

Changes associated with Na,K-ATPase in brain, kidney, heart and liver of the spontaneously diabetic Goto-Kakizaki rat

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Alterations in Na,K-ATPase activity, in isoenzyme expression and/or number of units of the pump present in the plasma membrane have been associated with diabetes. These changes were described in different organs and tissues such as brain, heart, kidney, among others, and may result from altered insulin levels. However, the vast majority of studies were conducted in animal models of chemically induced diabetes, which are not consensual models for type 2 diabetes (T2D). The major goal of this work was to investigate putative modifications in Na,K-ATPase enzymatic activity or expression in brain, kidney, heart and liver in T2D.

The Goto-Kakizaky rat (GK) strain was used as a model of spontaneously developed T2D, and Wistar rats as controls. Na,K-ATPase activity was assessed by the hydrolysis of ATP (Pi formed in the presence/absence of ouabain was measured using a colorimetric assay) and the isoenzymatic expression by Westernblot.

A decrease in Na,K-ATPase activity in renal and cardiac tissues from GK comparatively to controls (55.7% and 77.5%, respectively) was observed. The pump activity was similar in liver and brain tissues. In renal tissue, expression of $\alpha 1$ -Na,K-ATPase was similar between GK and controls but $\alpha 2$ - was 2.3x higher and $\alpha 3$ - was detected only in GK. Contrastingly a decreased expression of $\alpha 1$ - (49.5%) and $\alpha 2$ -isoforms (67.6%) was found in cardiac tissue. Despite similar Na,K-ATPase activity in liver and brain tissues, $\alpha 1$ -isoform expression was decreased (33.9%) in the liver from GK while in the brain an increase of $\alpha 1$ -isoform (~2x) together with a decrease of $\alpha 2$ -isoform (14.0%) expressions were observed.

It is unclear, except maybe for cardiac tissue where lower expression is potentially underlying the diminished pump activity in GK, whether the changes in isoenzyme expression is a key factor for differential Na,K-ATPase activity. Being responsive to complex regulation, other regulatory mechanisms may contribute to the impaired activity observed in kidney and heart.

These results have uncover changes in Na,K-ATPase activity and/or enzymatic expression in GK brain, kidney, heart and liver that may contribute to the undesirable conditions associated with T2D. This work highlight the relevance of further investigation about Na,K-ATPase regulation and role in physiopathology of T2D.

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Isozyme expression of isoforms $\alpha 1$, $\alpha 2$ and $\alpha 3$ of Na, K-ATPase were also analysed and changes were observed.

In the kidney, there was an expression of $0,009\pm 0,002$ and $0,021\pm 0,006$, in control rats and GK, respectively, or a significant increase in expression of the $\alpha 2$ isoform GK.

Already in the hepatic tissue, we observed a significant decrease in expression of the $\alpha 1$ isoform, registering an expression of $1,295\pm 0,095$ and $0,856\pm 0,084$, in control rats and GK, respectively.

In the brain, expression of the $\alpha 1$ isoform in the control and GK rats was $0,367\pm 0,021$ and $0,602\pm 0,073$, respectively, i.e. a significant increase in expression of this isoform in GK. In contrast, the $\alpha 2$ isoform, we observed a significant decrease in expression of GK, to which the recorded values of $0,043\pm 0,003$ and $0,037\pm 0,001$, in control rats and GK, respectively. Unlike isoform $\alpha 1$ and $\alpha 2$ to $\alpha 3$ -Na, K-ATPase is not significantly different between control and GK rats for which we observed an expression of $0,765\pm 0,047$ and $0,684\pm 0,044$, respectively. Finally, expression of the $\alpha 1$ isoform in heart and GK rats control was $0,782\pm 0,050$ and $0,465\pm 0,054$, respectively. The expression of the $\alpha 2$ isoform was $0,037\pm 0,011$ and $0,012\pm 0,004$, in control rats and GK, respectively. Therefore, in cardiac tissue both $\alpha 1$ and $\alpha 2$ isoform experienced a significant decrease in GK.

In summary, our results point to a significant decrease in the activity of Na, K-ATPase in GK in renal and cardiac tissue. These changes may underlie the changes in isoenzyme expression also observed isoforms $\alpha 1$ and $\alpha 2$ and $\alpha 3$ Na, K-ATPase, which makes sodium pump an important therapeutic target for T2D.

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