[Chem. Pharm. Bull. 36(9)3678—3682(1988)]

Glucose Release from Isolated, Perfused Liver of Alloxan-Diabetic Rats

TOSHIAKI NISHIHATA,*,[a] CHIE IKAWA,[b] YUKIKO SAITOH[b] and KIYOSHI SAKAI[b]

Pharmaceutical Chemistry Department, University of Kansas,[a] 2065 Constant Avenue, Lawrence, Kansas 66046, U.S.A. and Osaka University of Pharmaceutical Sciences,[b] 1–10–65 Kawai, Matsubara, Osaka 580, Japan

(Received February 1, 1988)

Glucose release from the isolated, perfused rat liver was observed to a greater extent in alloxan-diabetic rats, compared to normal rats. The addition of acetoacetate (an acetone body) to the perfusate increased the glucose release from the normal rat liver, suggesting that the greater glucose release from the alloxan-diabetic rat liver is due to functional disorder(s) of the liver induced by acetoacetate in the diabetic condition. It was observed that lipoic acid suppressed glucose release from the liver of alloxan-diabetic rats, and that the nonprotein thiol level in alloxan-diabetic rat liver was lower than that in normal rat liver. Addition of acetoacetate in the perfusate caused a decrease in nonprotein thiol level in normal rat liver; suggesting that nonprotein thiol loss may mediate the increase in glucose release caused by an increase in acetoacetate in alloxan diabetic rats.

Keywords——isolated perfused liver; alloxan-diabetic rat; glycogenolysis; phloretin; acetoacetate; lipoic acid; nonprotein thiol

The liver is a major organ in regulating blood glucose concentration, and the mechanism is insulin-independent,[1] though there is a mechanism by which an increase in portal insulin concentration increases the magnitude of net hepatic glucose uptake.[2] The liver stores glucose as glycogen when blood glucose concentration increases, and releases glucose predominantly by glycogenolysis when blood glucose concentration becomes too low. However, since depancreatized dogs displayed high blood glucose concentrations even after a 36-h fast,[3] it is likely that the liver is disfunctioning in regulating blood glucose concentration under diabetic conditions, independent of a mere insulin deficiency.

In the present study, we investigated the liver function of alloxan-diabetic rats in regulating glucose concentration by using the isolated, perfused rat liver.

Experimental

Materials——Phloretin and lithium acetoacetate were obtained from Sigma Chemicals Co. (St. Louis, U.S.A.). Lipoic acid was supplied by Fujisawa Pharmaceutical Industry (Osaka, Japan). Other reagents used were of analytical grade.

Isolated, Perfused Rat Liver Studies——Wistar male rats, 180 to 200 g, were fed *ad libitum*. Alloxan-diabetic rats were obtained by injection of alloxan subcutaneously at a dose of 100 mg/kg, and were used for experiments after three weeks. Plasma glucose concentration of alloxan-diabetic rats used was more than 200 mg/100 ml (glucose concentration in plasma was determined at 2 weeks after alloxan administration, and agsin on the day of the perfusion study).

The isolated, perfused rat liver was prepared according to the method of Blackmore *et al.*[4] The experimental procedures were as follows. Briefly, the liver was perfused with Krebs–Henseleit bicarbonate buffer (pH 7.4)[5] containing 1% bovine serum albumin (no glucose) for 5 min, and then perfused with the buffer containing various concentration of glucose. The flow rate was 28 ml/min at 34 °C according to the method of Kleineke and Soling,[6] and

liver weight (measured just after the perfusion study) was 7.2±0.3 g (the mean±S.D.) in rats used in this study. Perfusate through the liver was collected at 2 min intervals for 10 or 20 min to determine any change in glucose concentration in the perfusate.

In a separate study, after perfusion with the buffer for 5 min, the whole liver was excised and homogenized to assay nonprotein and protein thiols. The weights of livers used in this study were 7.4±0.6 g.

