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The α -isoforms of Na,K-ATPase and FXYD-peptides in small intestinal mucosa

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Na⁺,K⁺ATPase is an integral $\alpha\beta$ -heterodimer membrane protein present in almost all mammalian plasma membranes. The α -subunit exists in several distinct isoforms (α_1 - α_4) exhibiting individual affinities to Na⁺, K⁺ and ATP as well as a tissue-specific pattern of distribution. In the intestine Na⁺,K⁺ATPase creates the driving force for transepithelial transport of ions and solutes with a prevailing role of the α_1 -isoform. At present it is unknown whether the activity of intestinal Na⁺,K⁺ATPase can be modulated by a γ -peptide, the best characterized member of the FXYD-family of single-span membrane peptides. The aim of the present study was to quantify individual α -isoforms of Na⁺,K⁺ATPase in small intestinal mucosa and, secondly, to trace a hypothetical γ -peptide.

Homogenates of small intestinal mucosa from ad-libitum fed adult Wistar rats were used after humane killing. They were applied to SDS gels and, for calibration, in parallel lanes were run purified rat Na⁺,K⁺ATPase preparations with known isoform distribution and concentration. Western blots containing homogenate proteins and reference enzyme were incubated with isoform-specific monoclonal antibodies and radiolabelled secondary antibodies. The autoradiographic signals from adjacent α spots were used to quantify the individual isoforms (Hansen, 2001). A γ -peptide was traced by the

same method using a γ -specific polyclonal antibody and a radiolabelled secondary antibody. Measurements of enzymatic activity of Na⁺,K⁺ATPase were performed by a K⁺-dependent phosphatase assay using para-nitrophenyl phosphate as substrate.

Concentrations \pm S.E.M. of the α_1 -isoform containing Na⁺,K⁺ATPase of 54.9 \pm 9.4 and 35.2 \pm 6.0 pmol/mg protein or 4.6 \pm 0.8 and 2.9 \pm 0.5 nmol/g dry matter were found in jejunum and ileum, respectively ($P < 0.20$, each group $n=4$, Student's 2-tailed t test). Insignificant concentrations of α_2 and α_3 were found. The accumulated concentration of the ouabain-sensitive ($\alpha_2 + \alpha_3$)-isoforms seemed to represent less than 1 % compared to the ouabain-insensitive α_1 -isoform. A γ -peptide was not identified. Activities \pm S.E.M. of K⁺-pNPPase of 33.3 \pm 1.8 and 17.4 \pm 1.0 nmol/mg protein/minute were measured in non-detergent treated jejunal and ileal mucosal homogenates, respectively ($P < 0.0001$, each group $n=12$, Student's 2-tailed t test).

Rather high concentrations of the ubiquitous α_1 -isoform of Na⁺,K⁺ATPase with high or intermediate Na⁺-affinity are almost exclusively found in the small intestinal mucosa. This isoform pattern is similar to that seen in other tissues with transepithelial transport, such as the kidney. However, unlike the kidney the activity of Na⁺,K⁺ATPase in the small intestine does not seem to be modulated by a γ -peptide. The higher pump density and activity of K⁺-pNPPase in jejunum compared to ileum reflect the greater extent of sodium-coupled transport of e.g. sugars and amino acids in jejunum.

Hansen O (2001). *Acta Physiol Scand* 173, 335-341.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.