0014-4754/96/080827-04$1.50 + 0.20  

# Glucose formation from methylglyoxal in hepatocytes from streptozotocin-induced diabetic mice: the effect of insulin

M. P. Kalapos[a,*] P. Riba[b], T. Garzó[a] and J. Mandl[a]

[a] *First Institute of Biochemistry, Semmelweis University Medical School, Budapest (Hungary)*
[b] *Department of Pharmacology, Semmelweis University Medical School, Budapest (Hungary)*
*Received 21 March 1996; accepted 11 April 1996*

**Abstract.** Acetol and methylglyoxal are intermediates of the intrahepatic metabolism of acetone leading to pyruvate formation. In hepatocytes prepared from fasted streptozotocin-induced diabetic mice, net glucose production could be measured from methylglyoxal but not from acetone or acetol. Insulin increased glucose formation from methylglyoxal in a concentration-dependent manner, whereas it was ineffective when pyruvate was used as substrate. Drug oxidation, as evidenced by *p*-aminophenol formation from aniline, was enhanced by methylglyoxal, and insulin proved to be stimulatory in this case as well. It is concluded that insulin might be involved in the regulation of glucose formation from methylglyoxal, but its mode of action is not yet clear.
**Key words.** Acetone; acetol; methylglyoxal; pyruvate; gluconeogenesis; drug oxidation; insulin; hepatocyte (mouse).

The characteristic of diabetes mellitus is the disturbance of intermediary metabolism due to the absolute or relative absence of the effect of insulin. As a consequence of the higher rate of lipolysis, plasma ketone body (acetoacetate, β-hydroxybutyrate) concentrations are increased, which elevate acetone production as well. Acetone, the decarboxylated product of acetoacetate, not only is removed from the body in exhaled air or voided urine but can also be metabolized (for a review, see ref. 1). The metabolism of acetone yields pyruvate, and methylglyoxal (pyruvaldehyde) is an intermediate in this route[2].

In diabetes mellitus, glucose formation from acetone has been investigated both in vivo and in vitro, in human[3,4] and in animal[5,6] studies. However, little attention has been paid to glucose formation from methylglyoxal, especially in relation to diabetes mellitus, and few data are available. In starved rats, insulin hampered glycogen formation from methylglyoxal[7], while in rabbits, methylglyoxal failed to aid recovery from insulin-provoked hypoglycemia[8].

Since methylglyoxal is a product of cytochrome P450-catalyzed steps[2], and isozymes (cytochrome P450 IIE 1 gene subfamily) involved in these reactions are induced in diabetes (for a review, see ref. 9), the investigation of methylglyoxal metabolism under diabetic conditions is of importance. It is known that the supply of NADPH + $H^+$ for cytochrome P450s diverts intermediates from the gluconeogenic sequence (for a review, see ref. 10) and, at the same time, the cofactor supply for the conjugation phase of drug metabolism is diminished in the diabetic state[11].

In experiments, gluconeogenesis from methylglyoxal and its effect on drug oxidation (detected as phenol accumulation) were investigated in hepatocytes prepared from streptozotocin (STZ) induced diabetic mice. The effect of insulin on both processes was also examined. In this paper it is reported that the addition of insulin enhances glucose formation from methylglyoxal but not from pyruvate. On the other hand, when aniline is added, methylglyoxal increases phenol accumulation in the cells, and the amount of accumulated phenols depends on whether insulin is present or not.

## Materials and methods

Isolated hepatocytes were prepared by the collagenase perfusion method[12] from STZ-injected (single injection of 60 mg/body weight kg given 2 weeks before the experiments started) CFLP male mice, fasted for 24 h prior to the experiments. Viability of the cells determined by the Trypan blue exclusion test[13] was about 90–95% at the beginning of the experiments. Isolated cells ($2 \times 10^6$ cells/ml) were incubated in a Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 1% albumin and 2.5 mM $CaCl_2$ under constant bubbling of gas ($O_2$:$CO_2$, 95/5, v/v%) at 37 °C. In one series of experiments the Krebs-Henseleit bicarbonate buffer also contained not only amino acids necessary for protein synthesis (1 mM of each) but also 8.5 mM glucose and 2.5 mM pyruvate, referred to as supplemented medium in text.

* Corresponding author. Present address: Theoretical Biology Research Group, Dámvad utca 18, H-1029 Budapest (Hungary), Fax +36 1 1765499.

