Effect of clofibrate on the enzyme activity of rat liver plasma membranes

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Summary. The activity of 3 plasma membranes marker enzymes (5'-nucleotidase, Mg^{++}-ATPase and alkaline phosphodiesterase-I) was determined in plasma membranes isolated from liver of control and of clofibrate-treated rats. A complete identity of plasma membranes enzyme activity in the 2 groups of experimental animals was observed for the 3 enzymes studied.

Clofibrate administration to rats induces changes in hepatic metabolism[1] and ultrastructure[2]. We have shown recently that clofibrate treatment results in a 98% increase in the cytosolic content of the fatty acid-binding protein (FABP) and that isolated livers from clofibrate-treated rats take up free fatty acids (FFA) from the perfusate at a significantly higher rate (+76%) than control livers[3]. These results support the view that FABP plays a role in intracellular FFA transport in the liver cell.

Nevertheless, enhancement of FFA uptake by the liver could result from an indirect effect of the drug on plasma membranes properties and composition. In a previous study, following clofibrate administration, we excluded any modification of protein recovery and lipid composition of plasma membranes isolated from liver homogenates[3].

The present experiments were designed to confirm the identity of plasma membranes from an enzyme point of view in the control and clofibrate-treated rats.

Materials and methods. Male Sprague-Dawley rats weighing 200 g b.wt were given saline (control rats) or clofibrate (200 mg · kg^{-1}) (clofibrate-treated rats) via a gastric tube every 12 h for 4 days and were fed ad libitum until sacrifice. Plasma membranes were isolated from livers previously perfused through the portal vein with 50 ml ice-cold isotonic saline[4]. Enzyme activities of 5'-nucleotidase; EC 3.1.3.5.[4], glucose-6-phosphatase; EC 3.1.3.9.[4], Mg^{++}-ATPase; EC 3.6.1.3.[5], alkaline phosphodiesterase-I; EC 3.1.4.1.[6] and of succinate oxido-reductase; EC 1.3.99.1.[7] were determined on aliquot fractions of total homogenate and of purified plasma membranes.

Results and discussion. The enrichment of plasma membranes in 5'-nucleotidase (5'-ribonucleotide phosphohydrolase), an ecto-enzyme of the plasma membrane[8] was not modified after clofibrate administration (table 1). The plasma membrane enzyme activity of another ecto-enzyme[9,10], alkaline phosphodiesterase-I (oligonucleate 5'-nucleotidohydrolase), enriched 20 times over its activity in the total homogenate, remains unchanged after clofibrate treatment (table 1). The identity of Mg^{++}-ATPase activity was also observed in the 2 groups (table 1). The yield of enzyme activity recovered in the purified plasma membrane was identical in the control and clofibrate-treated rats for the 3 enzymes studied (table 2).

The differences of enrichment and recovery relative to the enzyme activity determined express the uneven distribution of each enzyme between the different subcellular organelles[11,12].

In addition, we have measured the microsomal and mitochondrial contamination of the plasma membranes: glucose-6-phosphatase activity of the plasma membranes represented 0.30±0.10% and 0.40±0.10% (n=6) for control and clofibrate-treated rats respectively of the total activity present in the total homogenate. Plasma membranes from control and clofibrate-treated rats were devoid of succinate oxido-reductase. These results show a very low microsomal contamination and no mitochondrial contamination of the plasma membrane studied.

In conclusion, our results exclude any effect of clofibrate treatment on FFA uptake by the liver resulting from modifications of the plasma membranes characteristics, and confirm the role of the fatty acid-binding protein (FABP) in the intracellular fatty acid transport.

Table 1. Effect of clofibrate treatment on the relative specific activity of plasma membranes enzymes

	5'-nucleotidase EC 3.1.3.5.	Alkaline phosphodiesterase-I EC 3.1.4.1.	Mg^{++}-ATPase EC 3.6.1.3.
Rats:			
Control	42.9±5.1*	20.5±1.7	16.0±1.3
Clofibrate-treated	44.3±4.2**	19.3±3.3**	13.7±1.1**

* Data are the mean±SEM from 6 plasma membranes preparations per group and represent for each enzyme the enrichment over its activity in the total homogenate (relative specific activity). The 2 experimental groups were compared according to the U-test of Mann-Whitney. ** not significant.

Table 2. Effect of clofibrate treatment on the plasma membranes enzymes recovery from total homogenate

	5'-nucleotidase EC 3.1.3.5.	Alkaline phosphodiesterase-I EC 3.1.4.1.	Mg^{++}-ATPase EC 3.6.1.3.
Rats:			
Control	27.1±2.5*	11.8±2.2	8.2±0.4
Clofibrate-treated	26.8±2.0**	10.3±2.2**	8.3±0.4**

* Data are the mean±SEM from 6 plasma membranes preparations per group and represent the percentage of total homogenate enzyme activity recovered in the plasma membranes. The 2 experimental groups were compared according to the U-test of Mann-Whitney. ** Not significant.

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