ACTA CHEMICA SCANDINAVICA 23 (1969) 237–243

The Influence of Ethanol on Glycerol Metabolism in Liver Slices from Fed and Fasted Rats

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The rate of glycerol uptake by rat liver slices was increased after fasting of the donor animals. Addition of ethanol to the incubation medium lowered glycerol uptake. The concentration of glycerophosphate in the slices was increased when the incubation medium contained glycerol, ethanol, or both. Under all the different experimental conditions the α-glycerophosphate level was highest in slices from fed animals. Dihydroxyacetone phosphate in the slices varied less than α-glycerophosphate. The concentration of AMP was increased by ethanol, but not by glycerol alone. The ATP × AMP/ADP^2 quotient varied between 1.2 and 0.3. The higher value was found when the incubation medium contained ethanol. It is concluded that the inhibition by ethanol of the glycerol uptake was caused by inhibition of the glycerokinase (EC 2.7.1.30) reaction by AMP.

Glycerol is metabolized predominantly in the liver.[1] The first step is presumably the phosphorylation of glycerol to α-glycerophosphate by ATP.[2–4] The metabolism of glycerol is strongly inhibited by the simultaneous metabolization of ethanol.[5,6] Grunnet and Lundquist [7] have proposed, that this effect of ethanol upon glycerol metabolism is due to an increase in AMP content in the liver. They have found that AMP is a potent inhibitor of glycerokinase, and previous experiments (Thieden, unpublished observations) have shown that ethanol causes an increase in AMP concentration in liver slices. In the present paper the effect of ethanol on glycerol metabolism in liver slices and on the concentrations of some intermediary metabolites is reported.

MATERIALS AND METHODS

Female Wistar rats weighing 100–200 g were used. The fasted rats were deprived of food for 20 h before use. The rats were killed by a blow on the head and decapitated. The livers were rapidly removed and chilled in ice. Slices 0.3 mm thick were cut with a McIlwain-Buddle tissue chopper and transferred to prepared Erlenmeyer flasks containing 10 ml of cold incubation medium. The amount of tissue is estimated by weighing the flasks before and after the addition of slices. Each flask contained 0.3–0.7 g of

tissue. The incubation medium was Krebs-Ringer bicarbonate.[8] This medium was prepared from the purest grade reagents and was used with the additions indicated in the tables. The flasks were gassed with oxygen for 5 min at the beginning of the incubation and shaken at 37°. Samples for the determination of glycerol were removed at 15 min intervals and the total incubation period was 60 min. The reaction was stopped by addition of $HClO_4$ and the content of glycerol determined.[9] To minimize changes in the concentrations of the rapidly reacting metabolites during manipulations after the completion of the incubations, these were terminated by rapid removal of the flasks from the water bath, discarding of 8 ml of the incubation mixture, cooling the flasks with the slices and the remainder in a mixture of ice and NaCl and addition of cold $HClO_4$ to give a final concentration of 11 % (w/v). After homogenization the material was centrifuged at low temperature. A measured sample of the supernatant was neutralized with KOH and the precipitate of $KClO_4$ removed.

The same liver was used in parallel assays and all results are referred to the wet weight of liver. The relative changes are used in determination of probability limits. Control samples without liver slices showed a small increase in glycerol concentration due to evaporation of water and a correction for this was applied. The pH did not change during the incubation.

The following determinations were done by the methods indicated: α-glycerophosphate,[10] dihydroxyacetonephosphate,[11] ATP,[12] ADP, and AMP [13] in the liver slices. In experiments in which these substances were measured the incubation time was 60 min, but some experiments were performed to determine the changes in the concentrations of ATP, ADP. and AMP during the first 30 min of the incubation period. Enzymes and coenzymes used were preparations from Boehringer & Soehne, Mannheim, Germany.

RESULTS

The uptake of glycerol. When liver slices from fed animals were incubated in buffer containing glycerol (5 mM) and glycose (11 mM) the uptake of glycerol was 32.5 μmoles per g wet wt. per hour. Addition of ethanol (10 mM) resulted in a 43 % decrease in glycerol uptake. The rate of glycerol uptake was increased by starvation, but addition of ethanol gave a similar (47 %) decrease in glycerol uptake (Table 1).

The addition of ethanol to the incubation medium caused a striking rise in AMP concentration (Table 2). This occurred whether glycerol was present

Table 1. Effect of ethanol on the rate of glycerol uptake.

