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# A study of sex difference in enzyme activities of rat liver plasma membranes

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SUMMARY

The specific activity and enrichment of several "plasma membrane marker enzymes" have been found to be significantly lower in liver plasma membranes of female than of male rats. The lipid composition showed no comparable sex difference. In brain synaptosomal or myelin fractions the same enzyme activities have not been appreciably enriched, and no sex difference was apparent.

Emmelot and Bos[1] reported recently that the alkaline phosphatase activities of plasma membranes isolated from rat liver were sex dependent. Enzyme activities were significantly higher in males than in females, and could be manipulated accordingly by castration and sex hormone administration. Their observation represented direct evidence of sex difference for a plasma membrane enzyme. Its significance is readily evident when considering the use of enzyme markers in the characterization of plasma membrane preparations[2]. Tissue and species differences in enzyme activity patterns may well be compounded by sex dependence. It seemed important to us to obtain additional data concerning this observation. To assess tissue differences, we included assays also on subcellular fractions of brain tissue.

Young male and female Sprague–Dawley rats, 130–170 g, were fasted overnight prior to sacrifice. The animals were decapitated by guillotine, the brain and liver were rapidly removed and placed in ice-cold homogenization media. All the steps of membrane isolation procedures were carried out at 0–4 °C.

Liver plasma membrane fraction was isolated according to the method of

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Neville[3]. On the basis of our previous experience with this method[4], the procedure was carried through only the first density gradient centrifugation and the resulting float (Fraction 4) was used in the enzyme assays. This membrane fraction was washed in 1 mM $NaHCO_3$ by high speed centrifugation, then resuspended in the same medium and stored frozen in smaller aliquots until use. From the brain, synaptosomal and myelin fractions were isolated according to the method of Whittaker *et al.*[5] as modified by Colburn *et al.*[6]. The fractions were washed in 1 mM $NaHCO_3$ and handled in the same manner as the liver membranes.

The enzyme assays were carried out at the pH specified, usually on the first or second day after the membrane isolation. Leucyl-β-naphthylamidase (EC 3.4.1.1) (pH 7.4), 5'-nucleotidase (EC 3.1.3.5) (pH 9.0), and *p*-nitrophenylphosphatase (EC 3.1.3.1) (pH 9.0) activities were measured as described previously[4]. The latter assay was carried out both with and without $K^+$ in the incubation medium ($K^+$-*p*-nitrophenylphosphatase and *p*-nitrophenylphosphatase, respectively). Alkaline glycerol phosphatase (EC 3.1.3.1) was measured in the same incubation medium and under the same conditions as *p*-nitrophenylphosphatase. The glycerol phosphatase assay was carried out in a final volume of 1 ml; the final concentration of the substrate, β-glycerol phosphate (Sigma), was the same (20 mM) as in the conventional assay[7]. The activity was measured with and without $K^+$ in the medium as in the case of *p*-nitrophenylphosphatase. The reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid. After centrifugation at high speed, aliquots of the supernate were analyzed for liberated inorganic phosphate by the method of Ames and Dubin[8].

Protein was determined by the method of Lowry *et al.*[9]. The total lipid, total cholesterol and total phospholipid content was determined, after extraction in chloroform–methanol (2:1, v/v), as described previously[10].

The yield of the liver plasma membrane fraction was identical in males and in females, *i.e.* 0.55 mg membrane protein per g wet weight liver. The membrane fractions for both sexes were remarkably constant in all lipid components assayed (mg lipid per mg membrane protein) as summarized in Table I. However, in the whole liver homogenate, the total protein content was slightly lower and the total lipid content was about 23% higher in the females.

The enzymatic data obtained in these experiments are tabulated according to the enzyme activities measured. In liver plasma membrane preparations, the specific activities of all the enzymes measured were consistently and significantly higher in males than in females. In the liver homogenate, only the *p*-nitrophenylphosphatases showed similar sex difference. Consequently, for most enzymes, the relative specific activities in male plasma membranes were significantly higher than in females.

