CORRESPONDENCE

To the Editor:

We read with interest the report by Kakimoto et al (*Metabolism* 44:825-832, 1995) who published their observations regarding altered lipid composition and enyzme activities of erythrocyte membranes in hepatic cirrhosis. In this well-designed study, the authors found an increased C/PI ratio and fluorescence polarization value in cholestatic cirrhotic patients. The Mg^{2+}-ATPase and LCAT activities were depressed. However, the Na^+,K^+-ATPase activity was unaltered. Recently, we measured the Na^+,K^+-ATPase activity of 22 children (6.1 ± 5.3 years) with definite chronic liver disease (CLD) (diagnoses: four extrahepatic biliary atresia, four congenital hepatic fibrosis, three Alagille syndrome, three glycogenosis type I/a, three idiopathic hepatitis in infancy, two biliary cirrhosis, one cystic fibrosis, one Byler disease, and one Wilson disease). Cholestasis was also present in 11 patients. The control group was formed by 19 age-matched patients undergoing minor surgical interventions. A 2-mL anticoagulated (EDTA) blood sample was drawn. The erythrocytes were washed three times (0.145 mol/L NaCl, 0.02 mol/L TRIS, pH 7.6: 1,500*g*, 3 · 10 minutes), then the red blood cells (hematocrit 90%) were mixed with the same volume of 1% Tween-20 (wt/vol) prepared in 0.25 mol/L saccharose, 0.02 mol/L TRIS, pH 7.6. The mixture was incubated at 4°C for 1 hour. For assessment of Na^+,K^+-ATPase in detergent, pretreated erythrocytes (DPE) were diluted fourfold in a reaction mixture (100 mmol/L NaCl, 10 mmol/L KCl, 50 mmol/L TRIS [pH 7.6], 5 mmol/L Na_2ATP, 0.5 mmol/L EDTA, 5 mmol/L $MgCl_2$, 37°C) in the presence or absence of 1 mmol/L ouabain. The final reaction volume was 0.4 mL. The reaction was stopped after 30 minutes by 0.2 mL 20% tricholoacetate (TCA). After a short centrifugation, the phosphate content of supernatant was assessed by Boehringer UV Phosphor kit, with a Hitachi 704 automatic system. One unit of Na^+,K^+-ATPase activity is expressed as 1 nmol Pi/mL erythrocyte/h.

The Na^+,K^+-ATPase activity in DPE was lower in children with CLD (1,823 ± 717 *v* control 3,183 ± 754 nmol Pi/mL EC/h, $P < .05$). Moreover, those children with cholestasis exhibited a significantly lower activity of Na^+,K^+-ATPase compared with noncholestatic patients (1,513 ± 786 *v* 2,134 ± 787 nmol Pi/mL EC/h, $P < .05$).

For the possible explanation of the difference between our findings and the results of Kakimoto et al, we suggest the following theory. In our model, detergent-pretreated erythrocytes were used, while Kakimoto et al examined hemoglobin-free erythrocyte membranes (ghosts). The probes of DPE may contain several intracellular and extracellular factors (eg, ions, hormones, and weakly membrane-associated enzyme regulators), which may affect the Na^+,K^+-ATPase activity. During the preparation phase of ghosts, these materials might have been washed out, so the activity of Na^+,K^+-ATPase in this model is influenced merely by membrane characteristics.

Concerning the decreased activity of sodium pump found in our study, we postulate that there are intracellular/extracellular factors present in CLD that may inhibit the enzyme. When they were washed out during the preparation procedure used by Kakimoto et al, the activity of Na^+,K^+-ATPase did not differ from that of controls.

The findings indicate that in CLD, some humoral factors inhibit Na^+,K^+-ATPase activity, while physicochemical properties of erythrocyte membrane per se do not affect it.

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REPLY

To the Editor:

Thank you for the opportunity to reply to the letter by Vásárhelyi et al. The authors describe 22 children with nine different liver diseases. Cholestasis was present in 11 patients, although their clinical data were not stated. However, there was no patient with cholestatic liver disease in our study. Furthermore, fluidity and lipid composition of erythrocyte membranes were not reported in the letter by Vásárhelyi et al. Therefore, it seems that the patients, methods, and purpose of their study were different from ours.

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