Assays——The glucose assay was performed with the aid of an analytical kit (Glucose Test Wako®, Wako Pure Chemicals Industry Ltd., Osaka, Japan) based on an enzymatic method. Assay of nonprotein thiol and protein thiol were performed by the method of Di Monte *et al.*,[7] using glutathione as the standard thiol.[8] Protein content in liver homogenate was measured by the method of Lowry *et al.*[9]

Results and Discussion

Since the perfusate used in the present studies did not contain any precursors for gluconeogenesis,[1] any glucose release from the isolated, perfused rat liver occurred by glycogenolysis.[6] Thus, in the present study, an increase in glucose concentration in the perfusate is predominantly due to glycogenolysis in the liver. As shown in Fig. 1, the glucose concentration in the perfusate after liver passage was maintained at a constant value from 4 to 20 min after starting the perfusion with the medium containing no glucose or 500 mg of glucose/100 ml, in both normal and alloxan-diabetic rats. Therefore, changes in glucose concentration during passage through the liver were determined 10 min after starting the perfusion. The changes in glucose concentration are expressed as the differences between the concentrations of glucose in the perfusate before and after liver passage (Table I).

When the buffer containing either no glucose or glucose at 100 mg/100 ml was perfused through the liver of normal rats, an increase in glucose concentration in the perfusate after liver passage was observed (Table I). However, net glucose uptake into the liver was observed when the buffer contained 500 mg of glucose/100 ml. Because the addition of phloretin (a known inhibitor of glucose uptake into hepatocytes) inhibited the decrease in glucose concentration in the perfusates containing 500 mg of glucose/100 ml, the isolated, perfused rat liver in the present study functioned normally with regard to glucose transport.

When the buffer containing various concentrations of glucose was perfused through livers of alloxan-diabetic rats, glucose concentrations in the perfusate were higher than those from normal rats (Fig. 1 and Table I). Since addition of phloretin in the buffer caused an increase in glucose concentration, even when the perfusate contained 500 mg of

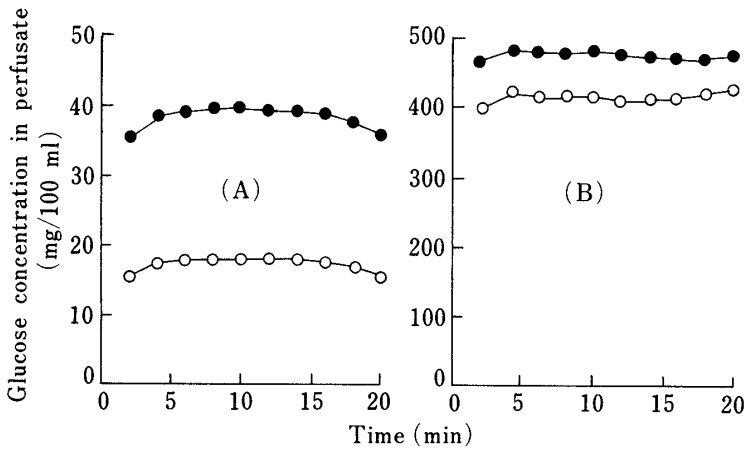

Fig. 1. Glucose Concentration in the Perfusate through the Isolated, Perfused Rat Liver in Normal (Open Symbols) and in Alloxan-Diabetic (Closed Symbols) Rats as a Function of Time after Perfusion with the Buffer (Containing No Glucose (A), or 500 mg of Glucose/100 ml (B)

Each value represents the mean±S.D. ($n=4$).

TABLE I. Glucose Concentration Changes in the Perfusate through Isolated, Perfused Rat Liver, after 10 min Perfusion with Medium Containing Various Concentrations of Glucose in Normal and Alloxan-Diabetic Rats

Perfusate before liver passage Concn. of glucose (mg/100 ml)	Change in glucose concentration in perfusate[a] (mg/100 ml)			
	Normal rats		Alloxan diabetic rats	
	Without phloretin[b]	With phloretin[b]	Without phloretin[b]	With phloretin[b]
No additive				
0	18.2± 2.4	18.6±1.7	39.6± 5.9[d]	38.7±4.4[d]
100	10.6± 3.1[e]	15.2±5.6	39.1± 7.6[d]	41.4±5.9[d]
500	−89.1±15.6[e]	5.7±5.6[e]	−21.3±12.6[d,e]	33.2±6.3[d]
250 μM acetoacetate[c] in the perfusate				
0	36.9± 4.2[f]			
2.5 μM lipoic acid[c] in the perfusate				
0	19.1± 2.7		23.6± 2.9[f]	
250 μM acetoacetate and 2.5 μM lipoic acid				
0	24.2± 4.9[g]			

