference between proglycosyn and Itu noticed so far is that proglycosyn has no effect on cyclic AMP levels, whereas Itu increased cyclic AMP. However, the experiments with cyclic AMP presented by Yamanouchi et al. [44] were performed with fed animals. Although proglycosyn and Itu have no obvious chemical analogies, the similar effects indicate that the two components or their metabolites may share a common, as yet not defined, mechanism of action.

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