

## Influence of sub-chronic diabetes mellitus on functional properties of renal $\text{Na}^+, \text{K}^+$ -ATPase in both genders of rats

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**Abstract.** For characterization of  $\text{Na}^+, \text{K}^+$ -ATPase, a key enzyme involved in maintenance of intracellular sodium homeostasis, expression of  $\alpha 1$  subunit and the ATP- and  $\text{Na}^+$ -binding properties were investigated by Western blot analysis and by enzyme kinetics, respectively. Previous studies documented time-dependent alteration of properties of renal  $\text{Na}^+, \text{K}^+$ -ATPase from its mobilization after 8 days to serious deteriorations after 16 weeks of diabetes in rats. Characterizing the critical period during development of the disease, when mobilization of  $\text{Na}^+, \text{K}^+$ -ATPase observed in the acute phase turns to its damage, we examined the enzyme properties after 8 weeks lasting diabetes which was induced by a single intraperitoneal administration of streptozotocin in a dose of  $65 \text{ mg} \cdot \text{kg}^{-1}$ . The unchanged expression of  $\text{Na}^+, \text{K}^+$ -ATPase  $\alpha 1$ -subunit in both genders indicates that 8 weeks represent the time when the mobilization of enzyme synthesis observed previously in acute diabetes is lost. In this time the renal  $\text{Na}^+, \text{K}^+$ -ATPase undergoes structural changes in the vicinity of  $\text{Na}^+$ -binding site resulting in worsened affinity to sodium in both genders as indicated by 13% and 18% increase of  $K_{\text{Na}}$  value in female and male rats, respectively. However, gender specific was the diabetes-induced decrease in affinity to ATP by 18% which occurred in female rats only.

**Key words:** Sodium pump — Kidney — Streptozotocine — Sex difference —  $\alpha 1$  subunit

**Abbreviations:**  $\text{Na}^+, \text{K}^+$ -ATPase, sodium/potassium-exchanging adenosine triphosphatase; FD, diabetic female rats; FC, control female rats; MD, diabetic male rats; MC, control male rats; STZ, streptozotocine.

### Introduction

Diabetes mellitus is a serious metabolic disorder characterized by many changes in organism such as hyperglycemia resulting from impairment in insulin production and is associated with long-term complications introducing deterioration of the eyes, heart failure, nerve damage, micro-vascular damage and chronic renal failure. Changes in electrolyte homeostasis, mainly altered sodium balance and hypertension were documented in both insulin-dependent and non-insulin-dependent diabetic patients

(O'Hare et al. 1985; Weidmann and Ferrari 1991; Landsberg 1994).

The primary mechanism for  $\text{Na}^+$  reabsorption in the kidney is represented by the  $\text{Na}^+, \text{K}^+$ -ATPase which is located on the basolateral membrane in proximal tubules of nephron. This enzyme is heterodimeric protein that uses the energy from hydrolysis of ATP to maintain the low intracellular sodium concentration and high intracellular potassium concentration common to most living cells (Jorgensen and Pedersen 2001). Renal  $\text{Na}^+, \text{K}^+$ -ATPase consists of three subunits:  $\alpha$ ,  $\beta$  and  $\gamma$ . The main isoforms in rat kidney are  $\alpha 1$  and  $\beta 1$  subunits (Martin-Vasallo et al. 1989). The subunit  $\alpha$  is a membrane multi-spanning protein that is responsible for the catalytic and transport properties of the enzyme. This subunit contains binding sites for the cations and ATP (Blanco and Mercer 1998). The subunit  $\beta$  is needed for sta-

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bility, functional maturation, and/or exit of  $\alpha$  subunit from the endoplasmic reticulum (McDonough et al. 1990). The third  $\gamma$  subunit, expressed mainly in kidney, is involved in regulation of the sensitivity of  $\text{Na}^+, \text{K}^+$ -ATPase to transported cations (Arystarkhova et al. 1999; Kuster et al. 2000).

Summarization of data from recent studies in streptozotocine-induced diabetic rats has shown that changes in the function of renal  $\text{Na}^+, \text{K}^+$ -ATPase depend on the development and the seriousness of the disease in time-dependent manner. In the acute phase of disease after 8–10 days, the activity and expression of the  $\text{Na}^+, \text{K}^+$ -ATPase was significantly higher in diabetic animals as compared to the control group (Lal et al. 2000; Javorková et al. 2009). In this model of acute diabetes, a significant gender specific increase in the  $\text{Na}^+, \text{K}^+$ -ATPase activity accompanied by significant increase in  $K_m$  value indicating a worsened affinity of ATP-binding site in female rats was shown (Javorková et al. 2009).

Prolongation of the streptozotocine (STZ)-induced diabetes mellitus to 6 weeks was followed by significant increases of  $\alpha 1$  and  $\beta 1$  subunits abundance associated with significant increase in  $\text{Na}^+, \text{K}^+$ -ATPase activity (Ng et al. 1993; Tsimaratos et al. 2001b). On the other hand, in a case of chronic diabetes lasting 10–16 weeks, decrease in the activity of the  $\text{Na}^+, \text{K}^+$ -ATPase was observed (Tsimaratos et al. 2001a,b; Unlucerci et al. 2001; Vrbjar et al. 2004, 2007). Correspondingly, the expression of  $\alpha 1$  and  $\beta 1$  subunits was decreased (Tsimaratos et al. 2001b).

