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CHANGES IN SOME MARKER ENZYME ACTIVITIES OF LIVER PLASMA MEMBRANES DURING REGENERATION AFTER PARTIAL HEPATECTOMY IN RATS

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SUMMARY

Liver plasma membranes were isolated from regenerating rat livers between 20 h and 10 days after partial hepatectomy in order to study the effect of partial hepatectomy on some membrane enzyme activities. Mg^{2+}-ATPase (EC 3.6.1.4) activity, but not $(Na^+ + K^+)$-ATPase activity, decreased slightly at 2 days, whereas leucyl-β-naphthylamidase (EC 3.4.1.1) and 5'-nucleotidase (EC 3.1.3.5) activities increased considerably at 1–2 and 3–5 days, respectively. These changes were not parallel to a sharp increase in mitotic activity of liver cells which occurred at 36 h.

INTRODUCTION

Some enzyme activities and components of rat liver plasma membranes have been reported to change under various conditions, such as in hepatoma [1–4], in regenerating liver [5], after bile duct ligation [5], following the administration of carbon tetrachloride [6–9] and other hepatotoxins [10, 11], or in essential fatty acid deficiency [12]. As the change in membrane enzyme activity may be associated with the function of liver cells, we made an attempt to follow the marker enzyme activities of liver plasma membranes during rapid regeneration after a great reduction of liver mass by partial hepatectomy. The effects of hepatectomy on Mg^{2+}-ATPase, $(Na^+ + K^+)$-ATPase, 5'-nucleotidase and leucyl-β-naphthylamidase activities of plasma membranes are presented together with other indices of regeneration.

METHODS

Female rats of Sprague-Dawley strain, 8–9 weeks old, were used throughout. Partial hepatectomy was performed as aseptically as possible according to the method of Higgins and Anderson [13], with approximately 69 % of total liver weight being resected. Animals were decapitated at intervals between 20 h and 10 days after surgery, and the livers were perfused in situ with cold 0.9 % saline via a portal vein, to remove

blood components. Liver plasma membranes were prepared basically according to the procedure described by Neville [14], except that instead of a discontinuous gradient, a linear continuous gradient of 25 ml of from 46.8 to 27.4 % sucrose was made using a Hitachi DGK-U automatic density gradienter. Plasma membrane fractions from normal and regenerating livers floated as a packed layer at density 1.165–1.170. The isolated membrane fraction was washed twice with 1 mM $NaHCO_3$ solution, suspended in 20 mM Tris/acetate buffer (pH 7.5), kept in ice and assayed for the enzyme activities within 1 day. Mg^{2+}- and $(Na^+ + K^+)$-ATPase and 5'-nucleotidase activities were measured at 37 °C in assay systems with the ionic composition described by Emmelot et al. [5]. Leucyl-β-naphthylamidase was assayed at 37 °C by the method of Goldbarg and Rutenburg [15]. Membrane protein was determined by the Lowry procedure [16] using crystalline bovine serum albumin as standard. DNA was determined by the method of Burton [17] after extraction by the method of Schneider [18]. Tissue protein was estimated by the biuret method after solubilization of the DNA-extracted residue with deoxycholate in alkaline solution. A portion of the liver was fixed in 10 % formalin, and paraffin sections were stained with hematoxylin and eosin to determine the number of mitoses per 1000 parenchymal cells. Each experiment was always paralleled with a normal or a sham-operated group. The values obtained from the sham-operated groups were within the normal range.

RESULTS AND DISCUSSION

Fig. 1 shows the changes in wet weight, protein and DNA content, and mitotic activity of the liver after partial hepatectomy. Liver weight increased linearly for 5

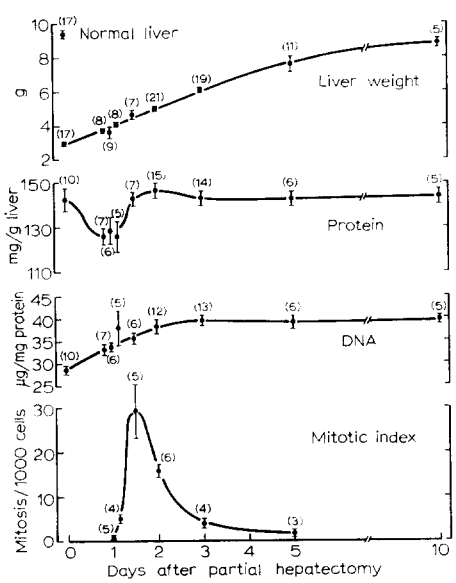

Fig. 1. Changes in wet weight, protein and DNA content, and mitotic index of the liver during regeneration after partial hepatectomy in rats. Points represent means ±S.E.M. (vertical lines). Figures in parentheses indicate the number of animals.

days, recovering approximately 80 % of the original weight. A slight protein content decrease at about 24 h may be attributed to the swelling of cells. A continuous increment in DNA content was observed between 20 h and 3 days and DNA content remained elevated after 10 days. Mitotic activity began to rise at 28 h, reached a peak at 36 h and then rapidly decreased.

Changes in marker enzyme activities of the membrane fraction are summarized in Fig. 2. Mg^{2+}-ATPase activity decreased slightly but significantly at 2 days. ($Na^+ + K^+$)-ATPase, however, was not affected. 5′-Nucleotidase activity increased considerably at 3–5 days, returning to the normal level within 10 days. Leucyl-β-naphthylamidase activity was also elevated during an early period of regeneration: the activity increased by 24 h, reached a maximum value of approximately double the normal activity at 48 h and then returned to the normal range between 3 and 5 days.

