ASPECTS OF HEPATIC HYPOXIA: OBSERVATIONS ON THE ISOLATED, PERFUSED PIG LIVER*

NIELS TYGSTRUP, M.D.

Division of Hepatology
Medical Department A and Surgical Department C
Rigshospitalet

Department of Clinical Physiology
Kommunehospitalet

Copenhagen, Denmark

THE liver represents only 2% of the body mass and consumes about 25% of the total resting oxygen uptake. Yet hypoxia is not considered to be a significant cause of clinical liver damage. This may be either because hypoxic liver damage is difficult to detect or because the human liver is relatively resistant to hypoxia.

Reduction of the hepatic oxygen supply to about 50% by clamping the hepatic artery[1] was not found to reduce the hepatic oxygen uptake in man, but the oxygen tension of the hepatic venous blood became so low (about 20 mm. Hg) that further reduction in supply presumably is incompatible with normal oxidative metabolism. Also, reduction of the hepatic inflow via the portal vein by means of vasopressin failed to affect hepatic oxygen uptake.[2] There is little doubt, however, that physiologic regulatory mechanisms exist. Thus, a sojourn at high altitude (3,550 m.) was accompanied by a significant rise in splanchnic blood flow as determined by an indirect method.[3]

The era of transplantation has renewed interest in the effect of hypoxia on the liver because the transport of the liver from the donor to the recipient inevitably involves a period of anoxia which may determine the viability of the organ.[4] The isolated, perfused liver offers good opportunities to study the metabolic effects of hypoxia more closely because the supply of oxygen can be predetermined

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within a wide range and because metabolic interactions with the body are avoided. However, the perfused liver usually will have been exposed to some degree of hypoxia during and after its removal from the donor animal and before the perfusion is established; therefore normal control values cannot be obtained. Further, the perfusion does not represent strictly physiologic conditions, even if flow, temperature, pressure, etc. are kept within physiologic limits.[5] For example, the suspension of the liver in the body, which may be important for the homogeneous distribution of flow in sinusoids, cannot be imitated completely.[6]

The Normal Oxygen Metabolism of the Isolated, Perfused Liver

The oxygen uptake of the isolated, perfused liver is smaller than that of the liver *in vivo*, as was first demonstrated by Lundsgaard,[7] who also showed that it could be restored to normal by cross-perfusion with the hind part of the animal. In our experiments with isolated perfused porcine livers the oxygen uptake was 1.3 mmol./min./kg. (standard deviation [SD] = 0.34) and it was almost twice as high (2.5 mmol./min./kg. liver [SD = 1.0]) when the isolated liver was cross-perfused with a hepatectomized pig or with a human patient in acute hepatic failure.[8] This, together with the fact that the ratio of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) in the isolated liver is similar to that of the pig liver *in vivo*,[5] may mean that the low uptake of oxygen is not primarily a result of impaired function. It is more likely that the oxygen requirements of the isolated organ are diminished, perhaps because some oxygen-consuming processes slow down or stop when the requirements of the body no longer have to be satisfied. These processes, which are of great theoretical and practical interest because they may represent the truly essential functions of the liver, have not been identified. It is known, however, that loading with certain substrates such as fructose[9] and probably also ammonia and certain amino acids may increase the uptake of oxygen by the isolated liver. We therefore think that the isolated liver may be a suitable model for the study of hepatic hypoxia.

Hypoxia in the Isolated, Perfused Pig Liver

The liver of the pig was chosen for several reasons. The function of this preparation is of interest because of its use as a hepatic adjuvant

in clinical liver failure[10-14] and because the porcine liver generally is believed to resemble the human liver metabolically. Technically, it is preferable to the liver of smaller animals because frequent sampling of perfusate and tissue may be performed without disturbing the perfusion. The main drawback of the preparation is its cost in equipment, manpower, and animals.

Experimental hypoxia was obtained by aeration of the perfusate with air mixtures containing 21 to 0% of oxygen, 5% of carbon dioxide, and nitrogen (74 to 95%).