Summarization of the literature data indicates that the period of 8 weeks represents approximately the middle between the upper limit of the acute phase and bottom limit of the chronic phase of experimental diabetes. Trying to characterize the critical period during the development of the disease, when the mobilization of the  $\text{Na}^+, \text{K}^+$ -ATPase observed in the acute phase turns to the damage of the enzyme, the present study was designed to investigate the influence of 8 weeks lasting sub-chronic diabetes on protein levels of  $\alpha 1$  subunit in male and female rats, as well as characterization of kinetic properties of this catalytic subunit of renal  $\text{Na}^+, \text{K}^+$ -ATPase.

## Materials and Methods

### *Animal model*

The experimental model of diabetes mellitus in male and female rats was induced by a single intraperitoneal application of STZ in a dose of  $65 \text{ mg} \cdot \text{kg}^{-1}$ . STZ was dissolved in  $0.1 \text{ mol} \cdot \text{l}^{-1}$  citrate buffer with pH 4.5. The animals were fasted overnight prior to STZ administration. The age of animals at the beginning of the experiment was eight weeks. Water and food were available immediately after dosing. Eight weeks after STZ administration, animals with plasma glucose level

higher than  $10 \text{ mmol} \cdot \text{l}^{-1}$  were considered diabetic and were included in this study. Control groups received a single dose of  $0.1 \text{ mol} \cdot \text{l}^{-1}$  citrate buffer. The rats were divided in four groups. Group FD consisted of diabetic female rats, FC group served as female controls. Group MD represented diabetic male rats and MC served as control male group. During the experiment, the animals were housed in groups of 3 in cages of the type T4 Velaz (Prague, Czech Republic) with bedding composed of wood shaving (exchanged daily). All rats were allowed free access to food and drinking water. The animal room was air-conditioned and the environment was continually monitored for the temperature of  $23 \pm 1^\circ\text{C}$  with relative humidity of  $55 \pm 10\%$ . All the experiments were terminated in the age of animals 16 weeks. At the end of experiment (after 8 weeks), rats were killed under thiopental anesthesia, glucose plasma level was measured by commercial glucose GOD 250 kit (PLIVA-Lachema, Brno, Czech Republic). The kidneys were immediately frozen in liquid nitrogen and stored for further investigations of  $\text{Na}^+, \text{K}^+$ -ATPase properties. All experiments were approved by the Veterinary Council of the Slovak Republic (Decree No. 289, part 139, July 9th 2003) and they conform to Principles of Laboratory Animals Care (NIH publication 83-25, revised 1985).

### *Preparation of plasmalemmal fraction for kinetic measurements*

The plasmalemmal membrane fraction from rat kidney was isolated according to Jorgensen (1974) with slight modifications. Briefly, the renal tissue was homogenized in cold isolation medium containing (in  $\text{mmol} \cdot \text{l}^{-1}$ ): 250 sucrose, 25 imidazol, 1 EDTA (pH 7.4) using a tissue disruptor ( $3 \times 10 \text{ s}$  at a setting of 4, Polytron PT-20). The homogenate was centrifuged at  $6000 \times g$  for 15 min. The sediment was re-homogenized and centrifuged again at  $6000 \times g$  for 15 min. The collected supernatants from both centrifugations were re-centrifuged at  $48\,000 \times g$  for 30 min and the final sediment was re-suspended in the isolation medium. An aliquot was removed for determination of proteins by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

### *Kinetic measurements of $\text{Na}^+, \text{K}^+$ -ATPase*

ATP-kinetics of  $\text{Na}^+, \text{K}^+$ -ATPase were estimated at a temperature of  $37^\circ\text{C}$  measuring the hydrolysis of ATP by  $10 \mu\text{g}$  plasmalemmal proteins in the presence of increasing concentrations of substrate ATP ( $0.16\text{--}8.0 \text{ mmol} \cdot \text{l}^{-1}$ ). The total volume of medium was  $0.5 \text{ ml}$  containing (in  $\text{mmol} \cdot \text{l}^{-1}$ ):  $4 \text{ MgCl}_2$ ,  $10 \text{ KCl}$ ,  $100 \text{ NaCl}$  and  $50 \text{ imidazole}$  (pH 7.4). After 20 min of pre-incubation in substrate-free medium, the reaction was started by addition of ATP and after 20 min the reaction was stopped by addition of  $0.3 \text{ ml}$  12% ice-cold solution of trichloroacetic acid. The liberated inorganic phosphorus