The rate of glycerol uptake was measured by incubating liver slices in 10 ml Krebs-Ringer bicarbonate buffer containing 5 mM glycerol at 37° with additions as indicated. The results are expressed as mean values ± S.E.M. with numbers of determinations given in parentheses. (Fisher's P values represent the probability of identity between the means (corresponding pairs)). Values of P greater than 0.05 are shown as N.S., *i.e.*, not significantly different.

Additions	Rate (μmoles of glycerol/g wet wt./h) Fed rats	Fasted rats
(1) Glucose (11 mM)	32.5 (7) ± 1.2	50.1 (5) ± 2.8
(2) Glucose (11 mM) + ethanol (10 mM)	18.8 (7) ± 0.8	26.6 (5) ± 2.3
Difference in rates	13.7 (7) ± 0.9	23.5 (5) ± 2.0
P values:		
(1) *versus* (2)	< 0.001	< 0.001

or not. Glycerol alone did not affect the AMP concentration. The increase in AMP could be as high as 200 %. The AMP was somewhat higher in slices from fed than from starved animals, but this difference was not significant. The elevation of AMP could be observed already 10 min after addition of ethanol (Fig. 1).

The incubation of the liver slices in different media did not cause significant changes in the ADP (Table 2). Addition of ethanol tended to reduce the ATP concentration. This fall was most pronounced, when the incubation medium contained both ethanol and glycerol (Table 2).

α-Glycerophosphate and dihydroacetonephosphate content in the liver slices. The concentration of α-glycerophosphate and of dihydroxyacetone phosphate were determined after the incubation of the slices in different media for 1 h. Incubation of the slices in medium containing ethanol caused an increase in α-glycerophosphate concentration. This has been observed previously.[14,15] Addition of glycerol in the incubation medium produced also an increase in α-glycerophosphate, but the highest concentrations were observed in the slices incubated in medium containing both glycerol and ethanol.

Under all the different experimental conditions the α-glycerophosphate concentrations were higher in slices from fed than from fasted animals.

Dihydroxyacetone phosphate concentration was decreased by fasting. When ethanol was present in the incubation medium the dihydroxyacetone phosphate concentration was increased and most markedly when also glycerol was present. Glycerol alone did not change the dihydroxyacetone phosphate concentration in the fasted rats.

The content of ATP, ADP, and AMP in the liver slices. The highest levels of all adenine nucleotides were found before the incubation (Fig. 1). The

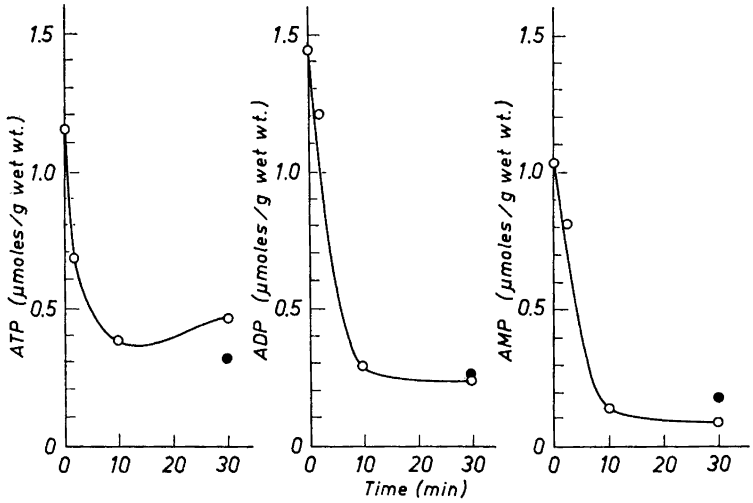

Fig. 1. Time-course of the adenine nucleotide content in rat liver slices during incubation in Krebs-Ringer bicarbonate containing 11 mM glucose. Experimental conditions as described in Methods. There were four observations for each point. ● indicates that ethanol in final concentration of 10 mM were added to the flasks after 20 min incubation.

Table 2. Effect of ethanol on the content of α-glycerophosphate, dihydroxyacetone phosphate, ATP, ADP, and AMP in the liver slices from fed and fasted rats.

The liver slices were incubated in Krebs-Ringer bicarbonate buffer for one hour. α-Glycerophosphate, dihydroxyacetone phosphate, ATP, ADP, and AMP were determined after this time. Statistical treatment was as in Table 1. The means compared are indicated by the numbers in the first column. Results are given as μmole/g wet wt.

