REFERENCES

1. Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. Am J Clin Nutr 1974;27:681-687.
2. Halliday JW, Powell LW. Ferritin metabolism and the liver. In: Powell LW, ed. Metals and the liver. New York: Marcel Dekker Inc. 1978:53-91.
3. Tsunetomi S, Ohto M, Iino Y, Shinagawa T, Kimura K, Morita M, Saisho H, et al. Diagnosis of small hepatocellular carcinoma by computed tomography: correlation of CT findings and histopathology. J Gastroenterol Hepatol 1989;4(5):395-404.
4. Bradbear RA, Bain C, Siskind V, Schofield FD, Webb S, Axelsen EM, Halliday JW, et al. Cohort study of internal malignancy in genetic hemochromatosis and other chronic non-alcoholic liver diseases. J Nat Canc Inst 1985;75:81-84.
5. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in non-cirrhotic patients with primary hemochromatosis. N Engl J Med 1985;313:1256-1262.
6. Ebara M, Ohto M, Shinagawa T, Sugiura N, Kimura K, Matsutani S, Morita M, et al. Natural history of minute hepatocellular carcinoma smaller than three centimeters complicating cirrhosis: a study in 22 patients. Gastroenterology 1986;90:289-298.

INTRACELLULAR ACIDOSIS: CAN IT DELAY THE INEVITABLE?

Gores G, Nieminen A-L, Wray BE, Herman B, Lemasters JJ. Intracellular pH during "chemical hypoxia" in cultured rat hepatocytes: protection by intracellular acidosis against the onset of cell death. J Clin Invest 1989;83:386-396.

ABSTRACT

The relationships between extracellular pH (pH_o), intracellular pH (pH_i), and loss of cell viability were evaluated in cultured rat hepatocytes after ATP depletion by metabolic inhibition with KCN and iodoacetate (chemical hypoxia). pH_i was measured in single cells by ratio imaging of 2',7'-biscarboxy-ethyl-5,6-carboxyfluorescein (BCECF) fluorescence using multiparameter digitized video microscopy. During chemical hypoxia at pH_o of 7.4, pH_i decreased from 7.36 to 6.33 within 10 min. pH_i remained at 6.1-6.5 for 30-40 min. (plateau phase). Thereafter, pH_i began to rise and cell death ensued within minutes, as evidenced by nuclear staining with propidium iodide and coincident leakage of BCECF from the cytoplasm. An acidic pH_o produced a slightly greater drop in pH_i, prolonged the plateau phase of intracellular acidosis, and delayed the onset of cell death. Inhibition of Na^+/H^+ exchange also prolonged the plateau phase and delayed cell death. In contrast, monensin or substitution of gluconate for Cl^- in buffer containing HCO_3^- abolished the pH gradient across the plasma membrane and shortened cell survival. The results indicate that intracellular acidosis after ATP depletion delays the onset of cell death, whereas reduction of the degree of acidosis accelerates cell killing. We conclude that intracellular acidosis protects against hepatocellular death from ATP depletion, a phenomenon that may represent a protective adaptation against hypoxic and ischemic stress.

COMMENTS

Hepatocytes possess membrane transport processes, such as Na^+-H^+ exchange (1) and Na^+-HCO_3^- cotransport (2), that maintain intracellular pH (pH_i) at levels well above that predicted by the Nernst equation. Numerous studies in other epithelia have demonstrated a relationship between pH_i and cellular function. Acidic cytoplasmic conditions are typically associated with a quiescent or dormant cellular state and intracellular alkalinization frequently accompanies cellular growth and activation (3, 4). In contrast, the relationship between pH_i and the structural and functional abnormalities that occur during ischemic and hypoxic hepatocellular injury are less clear.