was determined according to Taussky and Shorr (1953). In order to establish the  $\text{Na}^+, \text{K}^+$ -ATPase activity, the ATP hydrolysis that occurred in the presence of  $\text{Mg}^{2+}$  only was subtracted. The  $\text{Na}^+, \text{K}^+$ -ATPase kinetics for cofactor  $\text{Na}^+$  was determined by the same method, in the presence of increasing concentration of  $\text{NaCl}$  ( $2.0$ – $100.0 \text{ mmol}\cdot\text{l}^{-1}$ ) at constant amount of ATP ( $8 \text{ mmol}\cdot\text{l}^{-1}$ ). The kinetic parameters  $V_{\text{max}}$ ,  $K_m$ ,  $K_{\text{Na}}$  were evaluated from obtained data by direct nonlinear regression. The parameter  $V_{\text{max}}$  represents the maximal velocity,  $K_m$  and  $K_{\text{Na}}$  values represent the concentrations of ATP or  $\text{Na}^+$  necessary for half maximal activation of the enzyme. All results were expressed as mean  $\pm$  S.E.M. The significance of differences between the individual groups was determined with using of ANOVA and Bonferroni test. A value of  $p < 0.05$  was regarded as significant.

#### Preparation of tissue fractions for electrophoresis and immunochemical Western blot analysis

The tissue samples from kidneys were crushed in liquid nitrogen and consequently re-suspended in ice-cold buffer containing (in  $\text{mmol}\cdot\text{l}^{-1}$ ): 50 Tris-HCl, 250 sucrose, 1.0 dithiothreitol, 1.0 phenylmethylsulfonylfluoride (pH 7.4) and homogenized with a glass-teflon homogenizer. The homogenates were centrifuged at  $800 \times g$  for 5 min at  $4^\circ\text{C}$ , pellets after this centrifugation were discarded and the supernatants were centrifuged again at  $9300 \times g$  for 30 min. Following this second centrifugation the supernatants were discarded again and the pellets were re-suspended in homogenizing buffer supplemented with 0.2% Triton X-100 and centrifuged at  $9300 \times g$  for 1 min. The Triton X-100 soluble supernatants represented the particulate fractions. The protein concentrations were estimated by the method of Lowry et al. (1951).

#### Electrophoresis and immunochemical Western blot analysis

Samples of particular protein fractions containing equivalent amounts of proteins ( $90 \mu\text{g}$ ) per lane were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to Laemmli (1970). Total contents of  $\alpha 1$  subunits of  $\text{Na}^+, \text{K}^+$ -ATPase were estimated by Western blot analysis. For primary immunodetection of  $\alpha 1$  subunit of  $\text{Na}^+, \text{K}^+$ -ATPase, specific anti- $\alpha 1$  antibody (from Sigma) was used. After electrophoretic separation, proteins were transferred to nitrocellulose membrane (Western blot assays). As secondary antibodies the peroxidase-labelled anti-mouse immunoglobulins (Amersham Biosciences) were used. Bound antibodies were detected by the enhanced chemiluminescent method. Quantification of protein levels was performed using ImageJ program. Data were expressed as means  $\pm$  S.E.M. Statistical significance of differences between the groups was analyzed by the unpaired Student's  $t$ -test. Differences were considered as significant at  $p < 0.05$ .

## Results

### Body weight, kidney weight

In 8 weeks lasting sub-chronic diabetes we observed changes in all investigated weight parameters. Significantly lower gain in body weight was observed in diabetic animals of both genders amounting 30% and 22% decrease in males and females, respectively. The kidney weight increased significantly due to diabetes by 31–32% in both chronic diabetic groups as compared to adequate control groups. The kidney weight/body weight ratio was higher by 68% in FD and by 86% in MD as compared with controls (Tab. 1).

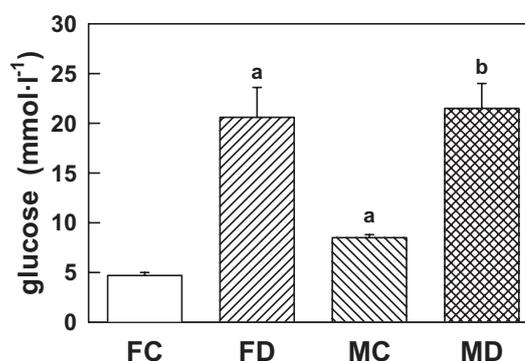
### Level of plasma glucose

In FD the level of plasma glucose increased by 338% as compared to the FC group. In male diabetic rats the plasma glucose level was higher by 153% as compared to corresponding controls (Fig. 1).

