Biochimica et Biophysica Acta, 1110 (1992) 119–122

BBAMEM 70710

Rapid Report

Hexose-specific inhibition in vitro of human red cell Ca^{2+}-ATPase activity

Mark R. Deziel [a,b], Richard S. Safeer [c], Susan D. Blas [c], Faith B. Davis [a,b] and Paul J. Davis [a,b]

[a] *Division of Molecular and Cellular Medicine, Department of Medicine, Albany Medical College, Albany, NY (USA),* [b] *the Stratton Veterans Administration Medical Center, Albany, NY (USA) and* [c] *the Department of Medicine, State University of New York at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY (USA)*

(Received 8 July 1992)

Key words: Red blood cell; ATPase, Ca^{2+}-; D-Glucose; (Human)

In a concentration-dependent manner (5.5–27.5 mmol/l), D-glucose incubated in vitro with human erythrocyte membranes at 37°C for 1 h inhibited membrane Ca^{2+}-ATPase activity by up to 75%. The IC_{50} was 11 mmol/l. L-Glucose was ineffective, as were 3-*O*-methylglucose, 2-deoxyglucose, sorbitol and *myo*-inositol. In contrast, D-fructose decreased Ca^{2+}-ATPase activity nearly as effectively as D-glucose and mannose and galactose at 11 mmol/l were less than 50% as effective as D-glucose. Tunicamycin (12 pmol/l), but not 10 mmol/l aminoguanidine, progressively antagonized in vitro the D-glucose effect on the enzyme. Erythrocyte membrane Ca^{2+}-ATPase activity may be regulated by glycosylation, rather than nonenzymatic glycation.

The nonenzymatic glycation of proteins is widely acknowledged to occur in vivo and in vitro under ambient glucose concentrations that are normal or increased [1–3]. Protein function and structure may be altered when excessive glycation occurs [4,5] and glycation has been postulated to contribute to the development of specific organ complications of diabetes mellitus [1,6]. Ca^{2+}-stimulated, Mg^{2+}-dependent adenosine triphosphatase (Ca^{2+}-ATPase) activity has been shown to be decreased in erythrocytes obtained from hyperglycemic diabetic patients [7–9]. We have reported that in vitro exposure of red blood cell membranes from normal subjects to ambient glucose concentrations above 5 mmol/l (90 mg/dl) for as little as 1 h at 37°C leads to significantly decreased membrane Ca^{2+}-ATPase activity [10]. Further, we showed that transient decreases in red cell Ca^{2+}-ATPase activity occur in vivo during short-term glucose administration [10]. Recently, Resnick et al. have reported that human erythrocyte Ca^{2+} content is increased in diabetic patients [11] and that red cell Ca^{2+} levels rise in vitro after short-term exposure of intact red cells to modestly elevated ambient glucose levels in the presence of physiological extracellular concentrations of Ca^{2+} [12]. In the present studies we have examined the effects of specific hexoses on human red cell Ca^{2+}-ATPase activity in vitro, as well as the effects of tunicamycin and aminoguanidine on the action of glucose in this model.

Human erythrocyte ghosts were prepared hypotonically, as previously described [13], from red cells obtained from normal subjects. The membranes were stored at −70°C until used within 24–72 h. D-Glucose, L-glucose, D-fructose, D-galactose, D-mannose, 3-*O*-methylglucose, 2-deoxyglucose, D-ribose, sorbitol, *myo*-inositol, aminoguanidine, ouabain, malachite green and tunicamycin were obtained from Sigma (St. Louis, MO).

Enzyme activity was measured by Na_2ATP hydrolysis as previously described [13] and enzyme activity expressed as μmol inorganic phosphate (P_i) liberated per mg membrane protein per 90 min assay time. Membrane protein was measured by the Lowry method [14] and P_i was quantitated by the malachite green method [15]. Membrane enzyme assays were conducted on duplicate samples and data presented reflect means ± S.E. of two or more separate experiments. Intra-assay and inter-assay coefficients of variation were 2 and 3%, respectively.

To study the effects of hexoses, aminoguanidine and tunicamycin on membrane Ca^{2+}-ATPase activity in

Correspondence to: P.J. Davis, Department of Medicine, A-57, Albany Medical College, Albany, NY 12208, USA.