Previous studies that examined the relationship between extracellular pH (pH_o) and hepatocyte injury suggested that acidosis may exert a protective effect (5, 6). The article under discussion extends these findings with the technological benefit (via multiparameter digitized video microscopy) of serial measurements of pH_i in single cells. The advantages of this technique are readily apparent in the figures demonstrating the cellular localization of the probe used, 2',7'-biscarboxyethyl-5, 6-carboxyfluorescein (BCECF), to the cytosol. This fluorescent probe, introduced into the cytosol in the form of the lipid-soluble acetoxymethyl ester where it is then cleaved by cytoplasmic esterases was assumed, in previous work, to be trapped in the cytosol. The cellular localization of BCECF is now confirmed by this elegant study using additional probes specific for lysosomes, endosomes and mitochondria and sequential dissolution of fluorescence by increasing concentrations of detergents.

Hypoxia was induced by the administration of KCN and iodoacetate to inhibit oxidative phosphorylation and glycolysis, respectively, and thereby deplete cultured hepatocytes of ATP. Maneuvers that prolonged and/or increased intracellular acidosis protected against cell death during ATP depletion, including inhibition of Na^+-H^+ exchange by amiloride and by Na^+ replacement and exposure of hepatocytes to acidic buffer. Conversely, cell death was accelerated by maneuvers that prevented a decrease in pH_i. However, there are two potential disadvantages to this form of "chemical hypoxia." The rapid decline in intracellular ATP levels (95% decrease within 5 min) that is observed may not duplicate the decrease that occurs *in vivo* during ischemic liver injury. Furthermore, the effect of iodoacetate-induced protein thiol alkylation is not addressed.

The authors speculate that the effect of intracellular acidosis is a suppression of autolytic degradative processes. In support of this hypothesis they discuss unpublished observations of a similar protective effect of acidosis against cystamine-induced hepatotoxicity, an injury thought to involve activation of neutral proteases (7). Alterations in cellular Ca^{2+} homeostasis have also been proposed to play a major role in ischemic cell injury, as recently reviewed (8), and cystamine hepatotoxicity has been demonstated to be mediated not only by activation of a nonlysosomal proteolytic system but

also by an inhibition of Ca^{2+} efflux (7). Although not discussed, these findings may provide a unifying theory of hepatocellular injury that incorporates both pertubations in intracellular Ca^{2+} levels and pH_i.

Loss of plasma membrane integrity is an important feature in most schema of cell injury and several studies have correlated plasma membrane blebbing with hepatocellular injury (8). It is, therefore, somewhat disconcerting that the authors observed that acidic pH_i does not prevent cell surface blebbing, scored with time-lapse video recordings of cultured hepatocytes.

Finally, these findings, as well as those of previous work (6), may have important clinical implications for tissue preservation before orthotopic liver transplantation. If the conclusions of this work are accepted, then storage of donor livers in acidic media should enhance organ viability. However, it should be noted that the cold storage solution in current use, the "UW" solution, is of pH 7.4 and has been reported to extend appreciably the preservation of donor livers for transplantation (9). Would a lower pH be better?

RICHARD H. MOSELEY, M.D.
Veterans Administration Medical Center and University of Michigan Medical Center
Ann Arbor, Michigan 48109

REFERENCES

1. Renner EL, Lake JR, Persico M, Scharschmidt BF. Na^+-H^+ exchange activity in rat hepatocytes: role in regulation of intracellular pH. Am J Physiol 1989;256:G44-G52.
2. Gleeson D, Smith ND, Boyer JL. Bicarbonate-dependent and -independent intracellular pH regulatory mechanisms in rat hepatocytes: evidence of Na^+-HCO_3^- cotransport. J Clin Invest 1989; 84:312-321.
3. Busa WB, Nuccitelli R. Metabolic regulation via intracellular pH. Am J Physiol 1984;246:R409-R438.
4. Frelin C, Vigne P, Ladoux A, Lazdunski M. The regulation of the intracellular pH in cells from vertebrates. Eur J Biochem 1988; 174:3-14.
5. Bonventre JV, Cheung JY. Effects of metabolic acidosis on viability of cells exposed to anoxia. Am J Physiol 1985;249:C149-C159.
6. Gores GJ, Nieminen A-L, Fleishman KE, Dawson TL, Herman B, Lemasters JJ. Extracellular acidosis delays onset of cell death in ATP-depleted hepatocytes. Am J Physiol 1988;255:C315-C322.
7. Nicotera P, Hartzell P, Baldi C, Svensson SA, Bellomo G, Orrenius S. Cystamine induces toxicity in hepatocytes through the elevation of cytosolic Ca^{2+} and the stimulation of a nonlysosomal proteolytic system. J Biol Chem 1986;261:14628-14635.
8. Thomas CE, Reed DJ. Current status of calcium in hepatocellular injury. HEPATOLOGY 1989;10:375-384.
9. Kalayoglu M, Sollinger HW, Stratta RJ, Dalessandro AM, Hoffmann RM, Pirsch JD, Belzer FO. Extended preservation of the liver for clinical transplantation. Lancet 1988;1:617-619.