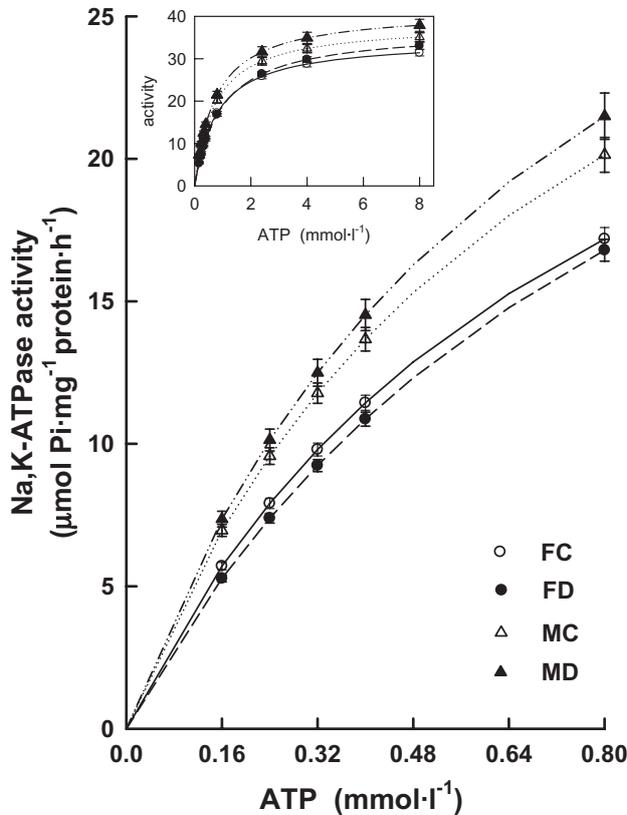
**Table 1.** Influence of the STZ-induced diabetes on weight parameters of rat males and females measured at the end of sub-chronic experiments (8 weeks)

Groups	Bw (g)	Kw (L+R) (mg)	Kw/Bw (mg/g)
FC	$208 \pm 4$	$1508 \pm 63$	$7.3 \pm 0.3$
FD	$164 \pm 6^*$	$1996 \pm 80^*$	$12.3 \pm 0.2^*$
MC	$374 \pm 17$	$2116 \pm 62$	$5.8 \pm 0.3$
MD	$263 \pm 8^*$	$2779 \pm 82^*$	$10.8 \pm 0.3^*$

Data represent means  $\pm$  SEM at the end of experiment,  $n = 7$  in all groups. MD, diabetic male rats; MC, control male rats; FD, diabetic female rats; FC, control female rats; Bw, body weight; Kw (L+R), kidney weight (left+right); Kw/Bw, kidney weight/body weight ratio; \*  $p < 0.005$  as compared to respective control.



**Figure 1.** Plasma level of glucose in female control rats (FC), in female rats with STZ-induced diabetes mellitus (FD), in male control rats (MC) and in male diabetic rats (MD). Data represent means  $\pm$  S.E.M,  $n = 9$  in each group. <sup>a</sup>  $p < 0.001$  as compared to the FC group; <sup>b</sup>  $p < 0.001$  as compared to the MC group.

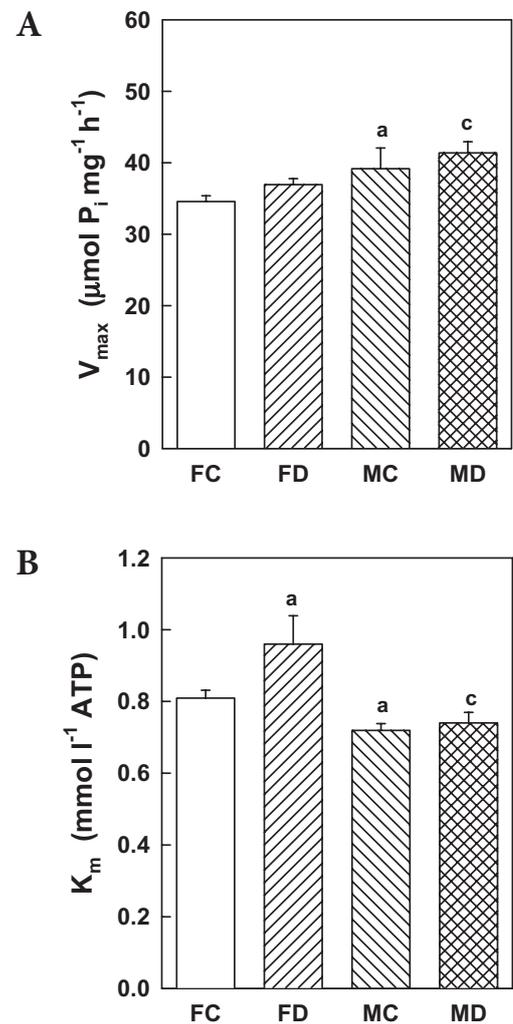


**Figure 2.** Activation of renal  $\text{Na}^+, \text{K}^+$ -ATPase by low concentrations of substrate ATP in female control rats (FC), in female rats with STZ-induced diabetes mellitus (FD), in male control rats (MC) and in male diabetic rats (MD). Insert: activation of the enzyme in the whole investigated concentration range of ATP.

