Transport of propionate in basolateral plasma membrane vesicles of sheep liver

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We have previously demonstrated that the uptake of propionate into isolated sheep hepatocytes is rapid, saturable and concentrative (1). The results of the study suggested that the uptake of propionate by sheep liver is mediated by specific membrane transport mechanism(s). However, definitive evidence for plasma membrane transport could not be obtained in intact hepatocytes due to rapid metabolism of propionate by the liver and due to lack of suitable inhibitors of propionate metabolism. In the present study we examined propionate transport in the absence of metabolism by using basolateral liver plasma membrane vesicles (blLPMV).

Six cross-bred wethers weighing 24-35 kg were used. Basolateral plasma membrane vesicles were isolated by a modification of the method of Blitzer and Donovan (2). Electron microscopy and enrichment of marker enzymes ascertained the purity of the blLPMV. Twenty microliters of blLPMV suspension containing 40-80 μg protein were preincubated for 5 minutes. Uptake of propionate was initiated by adding 80 μl of incubation medium containing appropriate amounts of [2-^{14}C]propionate. Uptake was terminated after 5 seconds by rapid vacuum filtration of the mixture through a 0.45 μm nitrocellulose filter. The filter was then transferred into a counting vial, mixed with 8 ml of scintillation liquid and the radioactivity was counted.

Uptake of propionate at equilibrium was proportional to osmotically sensitive space within the vesicles. Conversion of vesicles into sheets by the treatment of blLPMV with deoxycholate-EDTA resulted in abolition of uptake. The transport was not affected by the substitution of Na^+ by K^+. Uptake of propionate over the range of 0.05-1.0 mM was linear, and approached saturation at higher concentrations (Figure). Radiolabeled propionate transport demonstrated trans-stimulation when the vesicles were preloaded with unlabeled propionate. Under an outward-directed propionate gradient, [2-^{14}C]propionate transport exhibited saturation kinetics with an apparent k_m of 37.57 ± 4.75 mM and a V_{max} of 920.6 ± 62.04 nmol/mg protein/min. The results confirm that the uptake of propionate by sheep liver is carrier mediated.

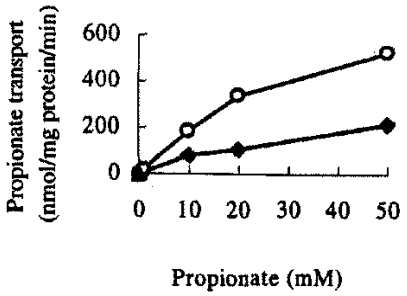

Figure. Propionate uptake in blPLMV preloaded with either a buffer containing 100 mM sodium propionate, 100 mM sucrose, and 10 mM HEPES-KOH, pH 7.5 (o) or a buffer containing 300 mM sucrose, and 10 mM HEPES-KOH, pH 7.5 (◆). The vesicles were incubated for 5 seconds in a medium containing 100 mM NaCl, 100 mM sucrose, and 10 mM HEPES-KOH, pH 7.5

1. Mohamed Ali A and Jois M. Uptake and metabolism of propionate in the liver isolated from sheep treated with glucagon. Br J Nutr 1997; 77:783-793.
2. Blitzer BL and Donovan CB. A new method for the rapid isolation of basolateral plasma membrane vesicles from rat liver . J Biol Chem 1984; 259:9295-9301.