Thus, during regeneration, these enzyme activities of the liver plasma membrane did not change with a constant proportion of enzyme activities similar to that found in normal plasma membranes, and it appears to take a certain period after cell division for all these activities to return to the normal range. In particular, leucyl-β-naphthylamidase activity had already increased before the mitotic activity started to increase, whilst 5′-nucleotidase activities increased after cell division had almost finished. Accordingly, the elevation of the former enzyme activity may be partly inducible independently of the initiation of cell proliferation, while that of the latter may be associated with cell proliferation or tissue organization.

Since there is a high concentration of these enzymes in the plasma membrane

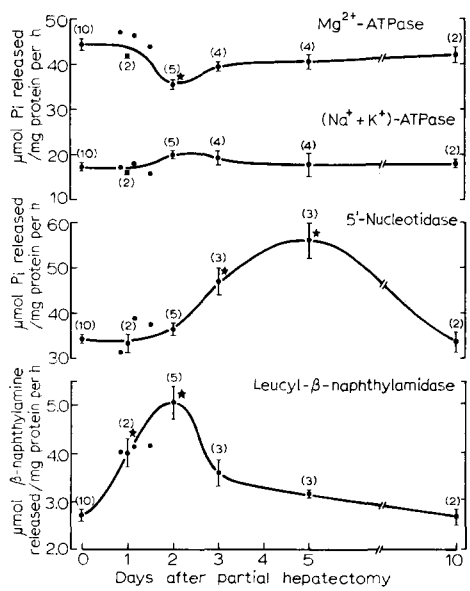

Fig. 2. Changes in Mg^{2+}- and ($Na^+ + K^+$)-ATPase, 5′-nucleotidase and leucyl-β-naphthylamidase activities of liver plasma membranes during regeneration after partial hepatectomy in rats. Points represent means ±S.E.M. (vertical lines). Figures in parentheses indicate the number of experiments. Marked (*) points are significantly different from the normal at $P < 0.01$.

lining bile canaliculi [19–22] and the plasma membrane fraction isolated by Neville's method is abundant in this portion of the membrane [3, 5, 14, 23], the changes in the enzyme activities appear to manifest the alteration of bile canalicular function during liver regeneration.

Although an electron microscopic study by Kimura [24] has revealed dilatation of bile canaliculi accompanying a disappearance of microvilli 2 days after partial hepatectomy, and a proliferation of Golgi apparatus close to the bile canalicular region at 5 days, and other studies [25, 26] have shown, in addition, that despite the great loss in liver mass, bile flow rate begins to increase 24 h after partial hepatectomy, it is still unknown how the changes of the activities of membrane enzymes tested in our experiments are related to these morphological and physiological changes.

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REFERENCES

1 Benedetti, E. L. and Emmelot, P. (1967) J. Cell Sci. 2, 499–512
2 Emmelot, P. and Bos, C. J. (1968) Biochim. Biophys. Acta 150, 354–363
3 Emmelot, P., Visser, A. and Benedetti, E. L. (1968) Biochim. Biophys. Acta 150, 364–375
4 Emmelot, P. and Bos, C. J. (1969) Int. J. Cancer 4, 705–722
5 Emmelot, P., Bos, C. J., Benedetti, E. L. and Rümke, P. (1964) Biochim. Biophys. Acta 90, 126–145
6 Masuda, Y., Kuchii, M., Yano, I., Yamamoto, H. and Murano, T. (1973) Jap. J. Pharmacol. 23, 627–637
7 Kuchii, M., Masuda, Y., Okada, N., Yamamoto, H. and Murano, T. (1973) Jap. J. Pharmacol. 23, 639–644
8 Le Page, R. N. and Dorling, P. R. (1971) Aust. J. Exp. Biol. Med. Sci. 49, 345–350
9 Kamath, S. A. and Rubin, E. (1974) Lab. Invest. 30, 494–499
10 Le Page, R. N. and Butler, W. H. (1967) Biochem. J. 105, 18–19p
11 Chandrasekhara, N. and Narayan, K. A. (1970) Cancer Res. 30, 2876–2880
12 Chandrasekhara, N. and Narayan, K. A. (1970) J. Nutr. 100, 477–480
13 Higgins, G. M. and Anderson, R. M. (1931) Arch. Pathol. 12, 186–202
14 Neville, D. M. (1960) J. Biophys. Biochem. Cytol. 8, 413–422
15 Goldbarg, J. A. and Rutenburg, A. M. (1958) Cancer 11, 283–291
16 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
17 Burton, K. (1956) Biochem. J. 62, 315–323
18 Schneider, W. C. (1957) in Methods in Enzymology (Colowick, S. P. and Kaplan, N. O., eds.), Vol. 3, pp. 680–684, Academic Press, New York
19 Wachstein, M. (1963) in The Liver (Rouiller, C. H., ed.), Vol. 1, pp. 137–194, Academic Press, New York
20 Gurd, J. W. and Evans, W. H. (1974) Arch. Biochem. Biophys. 164, 305–311
21 Monis, B. (1965) Nature 208, 587–588
22 Novikoff, A. B. and Essner, E. (1962) Fed. Proc. 21, 1130–1142
23 Emmelot, P. and Bos, C. J. (1966) Biochim. Biophys. Acta 120, 369–182
24 Kimura, A. (1969) Sapporo Med. J. 35, 343–360
25 Leong, G. F., Pessotti, R. L. and Brauer, R. W. (1959) Am. J. Physiol. 197, 880–886
26 Klaassen, C. D. (1974) J. Pharmacol. Exp. Ther. 191, 25–31