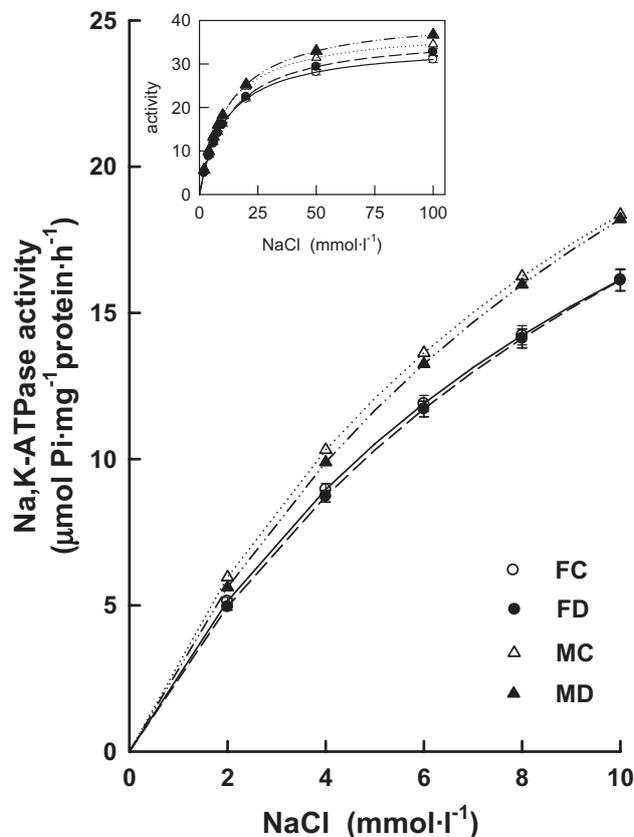
#### Kinetic measurements

Comparison of FC with MC group resulted in various activities of  $\text{Na}^+, \text{K}^+$ -ATPase. During activation of the enzyme with increasing concentration of substrate (ATP), the enzyme was more active in kidneys from male control rats. The difference was the highest in the presence of the lowest investigated concentration of ATP representing 21%. With increasing concentration of ATP the effect gradually decreased and at the presence of  $8 \text{ mmol}\cdot\text{l}^{-1}$  it was 12% (Fig. 2). Evaluation of the above data by the method of nonlinear regression resulted in statistically significant increase in  $V_{\max}$  value by 11% and the  $K_m$  value was decreased by 11% in MC group as compared to FC group (Fig. 3). Activation of the enzyme with increasing concentration of cofactor ( $\text{Na}^+$ ) resulted again in higher activities from MC as compared to FC group. The increase in activities of  $\text{Na}^+, \text{K}^+$ -ATPase varied in the range of 16% to 11% (Fig. 4). Evaluation of the kinetic parameters resulted in statistically significant increase in  $V_{\max}$  value by 11% and the  $K_{\text{Na}}$  value was remained (Fig. 5).

Diabetes in female rats was followed by 9% decrease of enzyme activity in the presence of lowest concentration of ATP. Increase of the ATP concentration was followed by continuous decrease of the effect. In the presence of  $2.4 \text{ mmol}\cdot\text{l}^{-1}$  of ATP the activities in FC and FD group were similar. Further increase of ATP concentration induced a gradual stimulation of the  $\text{Na}^+, \text{K}^+$ -ATPase in FD group (Fig. 2). Evaluation of the data resulted in similar  $V_{\max}$  values but the  $K_m$  value was higher significantly by 18% in FD vs. FC group (Fig. 3). When activating the enzyme with increasing concentration of  $\text{Na}^+$  we observed inhibition of the enzyme



**Figure 3.** Kinetic parameters of renal  $\text{Na}^+, \text{K}^+$ -ATPase during activation with substrate ATP in female control rats (FC), in female rats with STZ-induced diabetes mellitus (FD), in male control rats (MC) and in male diabetic rats (MD). The parameter  $V_{\max}$  represents the maximal velocity of enzyme reaction;  $K_m$  value refers to the concentration of ATP necessary for half maximal activation of the enzyme. Data represent means  $\pm$  S.E.M,  $n = 9$  in each group. <sup>a</sup>  $p < 0.001$  as compared to the FC group; <sup>c</sup>  $p < 0.01$  as compared to the FD group.



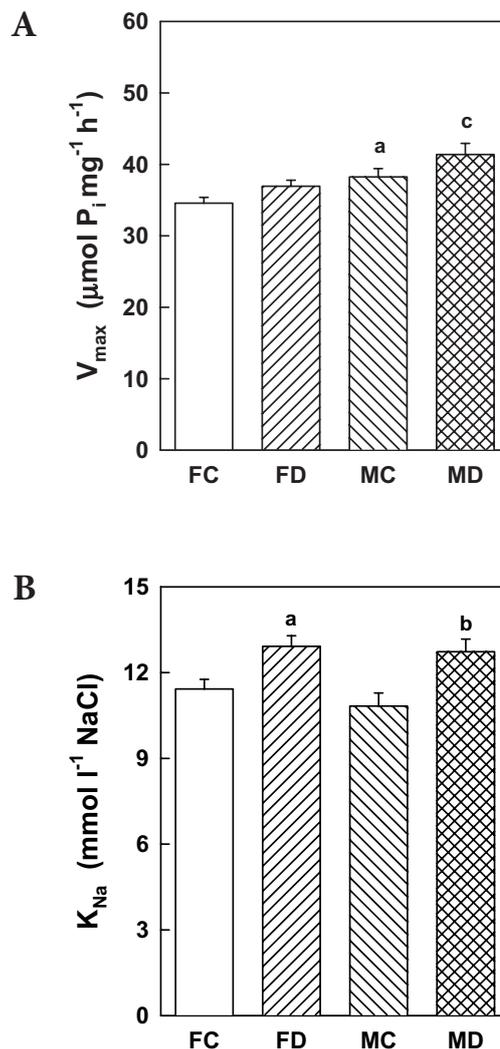
**Figure 4.** Activation of renal  $\text{Na}^+, \text{K}^+$ -ATPase by low concentrations of cofactor  $\text{Na}^+$  in female control rats (FC), in female rats with STZ-induced diabetes mellitus (FD), in male control rats (MC) and in male diabetic rats (MD). Insert: Activation of the enzyme in the whole investigated concentration range of NaCl.