"Alkaline phosphatase" activities are presented in Table II. In female liver plasma membrane preparations the specific activity of alkaline glycerol phosphatase was only 45% of that in the males. The liver homogenates showed no sex difference at all. The relative specific activity of this enzyme is much lower than that of all the other enzymes studied, particularly in females.

TABLE I

PROTEIN AND LIPID CONTENT OF PLASMA MEMBRANES FROM RAT LIVER

All data represent averages from four separate preparations, using 5 or 10 rats per preparation.

| *Protein or lipid* | *Fraction* | *Males, average ± S.E.* | *Females* | |
|---|---|---|---|---|
| | | | *Average ± S.E.* | *% of male value** |
| Total protein (mg) / Liver (g wet wt) | Homogenate | 154 ± 3.64 | 135 ± 4.01 | 88 |
| | Plasma membrane | 0.55 ± 0.045 | 0.55 ± 0.054 | 100 |
| Total lipids (mg) / Protein (mg) | Homogenate | 0.22 ± 0.0065 | 0.27 ± 0.0064 | 123 |
| | Plasma membrane | 0.56 ± 0.0090 | 0.60 ± 0.028 | 107 |
| Phospholipids (mg) / Protein (mg) | Homogenate | 0.17 ± 0.0050 | 0.17 ± 0.0044 | 100 |
| | Plasma membrane | 0.39 ± 0.0019 | 0.41 ± 0.019 | 105 |
| Cholesterol (mg) / Protein (mg) | Homogenate | 0.013± 0.0012 | 0.018± 0.0006 | 138 |
| | Plasma membrane | 0.12 ± 0.0016 | 0.10 ± 0.0048 | 83 |
| Phospholipids (mole) / Cholesterol (mole) | Homogenate | 6.5 ± 0.76 | 4.8 ± 0.24 | 74 |
| | Plasma membrane | 1.6 ± 0.055 | 2.1 ± 0.13 | 131 |

*Average of male values is taken as 100% and the average of the female value is shown accordingly.

Alkaline *p*-nitrophenylphosphatase and alkaline $K^+$-*p*-nitrophenylphosphatase activities were very similar quantitatively. Therefore, only $K^+$-*p*-nitrophenylphosphatase data are shown in Table II. In both sexes, the relative specific activity for $K^+$-*p*-nitrophenylphosphatase was somewhat higher than for *p*-nitrophenylphosphatase, *i.e.* 19.9 *vs* 17.8 in males, 11.5 *vs* 9.7 in females.

The present findings confirm the observation of Emmelot and Bos[1] that "alkaline phosphatase" activities are significantly lower in liver plasma membranes of female than in male rats. Emmelot and Bos[1] found no sex difference in $K^+$-*p*-nitrophenylphosphatase activities, neither did they measure the enzyme activities in the liver homogenates. The present data indicate that there is a sex difference in both *p*-nitrophenylphosphatase and $K^+$-*p*-nitrophenylphosphatase activities of both the liver homogenate and plasma membrane fractions. Alkaline glycerol phosphatase activities were lower only in the plasma membrane fractions of females than males. The relative specific activity of glycerol phosphatase was significantly lower than that of $K^+$-*p*-nitrophenylphosphatase. These facts suggest that the "alkaline phosphatase" assay employing *p*-nitrophenyl phosphate as substrate provides a better enzyme marker for rat liver plasma membranes than does the one with glycerol phosphate.