a) The change in glucose concentration = (concn. of glucose in the perfusate before liver passage) − (concn. of glucose in perfusate after liver passage). *b*) Phloretin was present in the medium at the concentration of 50 μM. *c*) The buffer containing acetoacetate and/or lipoic acid was perfused for 5 min before the glucose release study. Each value represents the mean ± S.D. ($n>4$). *d*) $p<0.05$ *versus* the result in normal rats. *e*) $p<0.05$ *versus* no glucose in normal rats and in alloxan-diabetic rats, respectively. *f*) $p<0.05$ *versus* the results in the absence of acetoacetate or lipoic acid. *g*) $p<0.05$ *versus* in the presence of acetoacetate alone.

glucose/100 ml, the liver does function in alloxan-diabetic rats for taking up glucose from the perfusate. However, glucose release from the liver of alloxan-diabetic rat is raised compared to that from the liver of normal rats.

In terms of the change in glucose concentration in the presence of phloretin, the reduction in glucose concentration from the perfusate containing glucose at 500 mg/100 ml was lost in the normal rat liver, with a change of glucose concentration of only 5.7 ± 5.6 mg/100 ml in the perfusate (Table I). Glycogenolysis in the normal rat liver apparently decreases with increasing glucose concentration in the perfusate. However, when phloretin was added to the perfusate of the alloxan-diabetic rat liver, the increase in glucose concentration in the perfusate after liver passage was significant, and the increased glucose concentration (about 30 mg/100 ml or more) was not influenced so much by the initial glucose concentration in the perfusate (Table I). These findings suggest that the liver of alloxan-diabetic rats in regulating glucose release predominantly by glycogenolysis is partly disfunctioning, with a high degree of glucose release even when high glucose concentrations are passed through the liver.

It has been reported that glycogenolysis in liver by phosphorylase is regulated by cytosolic Ca^{2+} concentration,[10,11] such that an increase in cytosolic Ca^{2+} concentration caused an increase in glycogenolysis. Thus, the activity of phosphorylase has often been measured to estimate cytosolic Ca^{2+} concentration in hepatocytes.[12,13] The addition of lipoic acid (2.5 μM) to the perfusate, which sequesters Ca^{2+} in the intracellular Ca^{2+} pools,[14] suppressed glucose release from the liver of alloxan-diabetic rats (Table I), but did not affect the normal rat liver. These results suggest that an increase in glucose release from the alloxan-diabetic rat liver may be due to the disorder of intracellular Ca^{2+} homeostasis.

Since it has been reported that an increase of acetone bodies in alloxan-diabetic rabbits caused a kidney disorder,[15] we examined the effect of adding acetoacetate (250 μM) to the

TABLE II. Concentrations of Nonprotein and Protein Thiols in the Isolated, Perfused Liver of Normal and Alloxan-Diabetic Rats

Treatment	Thiols (μmol/g-protein)	
	Nonprotein	Protein
(1) Normal rat liver after 5-min perfusion with the buffer	30.6 ± 2.9	111.4 ± 12.6
(2) Normal rat liver after 5-min perfusion with 250 μM acetoacetate	$22.2 \pm 3.8^{a)}$	102.4 ± 10.1
(3) Alloxan diabetic rat liver after 5-min perfusion with the buffer	$24.1 \pm 2.9^{a)}$	109.7 ± 11.5

Each value represents the mean $\pm$ S.D. ($n=4$ to 6). *a*) $p<0.05$ *versus* (1).

perfusate with respect to glucose release from the liver of normal rats. The concentration of acetoacetate used was a concentration which is often observed in the blood in alloxan-diabetic rabbits.[14] The addition of acetoacetate raised the concentration of glucose in the perfusate by about two-fold compared to that in the absence of acetoacetate (Table I). The addition of lipoic acid in the perfusate containing acetoacetate suppressed the increase in glucose release from the normal rat liver (Table I). These results suggest that an increase of acetoacetate as an acetone body in alloxan-diabetic rats may be involved in the mechanism of the high glucose release. However, it is not clear whether other acetone bodies such as 3-hydroxylactate increase glycogenolysis.