at low concentrations, which turned to stimulation at high concentrations of cofactor (Fig. 4). This biphasic effect in activities was reflected in significant increase of  $K_{\text{Na}}$  value by 13% accompanied with stable value of  $V_{\text{max}}$  (Fig. 5).

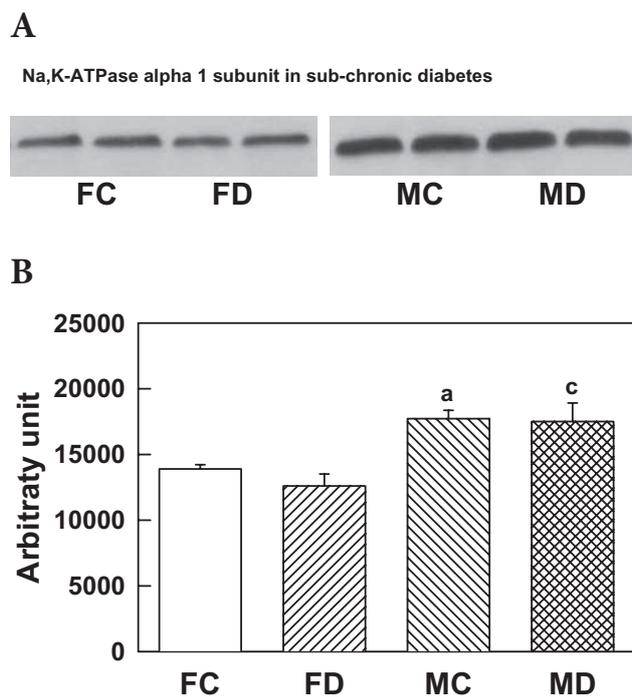
Diabetes in male rats induced a slight increase in enzyme activity throughout the investigated concentration range of ATP by 6–8% (Fig. 2). Consequently, kinetic parameters  $V_{\text{max}}$  and  $K_{\text{m}}$  did not change in MD group as compared to MC group (Fig. 3). When activating the enzyme with increasing concentration of cofactor we observed a biphasic effect on activity of  $\text{Na}^+, \text{K}^+$ -ATPase. In the presence of low concentration of  $\text{Na}^+$  the maximal inhibition reached 6% and in the presence of high concentration of  $\text{Na}^+$  the maximal stimulation reached 6% in the MD vs. MC group (Fig. 4). This biphasic effect in activities was reflected in significant increase of  $K_{\text{Na}}$  value by 18% in MD group accompanied with similar value of  $V_{\text{max}}$  in both male groups (Fig. 5).

Direct comparison of FD and MD groups showed higher stimulation (14–11%) of  $\text{Na}^+, \text{K}^+$ -ATPase activity in males

resulting in increased  $V_{\text{max}}$  value by 12% in the MD group (Fig. 2). The  $K_{\text{m}}$  value was lower in MD group by 23% (Fig. 3). Activation of the enzyme with increasing concentration of cofactor ( $\text{Na}^+$ ) resulted again in higher activities from MD as compared to FD group. The increase in activities of  $\text{Na}^+, \text{K}^+$ -ATPase varied in the range of 13% to 12% (Fig. 4). Evaluation of the above data resulted in statistically significant increase in  $V_{\text{max}}$  value by 12% without changes in the  $K_{\text{Na}}$  value (Fig. 5).



**Figure 5.** Kinetic parameters of renal  $\text{Na}^+, \text{K}^+$ -ATPase during activation with  $\text{Na}^+$  in female control rats (FC), in female rats with STZ-induced diabetes mellitus (FD), in male control rats (MC) and in male diabetic rats (MD). The parameter  $V_{\text{max}}$  represents the maximal velocity of enzyme reaction,  $K_{\text{Na}}$  value refers to the concentration of  $\text{Na}^+$  necessary for half maximal activation of the enzyme. Data represent means  $\pm$  S.E.M,  $n = 9$  in each group. <sup>a</sup>  $p < 0.001$  as compared to the FC group; <sup>b</sup>  $p < 0.001$  as compared to the MC group; <sup>c</sup>  $p < 0.01$  as compared to the FD group.