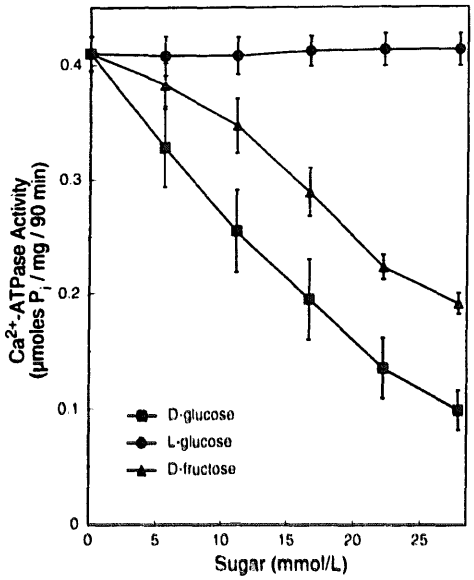

Fig. 1. Inhibition of erythrocyte membrane Ca^{2+}-ATPase activity by glucose and fructose. Erythrocyte membranes were incubated for 1 h at 37°C with D-glucose, L-glucose and D-fructose at the concentrations shown and then assayed for Ca^{2+}-ATPase activity.

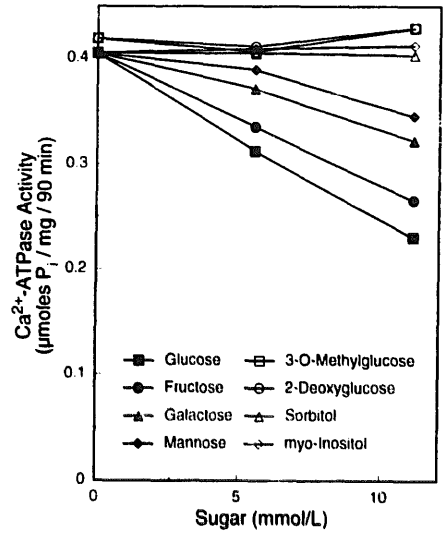

Fig. 2. Effect of sugar and sugar alcohols on erythrocyte membrane Ca^{2+}-ATPase activity. Erythrocyte membranes were incubated for 1 h at 37°C in the presence of the sugar and sugar alcohols shown at concentrations of 0, 5.5 or 11 mmol/l and assayed for Ca^{2+}-ATPase activity.

vitro, membranes were preincubated for 1 h at 37°C with various concentrations of the specific sugars or other reagents prior to enzyme assay. The buffer for these incubations was 10 mmol/l Tris-HCl (pH 7.4), as used in the ATPase assay. Control incubations of membranes were carried out in Tris-HCl.

Red cell Na^+/K^+-ATPase activity was measured as the difference in hydrolysis of Na_2ATP in the presence and absence of ouabain (1 mmol/l), as previously described [16].

As shown in Fig. 1, D-glucose in a concentration-dependent manner (5.5–27.5 mmol/l) progressively reduced membrane Ca^{2+}-ATPase activity. The reduction at 27.5 mmol/l was 75%, with 50% inhibition seen at 12 mmol/l. Washout of glucose after an preincubation period of 1 h, prior to enzyme assay incubation, did not restore enzyme activity (results not shown). Addition of 27.5 mmol/l D-glucose to membranes immediately prior to enzyme assay (i.e., with no glucose preincubation, but with glucose carried through the membrane ATPase assay) resulted in a decrease in enzyme activity of only 15%. In contrast to the D-glucose effect, L-glucose had no effect on membrane enzyme activity (Fig. 1). D-Fructose was slightly less potent than D-glucose in decreasing Ca^{2+}-ATPase activity, achieving at 27.5 mmol/l a 50% reduction in enzyme activity (Fig. 1).

3-*O*-Methylglucose, 2-deoxyglucose, sorbitol and *myo*-inositol at concentrations of up to 11 mmol/l had no effect on human red cell Ca^{2+}-ATPase activity (Fig. 2). D-Galactose and mannose at 11 mmol/l induced 20 and 15% decreases, respectively, in enzyme activity.

The inhibitory effect of glucose (11 mmol/l) on membrane Ca^{2+}-ATPase activity was prevented by coincubation of membranes with 12 pmol/l tunicamycin (Fig. 3, left panel). Aminoguanidine (10 mmol/l) did not antagonize the D-glucose effect on the ATPase (Fig. 3, right panel).

Incubations of human erythrocyte membranes with D-glucose, D-fructose, galactose and mannose did not affect Na^+/K^+-ATPase activity (results not shown).

