HEPATOLOGY *Elsewhere*

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FURTHER INSIGHTS INTO SINUSOIDAL ORGANIC ANION UPTAKE

Min AD, Johansen KL, Campbell CG, Wolkoff AW. Role of chloride and intracellular pH on the activity of the rat hepatocyte organic anion transporter. J Clin Invest 1991;87:1496-1502.

ABSTRACT

Previous studies in cultured rat hepatocytes revealed that initial uptake of sulfobromophthalein (BSP) was markedly reduced upon removal of Cl^- from the medium. In the present study, unidirectional Cl^- gradients were established in short-term cultured rat hepatocytes and their effect on BSP uptake was determined. These investigations revealed that BSP uptake requires external Cl^- and is not stimulated by unidirectional Cl^- gradients, suggesting that BSP transport is not coupled to Cl^- transport. In contrast, BSP transport is stimulated by an inside-to-outside OH^- gradient, consistent with OH^- exchange or H^+ cotransport. As the presence of Cl^- is essential for but not directly coupled to BSP transport, binding of ^{35}S-BSP to hepatocytes was determined at 4° C. This revealed an ~10-fold higher affinity of cells for BSP in the presence as compared to the absence of Cl^- (K_a + 3.2 ± 0.8 vs. 0.42 ± 0.09 μM^{-1}; $P < 0.02$). Affinity of BSP for albumin was Cl^--independent, and was ~10% of its affinity for cells in the presence of Cl^-. These results indicate that extracellular Cl^- modulates the affinity of BSP for its hepatocyte transporter.

COMMENTS

In comparison with investigations aimed at explaining the mechanisms of hepatic bile acid uptake, the study of the transport of nonbile acid organic anions such as bilirubin across the sinusoidal membrane seems to be an arduous undertaking to the outside observer. Transport studies are hampered by the low water solubility and the strong tissue-binding capacity of bilirubin. Despite these difficulties, uptake of bilirubin and sulfobromophthalein (BSP) has been shown to have features compatible with carrier-mediated transport, and at least three distinct membrane carriers for bilirubin have been described (1, 2). Previous work has demonstrated that bilirubin and BSP uptake by short-term cultured rat hepatocytes and isolated perfused rat livers is markedly impaired by the substitution of Cl^- in the medium with gluconate or bicarbonate but is unaffected by the substitution of Na^+ with other inorganic cations (3). An inwardly directed chloride gradient also stimulated BSP transport in isolated rat liver sinusoidal membrane vesicles (4), although uptake of bilirubin diglucuronide does not exhibit chloride dependency (5). In this study the nature of this chloride effect is examined, and additional insights into sinusoidal bilirubin transport are offered.

After demonstrating that transient unidirectional Cl^- gradients could be established in cultured hepatocytes by virtue of the slow influx and efflux of this anion, BSP uptake in the presence of an inwardly directed Cl^- gradient was examined and found to be no different from uptake under control conditions. These results suggest that BSP transport is not coupled to chloride flux. Instead, extracellular Cl^- was shown to modulate the affinity of BSP binding to hepatocytes without, however, an effect on the affinity of albumin for BSP. Not examined is the effect of extracellular nitrate on BSP binding to hepatocytes, because enhanced uptake of BSP over even that observed with a chloride gradient was observed in the presence of an inwardly directed nitrate gradient in this and previous work (3) but not in studies using sinusoidal membrane vesicles (4). In addition, the ability of iodide to adequately substitute for chloride was not discussed.

Besides addressing the chloride effect, additional features of BSP uptake were examined in this study. Although previous reports have suggested that BSP uptake is electrogenic (i.e., enhanced in the presence of a positive membrane potential) (4, 6), uptake of BSP in short-term cultured hepatocytes depolarized in a KCl medium was no different from uptake in a NaCl medium. However, perhaps the most intriguing finding was the demonstration that an outwardly directed OH^- gradient enhances BSP uptake. Although these results suggested that BSP uptake in part may be the result of H^+ cotransport or OH^- exchange, additional studies clearly are required. In sinusoidal membrane vesicles, an outwardly directed OH^- gradient stimulated BSP uptake, although uptake at equilibrium was also significantly greater, suggesting enhanced binding under these conditions (4). In fact, an outwardly directed H^+ gradient also stimulated BSP uptake without affecting equilibrium uptake values (4). In contrast, BSP has been shown to inhibit pH gradient-driven sulfate uptake by sinusoidal membrane vesicles (7), thereby lending support for the existence of a OH^-/BSP exchange mechanism.

In summary, this study demonstrated that BSP uptake does not involve, as initially suggested (3), either Cl^-/organic anion exchange or Cl^-/organic anion cotransport. Although the nature of the chloride effect on bilirubin transport has been partially clarified, the driving forces for nonbile acid organic anion uptake at the sinusoidal surface, however, remain incompletely defined. Functional reconstitution of the transport

protein involved into liposomes, as has been reported for the protein bilitranslocase (6) and work in progress at the molecular level, may be required for a better understanding of this critical hepatocellular function.