INCREASED ENDOGENOUS NEUROPEPTIDE LIGAND FOR BENZODIAZEPINE RECEPTORS IN HEPATIC ENCEPHALOPATHY

Rothstein JD, McKhann G, Guarneri P, Barbaccia ML, Guidotti A, Costa E. Cerebrospinal fluid content of diazepam binding inhibitor in chronic hepatic encephalopathy. Ann Neurol 1989;26:57-62.

ABSTRACT

The neuropeptide diazepam binding inhibitor (DBI) is an endogeneous allosteric modulator of gamma-aminobutyric acid (GABA) receptors at the benzodiazepine recognition site. Recent theories on the neurochemical cause for hepatic encephalopathy have implicated activation of inhibitory neurotransmitter GABA systems. In 20 patients with hepatic disease, blood and cerebrospinal fluid (CSF) levels of ammonia and amino acids were measured. As in previous studies there was a selective elevation of CSF amino acids as well as a correlation between CSF glutamine levels and encephalopathy. CSF DBI levels were maximally elevated 5-fold in patients with hepatic encephalopathy, but they were normal in those patients with liver disease not associated with changes in mental status and in patients with nonhepatic encephalopathy. Levels of DBI correlated with the clinical staging of hepatic encephalopathy. These data suggest that DBI may participate in the modulation of cerebral function in hepatic encephalopathy.

COMMENTS

The identification of specific receptors for the benzodiazepines in mammalian brain in the 1970s led to intensive efforts by several research groups to identify the endogenous neurohormone (or ligand) for these receptors. The 1980s saw an ever growing list of candidate compounds that included beta-carbolines, hypoxanthines, porphyrins, neuropeptides and many others. Benzodiazepine-like immunoreactivity has been found in human brain samples stored in paraffin since 1940, 10 yr before the first synthesis of a benzodiazepine and 20 yr before its introduction onto the pharmaceutical market. This finding has led to the suggestion that benzodiazepine receptor ligands may be synthesized *in situ* or may be of dietary origin (1).

In 1983, the polypeptide, diazepam binding inhibitor (DBI), which is capable of inhibiting 3-H-benzodiazepine binding, was isolated from rat brain (2). Behavioral pharmacological evaluation suggests that DBI acts as an inverse agonist of *central* benzodiazepine receptors, that is, brain benzodiazepine receptors that are functionally linked to the GABA-A receptor and chloride channels, producing anxiety and eliciting a proconflict response (i.e., a neurobehavioral test used in experimental animals to predict the anxiogenic or anxiolytic action of a drug). In the study of Rothstein et al. levels of DBI in cerebrospinal fluid (CSF) were measured in patients with hepatic encephalopathy (HE). A total of 20 patients were studied; 10 had biopsy-proven chronic active hepatitis, 7 had alcoholic cirrhosis, 2 had primary biliary cirrhosis and 1 had Wilson's disease. Thirteen of these patients had chronic recurrent encephalopathy; the remaining 7 patients had no signs of encephalopathy. The degree of encephalopathy was monitored both neurologically and by EEG. DBI content of CSF was found to be elevated threefold in patients with HE, and