Schaefer et al. [8] and others [7,9] have reported that red cells obtained from chronically hyperglycemic diabetic patients have decreased membrane Ca^{2+}-ATPase activity. We [10] and Gonzalez Flecha et al. [7]

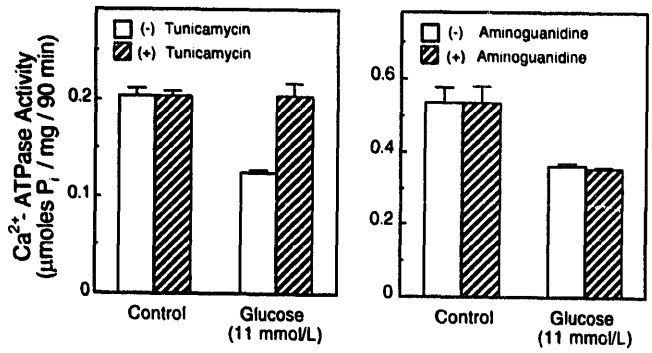

Fig. 3. Protection by tunicamycin and lack of protection by aminoguanidine of erythrocyte membrane Ca^{2+}-ATPase activity against inhibition by glucose. Membranes were incubated in buffer containing 0 or 11 mmol/l D-glucose in the absence or presence of 12 pmol/l tunicamycin (left panel) or 10 mmol/l aminoguanidine (right panel). After incubation for 1 h at 37°C, the membranes were assayed for Ca^{2+}-ATPase activity.

postulated that this decrease in Ca^{2+}-ATPase (calcium pump) activity would promote increased intracellular concentrations of Ca^{2+}. Resnick et al. have confirmed that Ca^{2+} content is indeed increased in red cells from hyperglycemic diabetic patients [11], as well as in normal human erythrocytes exposed in vitro to increased glucose concentrations [12]. This suggests that the glucose effect on Ca^{2+}-ATPase activity [7–10] has physiologic relevance. There was no hexose effect on membrane Na,K-ATPase activity, although hyperglycemia in vivo can elevate activity of this enzyme [10]. The in vivo observations of decreased Ca^{2+}-ATPase activity in the setting of hyperglycemia by Schaefer et al. [8] and others [7,11] did not provide a mechanism by which the enzyme inhibition might occur. The glucose effect on ATPase might be expressed at the genomic level [17], for example, were erythroid precursors to be exposed to high ambient glucose concentrations. Our previous studies, however, indicated that transient hyperglycemia in normal subjects promoted short-term, reversible decreases in Ca^{2+}-ATPase activity [10]. Further, incubation of membranes of mature red cells obtained from healthy subjects with D-glucose in vitro has led to substantially reduced Ca^{2+}-ATPase activity [7,10], indicating that the in vivo effect of glucose is largely, if not entirely, exerted at the plasma membrane. Because the red cell membrane exposed in vivo to glucose is unresponsive in vitro to the calmodulin Ca^{2+} complex [10,18], the endogenous activator of Ca^{2+}-ATPase [19], the glucose effect on the latter may involve the site on the enzyme that interacts with calmodulin.

The present studies are remarkable in their documentation of the stereo specificity of the hexose effect, the lack of effect of aminoguanidine on the action of glucose on Ca^{2+}-ATPase and the confirmation that tunicamycin antagonizes the effect of D-glucose on the enzyme. D- and L-glucose are equally effective in promoting glycation. The fact that L-glucose is ineffective in the Ca^{2+}-ATPase model indicates that nonenzymatic glycosylation (glycation) is in fact not the mechanism of the action of D-glucose on the ATPase. Further, aminoguanidine inhibits protein glycation in several experimental models [20–22], but was ineffective in vitro in the current studies of Ca^{2+}-ATPase activity. In results not included here, we have shown that two other inhibitors of glycation, sodium salicylate and acetylsalicylic acid [23] (10^{-8} to 10^{-4} M), were also ineffective in preventing the in vitro action of glucose (11 mM) on red cell Ca^{2+}-ATPase activity. The absence of influence of nonmetabolizable sugars – 3-*O*-methylglucose and 2-deoxyglucose – on the Ca^{2+}-ATPase activity in the present studies indicates either that hexose metabolism is required for inhibition by sugars of Ca^{2+}-ATPase or, more likely, that the 2- and 3-carbons in hexoses are critical to the effect.