Additions	Glycerophosphate	Dihydroxyacetone-phosphate	ATP	ADP	AMP
FED RATS					
(1) Glucose (11 mM)	0.94±0.09 (6)	0.041±0.010 (5)	0.500±0.038 (5)	0.354±0.075 (5)	0.052±0.023 (5)
(2) Glucose (11 mM)+ethanol (10 mM)	1.61±0.09 (6)	0.057±0.010 (5)	0.493±0.042 (5)	0.284±0.041 (5)	0.178±0.028 (5)
(3) Glucose (11 mM)+glycerol (5 mM)	1.92±0.17 (6)	0.054±0.010 (5)	0.541±0.040 (5)	0.330±0.058 (5)	0.075±0.018 (5)
(4) Glucose (11 mM)+ ethanol (10 mM)+ glycerol (5 mM)	3.89±0.22 (6)	0.080±0.007 (5)	0.354±0.044 (5)	0.232±0.023 (5)	0.153±0.021 (5)
P values:					
(1) *versus* (2)	<0.001	<0.05	N.S.	N.S.	<0.01
(1) *versus* (3)	<0.01	<0.01	<0.05	N.S.	N.S.
(1) *versus* (4)	<0.001	<0.01	<0.001	N.S.	<0.01
FASTED RATS					
(1) Glucose (11) mM)	0.27±0.06 (6)	0.020±0.005 (6)	0.537±0.027 (5)	0.273±0.016 (5)	0.043±0.006 (5)
(2) Glucose (11 mM)+ethanol (10 mM)	0.57±0.04 (6)	0.034±0.004 (6)	0.425±0.016 (5)	0.233±0.033 (5)	0.139±0.015 (5)
(3) Glucose (11 mM)+glycerol (5 mM)	0.73±0.08 (6)	0.031±0.004 (6)	0.514±0.025 (5)	0.290±0.030 (5)	0.051±0.005 (5)
(4) Glucose (11 mM)+ethanol (10 mM)+ glycerol (5 mM)	2.87±0.23 (6)	0.073±0.006 (6)	0.348±0.018 (5)	0.268±0.026 (5)	0.128±0.018 (5)
P values:					
(1) *versus* (2)	<0.001	<0.05	<0.05	N.S.	<0.01
(1) *versus* (3)	<0.01	N.S.	N.S.	N.S.	N.S.
(1) *versus* (4)	<0.001	<0.001	<0.01	N.S.	<0.01

Table 3. Effects of the different experimental conditions on the sum of adenine nucleotides, ATP/ADP ratio, and on the equilibrium in the adenylate-kinase system. The values given are calculated on the data from Table 2.

Additions	ATP+ADP+AMP μmole/g wet wt.	$\frac{ATP}{ADP}$	$\frac{ATP \cdot AMP}{ADP^2}$
FED RATS			
(1) Glucose (11 mM)	0.906±0.095 (5)	1.57±0.22 (5)	0.22±0.08 (5)
(2) Glucose (11 mM) Ethanol (10 mM)	0.955±0.077 (5)	1.81±0.19 (5)	1.17±0.20 (5)
(3) Glucose (11 mM) Glycerol (5 mM)	0.945±0.110 (5)	1.74±0.18 (5)	0.41±0.11 (5)
(4) Glucose (11 mM) Ethanol (10 mM) Glycerol (5 mM)	0.727±0.071 (5)	1.49±0.20 (5)	0.89±0.16 (5)
P values:			
(1) *versus* (2)	N.S.	N.S.	<0.01
(1) *versus* (3)	N.S.	N.S.	N.S.
(1) *versus* (4)	<0.01	N.S.	<0.01
FASTED RATS			
(1) Glucose (11 mM)	0.837±0.046 (5)	1.98±0.10 (5)	0.33±0.08 (5)
(2) Glucose (11 mM) Ethanol (10 mM)	0.797±0.051 (5)	1.94±0.023 (5)	1.16±0.17 (5)
(3) Glucose (11 mM) Glycerol (5 mM)	0.854±0.057 (5)	1.82±0.11 (5)	0.33±0.06 (5)
(4) Glucose (11 mM) Ethanol (10 mM) Glycerol (5 mM)	0.744±0.053 (5)	1.34±0.12 (5)	0.64±0.08 (5)
P values:			
(1) *versus* (2)	N.S.	N.S.	<0.05
(1) *versus* (3)	N.S.	N.S.	N.S.
(1) *versus* (4)	N.S.	<0.05	<0.05

first 10 min of incubation caused a rapid fall in all three nucleotides. The degradation is possibly due to 5′-AMP deaminase and 5′-nucleotidase.[16,17] The values determined after 30 min incubation did not differ from those determined after 1 h.