Oxygen Uptake and Adenine Nucleotides

In most cases a normal oxygen uptake was maintained until the hepatic venous oxygen tension fell below 30 mm. Hg, and below this level the relation was almost linear. ATP fell more gradually, and there probably is a positive intercept with the ordinate: i.e., the ATP concentration does not fall to zero. The concentration of ADP was independent of the hepatic venous oxygen tension, but the concentration of AMP rose steeply at oxygen tensions below 30 mm. Hg in the hepatic venous blood. Thus, both adenine nucleotide concentrations and the uptake of oxygen pointed to the same limit between a normoxic and hypoxic state.

The relation between the adenine nucleotides is largely determined by adenylate kinase (Enzyme Commission classification: 2.7.4.3.) which catalyzes the reaction 2 ADP $\rightleftarrows$ ATP + AMP, and a decrease in ATP accordingly will cause an increase in adenosine monophosphate (AMP) if the total concentration of adenine nucleotide and the equilibrium of the adenylate kinase reaction is maintained. It has been estimated that the relative changes in the concentration of ATP, ADP, and AMP during hypoxia might be accounted for by an equilibrium constant of the kinase system of 0.8.

The relatively large increase in the concentration of AMP resulting from this mechanism may be physiologically important in two respects. First, it delays the depletion of ATP during hypoxia (two ATP molecules provide three high-energy phosphate bonds). Second, it may enhance the metabolic reactions to hypoxia because AMP is a potent regulator of many processes (e.g., stimulation of glycolysis and inhibition of gluconeogenesis).

Metabolic Rates During Hypoxia

Metabolic rates presumably give a more relevant picture of the metabolic effects of hypoxia. The studies of our group have been concerned mainly with the uptake of galactose, which has been found to follow Michaelis-Menten kinetics.[15] The galactose-elimination capacity (an approximation to Vmax., i.e., the maximum metabolic rate during complete saturation with galactose) can be measured by the administration of physiological amounts of galactose.

The galactose-elimination capacity also was found to be sensitive to hypoxia, but the fall was not parallel to the reduction in the concentration of ATP. Normal galactose elimination was maintained when the hepatic venous oxygen tension was above 20 to 25 mm. Hg; at lower oxygen tensions it fell more steeply than the concentration of ATP. As a result of this, the relation between the galactose-elimination rate and ATP concentration was curvilinear. However, the relation between the reciprocals of these variables was linear within the analytical error of their estimates, and the observations therefore are compatible with a Michaelis-Menten relation. Since both galactose and ATP are substrates of the first step in hepatic galactose metabolism: i.e., the formation of galactose-1-phosphate, this would appear to be the simplest interpretation, although numerous other mechanisms are conceivable.

The estimates of K_m (i.e., the concentration where the elimination rate is one half of the elimination capacity) with respect to ATP derived from hypoxic livers with unsaturated galactose metabolism were not significantly different.[16] The data thus are compatible with the sequential, bisubstrate mechanism for the galactokinase reaction, as demonstrated *in vitro* by Ballard.[17]

The metabolic parameters may be assessed from plots of the intercepts and slopes of the curves. The absolute Vmax of galactose assessed in this manner was about four times greater than the galactose-elimination capacity, as determined during physiologic conditions. In other words, there appears to be an excess galactokinase activity in the liver. The K_m of the process in respect to ATP was much higher than the concentration of ATP. This may have a physiologic significance by depressing phosphorylation of galactose when ATP is low, conserving available ATP for more essential processes.

During aeration of the perfusate with oxygen-free air the inhibition of galactose elimination was stronger than could be accounted for by the low concentration of ATP. This may be of special interest as an indicator of hypoxic damage: i.e., structural and functional changes which cannot be ascribed to regulatory or adaptive mechanisms.

In conclusion, hepatic metabolism adapts well to hypoxia, and this is in agreement with the clinical impression that the liver is relatively resistant to hypoxic damage. The ultimate goal, to quantify the tolerance of the liver to hypoxia, is still far ahead, however. To attain this, a large number of the numerous metabolic processes of the liver must be studied under similar conditions and precise definitions of structural and functional hepatic damage must be formulated.

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