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Hepatocyte Anoxic Injury Is Prevented by High Concentration of Fructose

A. Gasbarrini, A.B. Borle, H. Farghali, P. Caraceni, S. Fagiuoli, A. Azzarone, G. Zetti, T.E. Starzl, and D.H. Van Thiel

HUMAN livers can only be stored for a short time before transplantation, thus a better understanding of potential pharmacologic and nutritional strategies that might protect the liver against anoxic damage may be important clinically. Fructose has been shown recently to prevent liver damage induced by warm ischemia as assessed by lactate dehydrogenase (LDH) release.[1] To determine whether fructose protects the liver from anoxic damage by production of glycolytic adenosine triphosphate (ATP), and whether an elevation of cytosolic free calcium (Ca_i^{2+}) is involved in anoxic liver cell injury, the intracellular ATP level in real time assessed by ^{31}P nuclear magnetic resonance (NMR) spectroscopy and intracellular free calcium determinations with aequorin using freshly isolated rat hepatocytes was studied. Lactate dehydrogenase release was measured to monitor liver cell damage.

MATERIALS AND METHODS

Hepatocytes

Freshly isolated hepatocytes were prepared from adult male Sprague-Dawley rats weighing between 200 and 250 g. The cells were isolated from fed animals with collagenase (Sigma type IV, Sigma Chemicals, St Louis, Mo) using a previously described perfusion method.[2] Cell viability assessed by trypan blue exclusion averaged 90% and was never less than 85%. The cells were imbedded in agarose gel threads and perfused at a rate of 0.6 mL/min with standard Krebs-Henseleit bicarbonate buffer (KHB) at 37°C. During the 1-hour control period, the KHB perfusate contained 1.3 mmol/L Ca^{2+}, 5 mmol/L glucose, and it was exposed to a gas phase of 95% O_2–5% carbon dioxide (CO_2). Anoxia was induced by saturating the perfusate for 2 hours with 95% N_2–5% CO_2. During the period of anoxia the medium contained glucose or fructose. After 2 hours of anoxia, reoxygenation was initiated by perfusing the cells with KHB containing glucose and 1.3 mmol/L Ca^{2+} saturated with 95% O_2–5% CO_2.

Intracellular ATP

Intracellular ATP was measured by ^{31}P-NMR spectroscopy in real time.[3] The ATP content was expressed as the ratio of the βATP peak to the peak of the external standard methylene diphosphonic acid contained in a sealed spherical glass bulb positioned within the agarose threads.

Cytosolic Ionized Calcium

Ca_i^{2+} was measured using the Ca^{2+}-sensitive photoprotein aequorin[4] incorporated into the hepatocytes by gravity-loading. The aequorin loaded cells were imbedded in agarose gel threads, placed in the cuvette of an aequorin luminescence photometer, and perfused at a rate of 0.6 mL/min with KHB at 37°C, as previously described.[4]

Lactate Dehydrogenase

Cell injury was monitored by measuring LDH release from the cells into the effluent perfusate before, during, and after the anoxic period. Because the concentration of perfused cells imbedded in the gel threads varied slightly between individual experiments, LDH release was expressed as the percent increase over the control value measured during the 1-hour control period of each experiment.

RESULTS

During the first hour of anoxia, ATP decreased faster and to a lower level with fructose than with glucose in the medium. On the other hand, the intracellular ATP concentration of the fructose group progressively increased during the second hour of anoxia. In contrast, the ATP content of the glucose exposed group continuously declined. The major surge in Ca_i^{2+} that occurred during anoxia was depressed 52% when glucose was replaced by fructose: Ca_i^{2+} reached only 0.7 ± 0.2 μmol/L instead of 1.45 ± 0.42 μmol/L ($P < .01$). Fructose did not eliminate the early transient increase in Ca_i^{2+} which is released from the mitochondria. Fructose completely prevented the release of LDH that occurs with anoxia. Indeed, when the cells were perfused with KHB containing glucose, LDH release increased sixfold within 2 hours of anoxia. In contrast, in the presence of fructose, LDH release declined 30%.

DISCUSSION

These results confirm the earlier reports of other investigators who found that the presence of high concentrations of fructose during anoxia protects liver cells against anoxic injury.[1] In the present experiments, fructose completely abolished the release of LDH in anoxic liver cells. However, the beneficial effect of fructose, during the first hour of anoxia, was not a result of a maintenance of higher ATP levels as proposed by several investigators. Indeed, with fructose the intracellular ATP concentration decreased faster and to a lower level than occurred in hepatocytes perfused with glucose. On the other hand, during the

From the Departments of Surgery (A.G., H.F., P.C., S.F., A.A., G.Z., T.E.S., D.H.VT.) and Physiology (A.B.B.), University of Pittsburgh, Pittsburgh, Pennsylvania; and Patologia Medica I (A.G., P.C.), University of Bologna, Bologna, Italy.

Address reprint requests to A. Gasbarrini, MD, Patologia Medica I, Policlinico Sant'Orsola, Via Massarenti, 40100 Bologna, Italy.

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second hour of anoxia, the concentration of ATP within the fructose exposed cells increased to 40% of the control value, while it continued to decline in the glucose-exposed group. This increase in ATP concentration can only be explained by a glycolytic formation of ATP that contributes to the protection against anoxia provided by fructose during the second hour of the 2 hours of experimental anoxia. Furthermore, fructose abolished the massive increase in Ca_i^{2+} that occurred during the second hour of anoxia which arises from extracellular source and is responsible for the activation of Ca^{2+}-sensitive degradative enzymes that produce liver cell death.

REFERENCES

1. Anundi I, King J, Owens DA, et al: Am J Physiol 253:G390, 1987

2. Studer RK, Borle AB: J Biol Chem 257:7987, 1982

3. Malloy CR, Cunnigham CC, Radda GK: Biochem Biophys Acta 885:1, 1985

4. Borle AB, Snowdowne KW: Methods Enzymol 124:90, 1986