Glucose content of the samples and blood glucose concentration of the animals were measured by the glucose oxidase/peroxidase method[14]. Since there was no measurable change in glucose content of the cells incubated without any gluconeogenic substrate, the actual glucose production of hepatocytes was calculated after subtraction of the glucose content of the samples measured at 0 min from that determined at 30 min. Just before the experiments, blood glucose concentration of mice was $12.3 \pm 1.2$ mM ($n = 10$) and $5.5 \pm 0.6$ mM ($n = 3$) in animals injected with STZ and in their normal littermates, respectively. Using $^{125}$I-labeled insulin, the ability of the hepatocytes to bind insulin was checked (Kalapos and Kovács, unpubl.). Lactoperoxidase-catalyzed iodination of insulin was performed as described by David and Reisfeld[15].

When aniline was added as a substrate for drug metabolism, *p*-aminophenol accumulation in the cells was determined according to the method of Schenkman et al.[16] DNA content of the cells was determined according to the method of Burton[17]. Methylglyoxal used was routinely purified by passage through a Dowex 1 ($Cl^-$, mesh 200/400) column, and methylglyoxal content was determined as described by Racker[18].

All results are expressed as mean ± SE. Statistical analysis of data was performed using Student's t-test (one-tailed).

Collagenase (type IV), glucose oxidase, *o*-dianisidine, STZ and methylglyoxal (40% aqueous solution) were purchased from Sigma, St. Louis, MO, USA. Peroxidase and NADPH + $H^+$ were bought from Reanal Fine Chemicals, Budapest, Hungary. Acetol was received from Aldrich-Chemie GmbH, Steinheim, Germany. Insulin was from Novo-Nordisk. All other chemicals were of analytical grade.

## Results and discussion

Net glucose formation in isolated hepatocytes from acetone, acetol or methylglyoxal, added as the sole substrates for gluconeogenesis, is presented in figure 1. There was no net glucose formation from acetone or acetol even when these were given at 20 mM concentration. However, methylglyoxal proved to be gluconeogenic; glucose formation from methylglyoxal increased when added up to 5 mM concentration, but above this aldehyde concentration glucose production, similar to the findings obtained in a study when hepatocytes were prepared from fasted mice[19], was diminished. In earlier studies net glucose formation was demonstrated from both acetone and acetol in hepatocytes from fasted rats[2], but not from fasted mice[20]. All these data raise the possibility of species differences and, at the same time, the role of other factors, such as cofactor supply for monooxygenases. This latter point seems to be supported by the fact that glucose production can

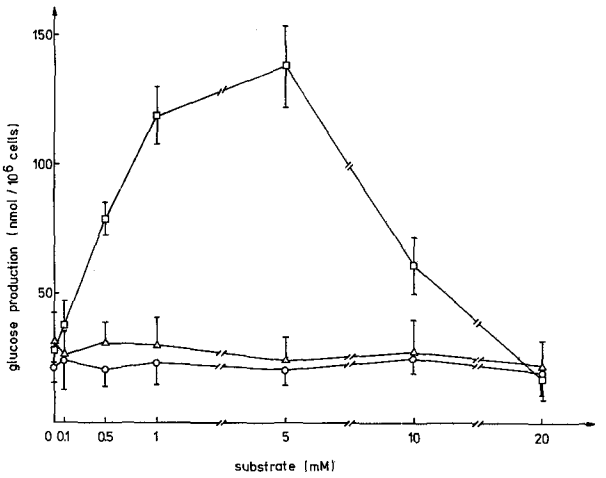


Figure 1. Glucose formation from acetone, acetol and methylglyoxal. Hepatocytes were prepared from 24 h-fasted STZ-treated mice and incubated in the presence of substrates at 37 °C for 30 min. Each point represents the mean ± SEM of 3 to 5 experiments. Acetone (○), acetol (△), methylglyoxal (□).

always be demonstrated when methylglyoxal – the first intermediate after the cytochrome P450-catalyzed steps in the gluconeogenic sequence starting from acetone[2] – is used, regardless of species or pretreatment of animals supplying hepatocytes (fig. 1 and refs 2 and 20).

Since methylglyoxal added at concentrations higher than 1 mM inhibited protein synthesis in isolated murine hepatocytes[19], 1 mM methylglyoxal was used to investigate the effect of insulin on glucose formation. As shown in the inset of figure 2, the addition of increasing insulin concentrations elevated glucose production when methylglyoxal was used, while it had no effect on glucose formation from pyruvate. To further substantiate the influence of insulin on gluconeogenesis from methylglyoxal, the accumulation of *p*-aminophenol – the hydroxylated product of aniline – was studied, since it is known that the conditions (e.g. diabetes mellitus, starvation) in which isozymes of the cytochrome P450 IIE 1 subfamily are induced, and the pools of cofactors needed for conjugation are reduced, favour phenol accumulation when aniline is added[21–23]. Since the NADPH + $H^+$ supply for cytochrome P450s burdens gluconeogenic sequence[10], better substrate provision for gluconeogenesis may promote drug oxidation. In hepatocytes incubated in the presence of 2 mM aniline with and without insulin, the maximal rate of *p*-aminophenol accumulation was achieved at 1 mM and 0.1 mM methylglyoxal, respectively (fig. 2). The addition of insulin also increased the amount of accumulated *p*-aminophenol (fig. 2). Data on phenol accumulation in experiments with hepatocytes incubated in a supplemented medium indicated that the supplementation of the medium did not have any influence on the position of the peak either in the presence or in the absence of