Emmelot and Bos[1] reported that no sex dependence of 5′-nucleotidase was apparent from their data. The discrepancy between their and the present findings might be attributed to the fact that there was a pH and ion concentration difference in the assay conditions. They assayed at pH 7.2 in the presence of $K^+$ in contrast to pH 9.0 and

TABLE II

"ALKALINE PHOSPHATASE" ACTIVITIES OF LIVER PLASMA MEMBRANES FROM MALE AND FEMALE RATS

All data represent averages of four separate observations. Glycerol phosphatase: incubation medium contained $Mg^{2+}$. $K^+$-*p*-nitrophenylphosphatase: incubation medium contained $Mg^{2+}$ and $K^+$.

| *Enzyme assay* | *Fraction* | *Males* | | *Females* | | |
|---|---|---|---|---|---|---|
| | | *Spec. act.** ± S.E.* | *Rel. spec. act.*** | *Spec. act.** ± S.E.* | *Rel. spec. act.*** | *Spec. act. (% of male value***)* |
| Glycerol phosphatase | Homogenate | 0.423 ± 0.027 | | 0.416 ± 0.028 | | 98 |
| | Plasma membrane | 2.36 ± 0.480 | 5.6 | 1.07 ± 0.350 | 2.6 | 45 |
| $K^+$-*p*-nitrophenyl-phosphatase | Homogenate | 0.115 ± 0.010 | | 0.083 ± 0.011 | | 72 |
| | Plasma membrane | 2.29 ± 0.19 | 19.9 | 0.953 ± 0.21 | 11.5 | 42 |

* Specific activity is expressed in μmoles of substrate metabolized per mg protein per h.

** Relative specific activity = specific activity of plasma membrane/specific activity of homogenate.

***Specific activity of male membranes is taken as 100% and the specific activity of female membranes is shown accordingly.

no added $K^+$ in the present study. The pH optimum for 5′-nucleotidase is at 9.0 and the ion effect varies with pH (ref. 11).

The data presented in Table III demonstrate that 5′-nucleotidase and leucyl-β-naphthylamidase show similar, though quantitatively lesser, sex difference to that of the "alkaline phosphatases" in liver plasma membranes. Emmelot and Bos[1] did not measure leucyl-β-naphthylamidase activity. All these enzymes are considered predominantly, if not exclusively, located in the membrane segments lining the bile canaliculi in the liver[12]. These areas may be characterized by special transport functions[13]. On the basis of their negative findings with 5′-nucleotidase, Emmelot and Bos[1] were inclined to think that the sex hormones exerted a more direct or specific control over a certain enzyme activity, *i.e.* alkaline glycerol phosphatase, than over the total structure and function of the membrane. The present findings suggest that this question should be left open. The effect of sex hormones on the differentiation and function of plasma membranes, or specific segments of it, appears to be an important and interesting problem to investigate. There have been indications of sex hormone effects related to metabolic functions of the microsomal membranes of the liver, such as drug metabolism[14,15], lipid and lipoprotein synthesis[16–18].

The lipid content of the brain homogenate and both brain fractions was higher than in the liver, except that the cholesterol level of the brain synaptosomal fraction and liver plasma membranes was essentially identical. The lipid composition showed no significant sex difference*.

In the brain, none of the enzyme activities measured showed any appreciable enrichment in the synaptosomal or myelin fractions. In the brain homogenate, all enzyme activities, with the exception of 5′-nucleotidase, were significantly higher than in the liver homogenates. No sex difference was apparent. In the myelin fraction only, the specific activity of $K^+$-*p*-nitrophenylphosphatase was about 50% higher than that of the *p*-nitrophenylphosphatase in both sexes*.

The enzyme activities measured in this study have been considered markers of plasma membranes. It has been pointed out previously[2,4] that the "plasma membrane marker enzyme" designation should not be used in general terms. This is supported by the data presented here. The species, the tissue and the sex are very important factors in determining whether a certain enzyme activity can be considered a marker of the plasma membrane or not. For example, alkaline glycerol phosphatase cannot be considered a characteristic marker of female rat liver plasma membranes as indicated by the very low relative specific activity. The negative findings regarding brain synaptosomal and myelin fractions both in terms of relative specific activity and the sex dependence of enzymes measured also stress this point.