**Figure 6.** Relative abundance of renal  $\text{Na}^+, \text{K}^+$ -ATPase  $\alpha 1$  subunit in female control rats (FC), in female rats with STZ-induced diabetes mellitus (FD), in male control rats (MC) and in male diabetic rats (MD). **A.** Immunoblot analysis. **B.** Relative densities of bands. <sup>a</sup>  $p < 0.001$  as compared to the FC group; <sup>c</sup>  $p < 0.01$  as compared to the FD group,  $n = 4$  in all groups.

#### Quantification of $\text{Na}^+, \text{K}^+$ -ATPase $\alpha 1$ subunit

Abundance of  $\alpha 1$  subunit of  $\text{Na}^+, \text{K}^+$ -ATPase was significantly higher by 28% in MC as compared to FC rats. Similarly, comparison of both diabetic group resulted in significant higher presence of  $\alpha 1$  subunit by 39% in the MD group. On the other hand, sub-chronic diabetes did not induce any significant changes in the relative quantity of  $\text{Na}^+, \text{K}^+$ -ATPase  $\alpha 1$  subunit neither in female nor in male rats (Fig. 6).

#### Discussion

In our study, in rats suffering STZ-induced diabetes for 8 weeks, significant decrease in body weight gain was observed in rats of both genders. Previously published studies documented that in acute STZ-induced diabetes lasting 7–10 days the reduction represented 10–22% in male rats (Davel et al. 2000; Javorková et al. 2009). Four-six weeks after the STZ treatment, body weight was significantly reduced by 34% (Michea et al. 2001) and after 9 weeks the reduction yielded 30% (Ziegelhoffer et al. 1996). Prolonga-

tion of STZ-induced diabetes to 16 weeks was followed by 56% decrease in body weight of males (Vrbjar et al. 2004). The present data of 30% lowering in body weight in diabetic males fits relatively well to the time course of development of the disease.

The relative kidney weight expressed as the Kw/Bw (kidney weight/body weight ratio) ratio is approximately doubled in diabetic male rats throughout the development of the disease in acute as well as in chronic phase (Tsimaratos et al. 2001a,b; Vrbjar et al. 2004; Javorková et al. 2009). Our present finding of increased Kw/Bw ratio is in agreement with above data and indicates that 8 weeks lasting diabetes induced a renal hypertrophy. This fact is caused not only by lowered body weight but by real hypertrophy of kidney as documented by significant increase of kidney weight in diabetic male rats.

The present study suggests a hypothesis about a probable gender specific influence in female rats as the loss in weight gain was significantly lower in female diabetic rats amounting 21% only. The explanation of the mechanism of the above effect remains unclear and needs further investigation. However, the kidneys in females were hypertrophied to similar extent like in males as suggested by similar increase of kidney weight as well as the Kw/Bw ratio in rats subjected to 8 weeks lasting diabetes.

Gender dependent were also the properties of the renal  $\text{Na}^+, \text{K}^+$ -ATPase in control animals as suggested by enhanced ability to bind ATP in male rats as documented by the decreased  $K_m$  value. This difference is relatively small but it was statistically significant in this study. The significantly higher  $V_{max}$  value indicates the higher presence of active enzyme molecules in the renal tissue of male rats. This hypothesis is also supported by quantification of  $\text{Na}^+, \text{K}^+$ -ATPase  $\alpha 1$  subunit. This finding is in agreement with data published in our previous study (Javorková et al. 2009). The above discussed gender specific differences of the  $\text{Na}^+, \text{K}^+$ -ATPase seem to be bound to kidney, as for other organs like heart and vascular smooth muscle contradictory data were published. In cardiac tissue higher presence of active  $\text{Na}^+, \text{K}^+$ -ATPase molecules was observed in female rats (Liu et al. 2007). The  $\text{Na}^+, \text{K}^+$ -ATPase in kidney revealed two qualitative differences as compared to the cardiac enzyme. The first difference is in the affinities of the ATP binding site. The better affinity to substrate in kidney from males presented in this study differs from this property in heart while the cardiac  $\text{Na}^+, \text{K}^+$ -ATPase revealed similar affinity to ATP in both genders (Vlkovicova et al. 2005). The second qualitative difference concerns the sodium binding site. The renal  $\text{Na}^+, \text{K}^+$ -ATPase showed similar affinities to sodium in males and females as documented in the present study. However, the enzyme in female hearts seemed to be naturally adapted to higher intracellular concentration of sodium because the cardiac  $\text{Na}^+, \text{K}^+$ -ATPase in females was

able to increase its activity in the range where the enzyme in male rats is already saturated with  $\text{Na}^+$  (Vlkovicova et al. 2005).