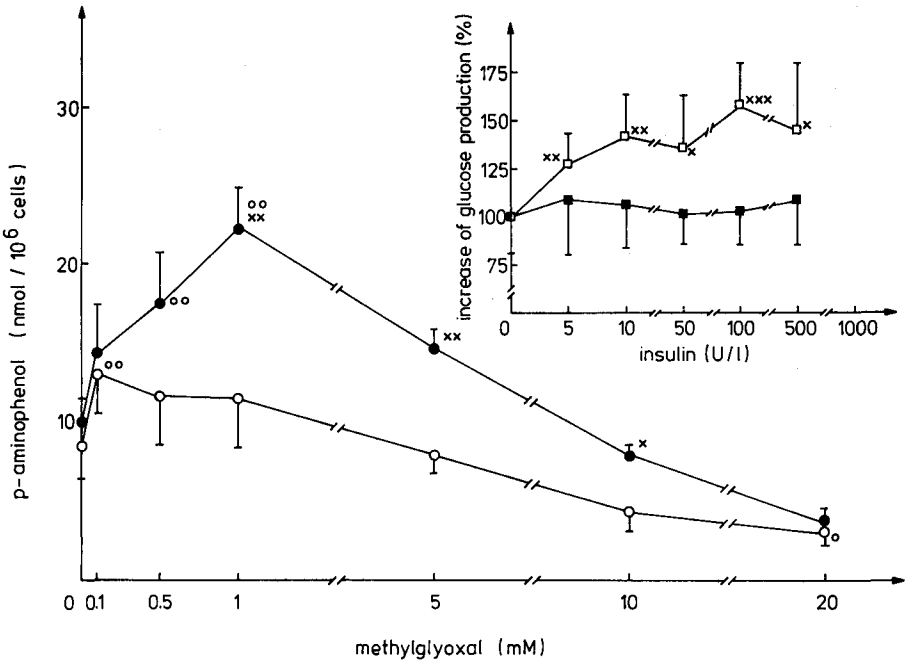


Figure 2. The influence of insulin on glucose formation from methylglyoxal and pyruvate, and on *p*-aminophenol accumulation in cells. Hepatocytes were prepared from 24 h-starved STZ-treated mice. The cells were incubated at 37 °C for 30 min in the presence of 2 mM aniline, and increasing concentrations of methylglyoxal were given to the medium with (●) and without (○) addition of 500 U/l insulin. Each point represents the mean ± SE of three experiments. Significantly different from the corresponding control: $^{o}p < 0.1$, $^{oo}p < 0.05$, and statistical difference of *p*-aminophenol accumulation between the two incubation conditions at $^{x}p < 0.1$ and $^{xx}p < 0.05$. Inset: The cells were incubated at 37 °C for 30 min in the presence of 1 mM methylglyoxal (□) or pyruvate (■), and an increasing amount of insulin was added. Glucose production is expressed as the percentage of the control values, mean ± SEM of 6 experiments. Hundred percent values are 85.7 ± 14.3 nmol/$10^6$ cells and 201 ± 41.7 nmol/$10^6$ cells for methylglyoxal and pyruvate, respectively. Significantly different from the corresponding control: $^{x}p < 0.1$, $^{xx}p < 0.05$ and $^{xxx}p < 0.02$.