No adenine nucleotides could be detected in the incubation medium.

The concentrations of ATP after 1 h incubation were somewhat higher, but the concentrations of ADP and of AMP were considerably lower, than those found by Puddu, Calderera and Marchetti,[18] who incubated liver slices in calcium free phosphate buffer. This may be explained by the protective effect of phosphate, as both 5′-AMP deaminase and 5′-nucleotidase are known to be inhibited by P_i.[19]

DISCUSSION

The addition of ethanol to the incubation medium produced an increased AMP concentration in the liver slices. This increase occurred whether glycerol was added or not. Oura, Räihä and Suomalainen [20] in their investigation on the effect of ethanol on the adenine nucleotide concentrations *in vivo*, did not find this effect. Their observations might offer, however, an explanation for this discrepancy. They have used relatively high ethanol concentrations, 4 mg/g body wt., and ethanol in this concentration had the same effects on AMP concentrations in mice as other alcohols, non metabolizable narcotics such as ethyl ether, and even hypnotics. All these substances decreased AMP slightly.

In vivo experiments (Thieden [29]) have shown, that low concentrations of ethanol increased the AMP. The AMP concentration in rat liver was 0.249 ± 0.025 μmoles/g wet wt. if 4 μmoles ethanol per g body wt. were infused intravenously, whereas infusion of saline resulted in 0.131 ± 0.014 μmoles/g wet wt.

The increased AMP concentration in the liver slices during ethanol oxidation may explain the inhibitory effect of ethanol on glycerol uptake. The glycerokinase reaction is strongly inhibited by AMP.[7] The increased α-glycerophosphate concentration during ethanol oxidation may act in the same direction [21] although other workers have not found evidence for the inhibition of the glycerokinase by glycerophosphate.[7] The liver slices from fasted rats showed considerably increased glycerol uptake when compared with slices from fed animals. This effect has been observed in kidney slices too.[22] As the glycerokinase reaction is irreversible [3] this must be explained either by the greater glycerokinase activity in the fasted rat or by an inhibition of the glycerokinase reaction in the fed rat. The elevated α-glycerophosphate concentration in the slices from fed animals might inhibit this reaction or perhaps a higher AMP concentration, although the differences observed between AMP content in fed and fasted rats are not significant.

The total adenine nucleotide content was somewhat decreased when both ethanol and glycerol were present in the incubation medium, but only in the fed rats was this decrease significant. Fasting apparently did not affect the sum of adenine nucleotides.

The ATP/ADP ratio remained virtually unchanged during the different experimental conditions.

The concentrations of AMP and ATP are governed by the equilibrium constant $K=(\mathrm{AMP})(\mathrm{ATP})/(\mathrm{ADP})^2$ of the adenylate kinase reaction, if we assume that the activity of this enzyme is sufficiently high to maintain the concentrations of the three reactants near equilibrium.[23] The equilibrium constant is reported to vary between 0.4 and 0.9 depending on the Mg^{2+} concentration.[17] In the present investigation the value of the quotient $(\mathrm{AMP})(\mathrm{ATP})/(\mathrm{ADP})^2$ calculated from the data obtained with and without ethanol varied between 1.2 and 0.3. Ethanol caused a striking increase in the quotient. The reason for this is not known, see however, Ref. 29.

The effect of ethanol on the concentration of glycerophosphate might be due to the increased $NADH/NAD^+$ ratio which occurs during ethanol oxida-

tion.[24,25] The increased NADH/NAD$^+$ ratio will cause an increased reduction of dihydroxyacetone phosphate to glycerophosphate, as we may assume that a steady state exists between these two red/ox systems.[26–28]

The glycerophosphate and dihydroxyacetone phosphate concentrations were lowest in liver slices from fasted animals. This effect could be caused by the greater gluconeogenetic capacity in this condition.

This work was supported by grants from *Statens almindelige Videnskabsfond* and from *Fonden til lægevidenskabens fremme*. The skilled technical assistance of Miss A. Severinsen and Miss M. Aarup is gratefully acknowledged.

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Received May 21, 1968.