Relating the quantitative properties, there is also a gender specific difference between the renal and cardiac  $\text{Na}^+, \text{K}^+$ -ATPase. In the renal tissue the enzyme shows slightly higher presence in male rats as documented in the present study, whilst in the cardiac tissue the enzyme is more abundant in females (Vlkovicova et al. 2005). Thus, beside the improved ability to extrude the excessive  $\text{Na}^+$  out from the cells, the higher presence of active enzyme molecules in the heart also implies the better functionality of the  $\text{Na}^+, \text{K}^+$ -ATPase in the cardiac tissue as compared to the renal tissue. The above gender specific differences between the renal and cardiac  $\text{Na}^+, \text{K}^+$ -ATPase may be ascribed to the organ specific action of estradiol. It was shown that in cardiac cells 17- $\beta$ -estradiol enhanced the activity of the enzyme (Dzurba et al. 1997; Liu et al. 2007), as well as the expression of the  $\text{Na}^+, \text{K}^+$ -ATPase  $\beta$  subunit (Liu et al. 2007). Also in vascular smooth muscle cells estradiol stimulated the  $\text{Na}^+, \text{K}^+$ -ATPase activity and its expression of  $\alpha 1$  subunit (Sudar et al. 2008). In renal tissue the estradiol acts on the enzyme indirectly *via* inhibiting the soluble adenylyl cyclase resulting in the decrease of catalytic activity of the  $\text{Na}^+, \text{K}^+$ -ATPase activity (Hallows et al. 2009).

The active number of  $\text{Na}^+, \text{K}^+$ -ATPase molecules in renal tissue of rats subjected to 8 weeks lasting diabetes was similar as indicated by unchanged  $V_{\max}$  values in male as well as in female rats as compared to their adequate controls. This supposal is confirmed also by similarities in immunoblot analysis of  $\alpha 1$  subunit expression of  $\text{Na}^+, \text{K}^+$ -ATPase in diabetic rats of both genders. These data are different from those observed in 8 days lasting acute diabetes, where mobilization of synthesis of  $\text{Na}^+, \text{K}^+$ -ATPase molecules was documented (Javorková et al. 2009). On the other hand, previous studies documented that chronic diabetes lasting 10–16 weeks decreased the activity of the  $\text{Na}^+, \text{K}^+$ -ATPase in male rats (Tsimaratos et al. 2001a,b; Unlucerci et al. 2001; Vrbjar et al. 2004, 2007). Correspondingly, the expression of  $\alpha 1$  and  $\beta 1$  subunits was decreased (Tsimaratos et al. 2001b). So, our data of similar amount of  $\text{Na}^+, \text{K}^+$ -ATPase in diabetic and control rats suggest that 8 weeks lasting sub-chronic diabetes may represent the critical period during the development of the disease, when the mobilization of the  $\text{Na}^+, \text{K}^+$ -ATPase observed in the acute phase turns to the damage of the enzyme.

Concerning the qualitative properties of  $\text{Na}^+, \text{K}^+$ -ATPase its  $\text{Na}^+$ -binding site starts to be damaged as suggested by decreased affinity as implied by increased  $K_{\text{Na}}$  value in diabetic rats of both genders. After acute form of diabetes the affinity to sodium remained unchanged (Javorková et al. 2009), while chronic 16 weeks lasting diabetes induced even more intensive deterioration of  $\text{Na}^+$ -binding properties (Vr-

bjar et al. 2004). So, from the point of view of  $\text{Na}^+$ -binding, the 8 weeks lasting diabetes represents the critical interval where the enzyme is already deteriorated what may result in decreased activity as well as in diminished transport of excessive sodium out from the cell.

The second investigated qualitative property of the  $\text{Na}^+, \text{K}^+$ -ATPase, it means the ability to bind substrate ATP revealed gender specific changes as a consequence of 8 weeks lasting diabetes. In female rats, hyperglycemia induced deterioration of the affinity of ATP binding site in the enzyme molecule as indicated by increased  $K_m$  value. In male rats, the ATP binding site of the enzyme was resistant to diabetes-induced complications as suggested by unchanged value of  $K_m$ . This finding is in agreement with our previous observation that during acute diabetes, the ATP binding site was affected already after 8 days in female rats in contrary to unaffected males (Javorková et al. 2009). In this connection, seems to be interesting the fact that in female rats the same dose of STZ was followed by much higher relative increase in blood glucose level in both experimental models (8 days and 8 weeks lasting diabetes) as compared to male rats. In acute model the increase in glucose represented 351% in FD vs. 159% in MD and in sub-chronic model it represented 338% in FD vs. 153% in MD. Thus, similar extent of hyperglycemia in both experimental models was followed by similar gender specific deterioration of ATP binding site during various stages of development of disease. The relatively higher hyperglycemia in females probably injured the ATP binding site already in acute phase of diabetes and this effect persisted also in later phase of the disease. On the other hand, in males the relative increase of glucose level was lower in diabetic rats, without any effect on the ATP binding site during the development of the disease.

## Conclusion

In summary, our present results bring new more detailed insight about functional alterations of renal  $\text{Na}^+, \text{K}^+$ -ATPase during development of STZ-induced diabetes with emphasis on gender specificity in rats. The period of 8 weeks represents the time when the mobilization of enzyme synthesis observed in acute diabetes is lost. In this time the renal  $\text{Na}^+, \text{K}^+$ -ATPase probably undergoes structural changes in the vicinity of  $\text{Na}^+$ -binding site as indicated by worsened affinity to sodium in both genders. Gender specific was the diabetes-induced decrease in affinity to substrate ATP which occurred in female rats only.

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