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REFERENCES

1. Berk PD, Potter BJ, Stremmel W. Role of plasma membrane ligand-binding proteins in the hepatocellular uptake of albumin-bound organic anions. HEPATOLOGY 1987;7:165-176.
2. Tiribelli C, Lunazzi GC, Sottocasa GL. Biochemical and molecular aspects of the hepatic uptake of organic anions. Biochim Biophys Acta 1990;1031:261-275.
3. Wolkoff AW, Samuelson AC, Johansen KL, Nakata R, Withers DM, Sosiak A. Influence of Cl^- on organic anion transport in short-term cultured rat hepatocytes and isolated perfused rat liver. J Clin Invest 1987;79:1259-1268.
4. Potter BJ, Blades BF, Shepard MD, Thung SM, Berk PD. The kinetics of sulfobromophthalein uptake by rat liver sinusoidal vesicles. Biochim Biophys Acta 1987;898:159-171.
5. Adachi Y, Roy-Chowdhury J, Roy-Chowdhury N, Kinne R, Tran T, Kobayashi H, Arias IM. Hepatic uptake of bilirubin diglucuronide: analysis by using sinusoidal plasma membrane vesicles. J Biochem 1990;107:749-754.
6. Sottocasa GL, Baldini G, Sandri G, Lunazzi G, Tiribelli C. Reconstitution *in vitro* of sulfobromophthalein transport by bilitranslocase. Biochim Biophys Acta 1982;685:123-128.
7. Hugentobler G, Meier PJ. Multispecific anion exchange in basolateral (sinusoidal) rat liver plasma membrane vesicles. Am J Physiol 1986;251:G656-G664.

YET ANOTHER ROLE FOR THE "GOOD" MATRIX PROTEIN: LAMININ IN REGENERATING LIVER

Martinez-Hernandez A, Delgado FM, Amenta PS. The extracellular matrix in hepatic regeneration: localization of collagen types I, III, IV, laminin, and fibronectin. Lab Invest 1991;64:157-166.

ABSTRACT

After partial hepatectomy, the liver is capable of complete regeneration, restoring normal hepatic size, architecture, and function. To study the role of the extracellular matrix in regeneration, the temporal and spatial sequence of deposition of several of its components, including collagen types I, III, and IV, laminin, and fibronectin, in rat liver, after an 80% hepatectomy, was characterized by light microscopy immunohistochemistry. A minimum of five animals were studied for each date. In agreement with previous reports, subsequent to 80% hepatectomy, there was a brisk mitosis of hepatocytes. The mitotic activity was maximal at 48 hours, primarily in the periportal and centrilobular zones, and resulted in the formation of hepatocyte clusters and widening of the hepatic plates. Of the extracellular matrix components studied, laminin was the one demonstrating the most dramatic changes. By 24 hours, laminin appeared in the hepatic sinusoids reaching a maximum staining intensity at 48 hours. Intracellular laminin was prominent in numerous nonparenchymal cells, with many having the morphology, location, and desmin content characteristic of Ito cells. Laminin staining decreased in the sinusoids at 4 days; however, some intracellular staining of Ito cells was present even at 8 days after hepatectomy. At the completion of regeneration, there was no evidence of any substantial change in the ratio: extracellular matrix/cell mass. The results indicate that: (a) hepatocytes can divide without prior removal of the subsinusoidal extracellular matrix; (b) during regeneration, hepatocyte division precedes sinusoidal formation; (c) during hepatic regeneration, and in spite of the presence of laminin in Ito cells, no basement membranes are formed; (d) the prominent expression of laminin and its proposed functions in morphogenesis suggest a critical role for this matrix component in the formation and reorganization of the regenerating liver.

COMMENTS

For most of us, the term hepatic regeneration brings to mind a tightly controlled process of liver cell growth. Proliferation of hepatocytes is certainly the most obvious aspect of regeneration, but it represents only one of a number of events that take place after liver injury or resection, all of which are essential to the restoration of functioning liver mass. One prerequisite for successful regeneration is that newly proliferated parenchymal and nonparenchymal cells must arrange themselves into hepatic lobules. Restructuring of the lobule does not occur automatically as liver cells proliferate; repair after liver injury can at times be disorganized, resulting in nodule formation. The program that controls hepatic regeneration, therefore, must contain specific information that permits restoration of normal lobular architecture.

Remodeling of the extracellular matrix may be the signal that facilitates lobular reorganization during liver regeneration. We know from cell culture experiments that the extracellular matrix, which comprises the reticulin framework of the liver, can actively influence the cells it supports (1, 2). The composition of the matrix appears to be of particular importance as a determinant of hepatocellular structure and differentiation (1, 2). Whether specific changes in matrix composition take place during regeneration has not previously been established.

Martinez-Hernandez and colleagues in this article have addressed this question by examining the fate of individual extracellular matrix proteins during hepatic regeneration. Using an immunohistochemical approach, they monitored the abundance and lobular distribution of several matrix elements, including (plasma) fibronectin; collagen types I, III and IV; and laminin, for 6 days after partial hepatectomy in the rat. All observations were made at the light microscopic level, with specific attention to the spatial relationships between individual matrix elements and proliferating liver cells.

The most striking observation in these experiments was that regeneration was accompanied by a pro-