It has been suggested previously[2] that many of the currently used assay procedures were not sufficiently refined to measure enzyme activities of the plasma membranes exclusively. The fact that the enzyme activities of liver homogenates have not

*Data are not shown here because they are negative results from the point of view of this paper, but are obtainable from the authors.

TABLE III

5'-NUCLEOTIDASE AND LEUCYL-β-NAPHTHYLAMIDASE ACTIVITIES IN LIVER PLASMA MEMBRANES FROM MALE AND FEMALE RATS

All data represent averages of four separate observations.

| *Enzyme assays* | *Fraction* | *Males* | | *Females* | | |
|---|---|---|---|---|---|---|
| | | *Spec. act.*± S.E.* | *Rel. spec. act.*** | *Spec. act.*± S.E.* | *Rel. spec. act.*** | *Spec. act. (% of male value***)* |
| 5'-Nucleotidase | Homogenate | 2.41 ± 0.076 | | 2.52 ± 0.075 | | 104 |
| | Plasma membrane | 36.7 ± 0.860 | 15.2 | 25.5 ± 2.23 | 10.1 | 70 |
| Leucyl-β-naphthyl-amidase | Homogenate | 0.344± 0.034 | | 0.388± 0.039 | | 113 |
| | Plasma membrane | 5.12 ± 0.630 | 14.9 | 3.19 ± 0.290 | 8.2 | 62 |

* Specific activity: μmoles of substrate metabolized per mg protein per h.

** Relative specific activity: specific activity of plasma membrane/specific activity of homogenate.

***Specific activity of male membranes was taken as 100% and the specific activity of female membrane is shown accordingly.

shown the sex dependence, with the exception of *p*-nitrophenylphosphatases, seem to point to this methodological difficulty.

REFERENCES

1 Emmelot, P. and Bos, C.J. (1971) *Biochim. Biophys. Acta* 249, 293
2 Solyom, A. and Trams, E.G. (1973) *Enzyme*, in the press
3 Neville, Jr, D.M. (1968) *Biochim. Biophys. Acta* 154, 540
4 Lauter, C.J., Solyom, A. and Trams, E.G. (1972) *Biochim. Biophys. Acta* 266, 511
5 Whittaker, V.P., Michaelson, I.A. and Kirkland, R.J.A. (1964) *Biochem. J.* 90, 293
6 Colburn, R.W., Goodwin, F.K., Murphy, D.L., Bunney, Jr, W.E. and Davis, J.M. (1968) *Biochem. Pharmacol.* 17, 957
7 Heppel, L.A. (1955) in *Methods in Enzymology* (Colowick, S.P. and Kaplan, N.O., eds), Vol. 2, p. 530, Academic Press, New York
8 Ames, B.N. and Dubin, D.T. (1960) *J. Biol. Chem.* 235, 769
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.* 193, 265
10 Solyom, A., Lauter, C.J. and Trams, E.G. (1972) *Biochim. Biophys. Acta* 274, 631
11 Franklin, J.E. and Trams, E.G. (1971) *Biochim. Biophys. Acta* 230, 105
12 Emmelot, P., Bos, C.J., Benedetti, E.L. and Rumke, P. (1964) *Biochim. Biophys. Acta* 90, 126
13 Emmelot, P. and Visser, A. (1971) *Biochim. Biophys. Acta* 241, 273
14 Quinn, G.P., Axelrod, J. and Brodie, B.B. (1958) *Biochem. Pharmacol.* 1, 152
15 Murphy, S.D. and DuBois, K.P. (1958) *J. Pharmacol. Exp. Therap.* 124, 194
16 Alfin-Slater, R.B. and Aftergood, L. (1971) *Lipids* 6, 693
17 Young, D.L. (1971) *J. Lipid Res.* 12, 590
18 Solyom, A. (1972) *Lipids* 7, 100