insulin, and the amount of accumulated *p*-aminophenol was not modulated either (data not shown). Methylglyoxal enters the known pathway of gluconeogenesis through pyruvate[2]. Since a crucial role is assigned to pyruvate concentration in gluconeogenesis[24], and the effect of A23187 calcium ionophore antibiotic on glucose formation starting from pyruvate depends on the concentration of pyruvate used[25], it seems plausible to suggest that insulin enhances glucose production from methylglyoxal in such a manner that pyruvate supply is increased. The ratio of glucose formation from methylglyoxal and pyruvate added at the same concentration is approximately 0.4 (see fig. 2, inset). Therefore, if insulin promotes the conversion of methylglyoxal to pyruvate through one or another of the well-known routes, the more efficient pyruvate supply can cause the higher rate of gluconeogenesis starting from methylglyoxal in the presence of insulin. Nevertheless, based on the phenol accumulation experiments in supplemented medium, it is not clear why supplementation of the incubation medium does not have any influence on phenol accumulation. Using hepatocytes isolated from acetone-pretreated fasted mice, it has been demonstrated that supplementation of the medium lowered the methylglyoxal concentration at which the rate of phenol accumulation was maximal[19]. It was concluded that this shift was due to the less effective metabolism of methylglyoxal, which in the presence of nutrients became toxic at lower concentrations[19]. In the present study this phenomenon is not seen, and insulin proved to be ineffective on glucose formation from pyruvate. Insulin is known to increase glycolysis and glycogen synthesis (for reviews, see refs 26 and 27). In diabetes mellitus the changed enzyme pattern in the liver favours gluconeogenesis and glycogenolysis (for a review, see ref. 28). As presented (fig. 2), insulin elevated glucose formation from methylglyoxal, and it can be postulated that by increasing the conversion of this aldehyde to pyruvate, insulin increases glucose production.

Acknowledgements. Thanks are due to Mrs Gizella Ferencz for her excellent assistance during the experiments. This work was supported by the Ministry of Welfare, Hungary.

1 Argiles, J. M., Trends Biochem. Sci. *11* (1986) 61.
2 Casazza, J. P., Felver, M. E., and Veech, R. L., J. Biol. Chem. *259* (1984) 231.
3 Owen, O. E., Trapp, V. E., Skutches, C. L., Mozzoli, M. A., Hoeldtke, R. D., Boden, G., and Reichard, G. A., Diabetes *31* (1982) 242.
4 Reichard, G. A., Skutches, C. L., Hoeldtke, R. D., and Owen, O. E., Diabetes *35* (1986) 668.

5 Hetényi, G., Byers, M., and Ferrarotto, C., Hormone Metabol. Res. *19* (1987) 143.
6 Kosugi, K., Chandramouli, V., Kumaran, K., Schumann, W. C., and Landau, B. R., J. Biol. Chem. *261* (1986) 13179.
7 Stöhr, R., Biochem. Zeitschr. *240* (1936) 26.
8 Kermack, W. O., Lambil, C. G., and Slater, R. H., Biochem. J. *21* (1927) 40.
9 Gonzalez, F. J., Pharmac. Rev. *40* (1989) 243.
10 Thurman, R. G., and Kauffman, F. C., Pharmac. Rev. *31* (1980) 229.
11 Grant, H. M., and Duthie, S. J., Biochem. Pharmacol. *36* (1987) 3647.
12 Mandl, J., Garzó, T., Mészáros, K., and Antoni, F., Biochim. Biophys. Acta *586* (1979) 560.
13 Jauregui, H. O., Hayner, N. T., Driscoll, J. L., Williams-Holland, R., Lipsky, M. H., and Galletti, P. M., In vitro *17* (1980) 1100.
14 Bergmeyer, H. U., and Bernt, E., in: Methods in Enzymatic Analysis, p. 123. Ed. H. U. Bergmeyer. Academic Press, New York 1963.
15 David, G. S., and Reisfeld, R. A., Biochemistry *13* (1974) 1014.
16 Schenkman, J. B., Remmer, H., and Estabrook, R. W., Molec. Pharmac. *3* (1967) 113.
17 Burton, K., Biochem. J. *62* (1956) 315.
18 Racker, E., J. Biol. Chem. *190* (1951) 685.
19 Kalapos, M. P., Garzó, T., Antoni, F., and Mandl, J., Biochim. Biophys. Acta *1092* (1991) 284.
20 Kalapos, M. P., Mandl, J., Bánhegyi, G., Antoni, F., and Garzó, T., Int. J. Biochem. *26* (1994) 1069.
21 Bánhegyi, G., Garzó, T., Antoni, F., and Mandl, J., Biochem. Pharmac. *37* (1988) 4157.
22 Dicker, E., McHugh, T., and Cederbaum, A. I., Biochim. Biophys. Acta *1035* (1990) 249.
23 Kalapos, M. P., Schaff, Z., Garzó, T., Antoni, F., and Mandl, J., Toxic. Letters *58* (1991) 181.
24 Groen, A. K., van Roermund, C. W. T., Vervoorn, R. C., and Tager, J. M., Biochem. J. *237* (1986) 379.
25 Kalapos, M. P., Riba, P., Garzó, T., Antoni, F., and Mandl, J., Int. J. Biochem. *24* (1992) 211.
26 Hers, H. G., A. Rev. Biochem. *45* (1976) 167.
27 Pilkis, S. J., El-Maghrabi, M. R., and Claus, T. H., A. Rev. Biochem. *57* (1988) 755.
28 Granner, D., and Pilkis, S. J., J. Biol. Chem. *265* (1990) 10173.