We have recently reported that losses of nonprotein thiol and/or protein thiol in the isolated, perfused liver of normal rats caused the increase in glucose release,[16] and that nonprotein thiol loss was observed in the intestinal epithelium of alloxan-diabetic rats.[17] Thus, the contents of the thiols in the isolated, perfused rat liver were measured 5 min after starting perfusion of the burffer in the absence of glucose.

Nonprotein thiol content in liver from alloxan-diabetic rats was lower than that in normal rat liver, even though there was no difference in the levels of protein thiol (Table II). A decrease in nonprotein thiol level in the normal rat liver was also observed after the addition of acetoacetate to the perfusate (Table II).

It has been reported[18] that nonprotein thiol loss induced an increase in Ca^{2+} liberation by inhibition of Ca^{2+} uptake into the intracellular Ca^{2+} pool in an *in vitro* isolated hepatocyte study. Thus, the results obtained in the present study suggest that nonprotein thiol in the isolated, perfused rat liver may also induce the liberation of Ca^{2+} in cytosol, though functional differences between the isolated perfused rat liver and isolated hepatocytes have been reported.[6]

Acknowledgement This work was supported in part by a grant from R. P. Scherer Co., Ltd. (Florida, U.S.A.).

References

1) H. F. Woods, W. A. Stubb, J. Johnson, and K. G. M. Albert, *Clin. Exper. Pharmacol. Physiol.*, **1**, 535 (1974).
2) R. N. Berman and R. J. Bicolo, *Am. J. Physiol.*, **227**, 1314 (1974).
3) T. Nishihata, Y. Okamura, A. Kamadaq, T. Higuchi, T. Yagi, R. Kawamori, and M. Shichiri, *J. Pharm. Pharmacol.*, **37**, 22 (1985).
4) P. F. Blackmore, J. P. Dehaye, and J. H. Exton, *J. Biol. Chem.*, **254**, 6945 (1979).
5) H. A. Krebs and H.-S. Henseleit, *Hoppe-Seyler's Z. Physiol. Chem.*, **210**, 33 (1932).
6) J. Kleineke and H.-S. Soling, *J. Biol. Chem.*, **260**, 1040 (1985).
7) D. Di Monte, G. Bellomo, H. Thor, P. Nicotera, and S. Orrenius, *Arch. Biochem. Biophys.*, **235**, 343 (1984).
8) S. Szabo, J. S. Trier, and P. W. Frankel, *Science*, **214**, 200 (1981).
9) O. H. Lowry, N. J. P. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

10) J. R. Exton, *Mol. Cell. Endocr.*, **23**, 233 (1981).
11) J. R. Willson, R. H. Cooper, and J. B. Hoek, *Biochim. Biophys. Acta*, **639**, 243 (1981).
12) M. Moore, H. Thor, G. Moore, P. Moldeus, and S. Orrenius, *J. Biol. Chem.*, **260**, 13035 (1985).
13) P. Cohen, A. Burchell, G. Foulken, P. V. W. Cohen, T. C. Vananum, and A. C. Nairm, *FEBS Lett.*, **92**, 287 (1978).
14) H. Thor, P. Hurtzell, S.-A. Svensson, and S. Orrenius, *Biochem. Pharmacol.*, **34**, 3717 (1985).
15) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.*, **26**, 2238 (1978).
16) T. Nishihata, C. Ikawa, Y. Saitoh, and K. Sakai, *J. Pharm. Pharmacol.*, **40**, 333 (1988).
17) T. Nishihata, B. T. Nghiem, H. Yoshitomi, C.-S. Lee, M. Dillsaver, T. Higuchi, R. Choh, T. Suzuka, A. Furuya, and A. Kamada, *Pharm. Res.*, **3**, 345 (1986).
18) S. A. Jewell, G. Bellomo, H. Thor, S. Orrenius, and M. T. Smith, *Science*, **217**, 1257 (1982).