Tunicamycin antagonizes the D-glucose effect and is acknowledged in intact cells to be an inhibitor of enzymatic glycosylation [24], usually, glycosylation of nascent proteins which are being processed for secretion or insertion in membranes. We postulate that even in the broken cell system we have studied, tunicamycin is acting to inhibit the glycosylation of a specific protein – either Ca^{2+}-ATPase or an associated moiety critical to enzyme activation.

Extrapolation of these observations in red cells to other tissues is incomplete. However, rabbit myocardial sarcoplasmic reticulum (SR) Ca^{2+}-ATPase activity is subject to inhibition by D-glucose (Davis, F.B., Warnick, P.B. and Davis, P.J., unpublished observations).

Acknowledgement

This work was supported in part by Veterans Administration Merit Review funding (P.J.D.).

References

1 Brownlee, M., Cerami, A. and Vlassara, H. (1988) New. Engl. J. Med. 318, 1315–1321.
2 Krantz, S., Lober, M. and Henschel, L. (1986) Exp. Clin. Endocrinol. 88, 257–269.
3 Garlick, R.L., Bunn, H.F and Spiro, R.G. (1988) Diabetes 37, 1144–1150.
4 Brownlee, M., Vlassara, H. and Cerami, A. (1984) Ann. Intern. Med. 101, 527–537.
5 Kowluru, R.A., Heidorn, D.B., Edmondson, S.P., Bitensky, M.W., Kowluru, A., Downer, N.W., Whaley, T.W and Trewhella, J. (1989) Biochemistry 28, 2220–2228.
6 Makita, Z., Radoff, S., Rayfield, E.J., Yang, Z., Skolnick, E., Delaney, V., Friedman, E.A., Cerami, A. and Vlassara, H. (1991) New Engl. J. Med. 325, 836–842.
7 Gonzalez Flecha, F.L., Bermudez, M.C., Cedola, N.V., Gagliardino, J.J. and Ross, J.P. (1990) Diabetes 39, 707–711.
8 Schaefer, W., Priessen, J., Mannhold, R. and Gries, F.A. (1987) Klin. Wochenschr. 65, 17–21.
9 Zemel, M.B., Bedford, B.A., Zemel, P.C., Marwah, O. and Sowers, J.R. (1988) J. Hypertens. 6 (Suppl.), S228–S230.
10 Davis, F.B., Davis, P.J., Nat, G., Blas, S.D., MacGillivray, M., Gutman, S. and Feldman, M.J. (1985) Diabetes 34, 639–646.
11 Resnick, L.M., Gupta, R.K., Bhargava, K.K., Gruenspan, H., Alderman, M.H. and Laragh, J.H. (1991) Hypertension 17, 951–957.
12 Resnick, L.M., Barbagallo, M., Gupta, R.K and Laragh, J.H. (1991) Proceedings of the 45th Annual Fall Conference of the Council for High Blood Pressure Research, American Heart Association, Chicago, September 24–27, Abstract 85.
13 Davis, P.J and Blas, S.D. (1981) Biochem. Biophys. Res. Comm. 99, 1073–1080.
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
15 Chan, K.M., Delfert, D. and Junger, K.D. (1986) Anal. Biochem. 157, 375–380.
16 Davis, P.J. and Bernardis, L.L. (1984) Metabolism 33, 591–595.
17 Cerami, A., Vlassara, H. and Brownlee, M. (1988) Diabetes Care 11 (Suppl. 1), 73–79.
18 Schaefer, W., Beeker, J. and Gries, F.A. (1988) Klin. Wochenschr. 66, 443–446.

19 O'Neil, K.T and DeGrado, W.F. (1990) Trends Biochem. Sci. 15, 59–64.
20 Brownlee, M., Vlassara, H., Kooney, A., Ulrich, P. and Cerami, A. (1986) Science 232, 1629–1632.
21 Lewis, B.S and Harding, J.J. (1990) Exp. Eye. Res. 50, 463–467.
22 Khatami, M., Suldan, Z., David, I., Li, W. and Rockey, J.H. (1988) Life Sci. 43, 1725–1731.
23 Yue, D.K., McLennan, S., Handelsman, D.J., Delbridge, L., Reeve, T. and Turtle, J.R. (1984) Diabetes 33, 745–751.
24 Kuo, S.C. and Lampen, J.O. (1974) Biochem. Biophys. Res. Comm. 58, 287–292.