

Total β -Adrenoceptor Deficiency Results in Cardiac Hypotrophy and Negative Inotropy

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Summary

The present study investigated cardiac function in hearts of mice with total deficiency of the β_1 -, β_2 - and β_3 -adrenoceptors (TKO) in comparison to wildtype mice (WT). We investigated cardiac morphology and echocardiographic function, measured protein expression of Ca^{2+} -regulatory proteins, SERCA 2a activity, myofibrillar function, and performed running wheel tests. Heart weight and heart-to-body weight ratio were significantly smaller in TKO as compared to WT. This was accompanied by a decrease in the size of the cardiomyocytes in TKO. Heart rate and ejection fraction were significantly diminished in TKO as compared to WT. Protein expressions of SERCA 2a, ryanodine receptor and $\text{Na}^+/\text{Ca}^{2+}$ -exchanger were similar in TKO and WT mice, but phospholamban protein expression was increased. PKA-dependent phosphorylation of phospholamban at serine 16 was absent and CaMKII-dependent phosphorylation at threonine 17 was decreased in TKO. All alterations were paralleled by a decrease in SERCA 2a-activity. A similar maximal calcium-dependent tension but an increased myofibrillar calcium-sensitivity was measured in TKO as compared to WT. We did not observe relevant functional impairments of TKO in running wheel tests. In the absence of β -agonistic stimulation, SERCA 2a activity is mainly regulated by alterations of phospholamban expression and phosphorylation. The decreased SERCA 2a activity following

β -adrenoceptor deficiency may be partly compensated by an increased myofibrillar calcium-sensitivity.

Key words

Beta-1 adrenoceptor • Beta-2 adrenoceptor • Beta-3 adrenoceptor • Knockout • Mice

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Introduction

β -adrenergic stimulation is one of the main neurohumoral mechanisms modulating cardiac function in physiological (e.g. exercise) as well as pathophysiological conditions (e.g. heart failure). Three β -adrenoceptor (β -AR) subtypes have been described with the β_1 - and β_2 -adrenoceptors mediating positive inotropic reactions mainly by stimulating the G_s -protein/adenylate cyclase/protein kinase A pathway (for review see Wallukat 2002), whereas β_3 -adrenergic

stimulation is coupled to a cardiac release of nitric oxide *via* activation of the endothelial NO synthase (Brixius *et al.* 2004, Gauthier *et al.* 1998, Gauthier *et al.* 1996, Moniotte *et al.* 2001), the functional implication of which has still to be clarified.

Beta-adrenergic positive inotropic effects are paralleled by an increase of intracellular systolic Ca^{2+} concentrations due to an increase in the Ca^{2+} influx through the L-type Ca^{2+} channels (Bers 2002), which is subsequently followed by an increase in the Ca^{2+} release out of the sarcoplasmic reticulum, as well as a decrease in the myofibrillar Ca^{2+} sensitivity (Brixius *et al.* 2002, van der Velden *et al.* 2003) and an increase in the Ca^{2+} accumulation by the sarcoplasmic reticulum (Bers 2002). The latter can be attributed to an increase in the activity of the sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA 2a) due to an increased phosphorylation of serine 16 (PLB-Ser16) and threonine 17 (PLB-Thr17) phospholamban (PLB) (Kranias and Solaro 1982, MacLennan and Kranias 2003).

Only recently, a transgenic mouse model was described with chronic deficiency of all three β -adrenoceptor subtypes (Bachman *et al.* 2002, 2004, Jimenez *et al.* 2002, Lee *et al.* 2008). The mice are viable and are characterized by cold intolerance due to impaired adaptive responses of the brown adipose tissue to cold exposure. We were interested in the regulation of the intracellular Ca^{2+} -homeostasis and myofibrillar function under conditions of chronic β -adrenergic deficiency. Therefore, we investigated cardiac morphology and echocardiographic function in total β -adrenoceptor knockout mice in comparison to their isogenic littermates. In addition, we measured protein expression of Ca^{2+} regulatory proteins, SERCA 2a activity, myofibrillar function, and performed running wheel tests.

Materials and Methods

Transgenic animals

Studies were performed on 12-week-old female $\beta_{1/2/3}$ -AR-KO mice (TKO = $\beta_{1/2/3}$ -KO = $\beta_1^{-/-}\beta_2^{-/-}\beta_3^{-/-}$) compared to the respective wildtype mice (WT = $\beta_1^{+/+}\beta_2^{+/+}\beta_3^{+/+}$). Creation of the TKO-mice has been described before (Jimenez *et al.* 2002). Mice were maintained at an artificial light/dark cycle at 20-22 °C with free access to water and standard laboratory chow diet. Following cervical dislocation the hearts were immediately taken out for further experiments and prepared as described below. The investigation conforms with the Guide for the

Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). The experiments were approved by the local animal ethics committee.

Morphological analysis

After being weighed the hearts were fixed with 4 % paraformaldehyde, dehydrated with alcohol, embedded in paraffin, and cut into 7 μm slices, which were stained with hematoxylin/eosin. For each group four slices of the left anterior wall were obtained and five photographs were taken at 400fold magnification (Leica DC500) respectively. Cross-sectional areas of five cardiomyocytes were determined by digitizing the images and computerized pixel counting including only nucleated myocytes for the analysis as described by Engelhardt *et al.* (1999).

Echocardiography

Transthoracic echocardiography was performed in 3-month-old mice as previously described (Tiemann *et al.* 2003). Mice were anesthetized with enflurane (3 % for induction and 0.8-1 % for echocardiography) in 50 % nitrous oxide and 50 % oxygen by face mask. The degree of anesthesia was adapted to minimize cardiodepressive effects. Thus the heart rate during anesthesia was maintained in the physiological range of conscious mice under resting conditions. The core temperature was maintained at 37 °C using a feedback-controlled heating pad. Echocardiography was performed using a commercially available ultrasound system equipped with a linear array transducer operating at an emission frequency of 15 MHz (harmonic-mode) with frame rates up to 280 Hz (HDI-5000, Philips Medical Systems, Bothell, WA, USA). Analysis included heart rate, left ventricular end-systolic and end-diastolic volume. For calculation of volumes the area-length method was used (Ghanem *et al.* 2006, Tiemann *et al.* 2003).

Tissue preparation

For the preparation of Western blot samples tissue of each heart was prepared as previously described (Munch *et al.* 1998). Briefly, 25-35 mg of left ventricular myocardial samples were thawed on ice in threefold volume of chilled preparation buffer (in mmol/l: sucrose 300, phenyl methyl sulfonyl fluoride 1, piperazine-N,N-bis(2-ethane sulfonic acid) 20, EDTA 1, P_i 10, pH 7.4). Care was taken to dissect myocardial tissue from

connecting tissue, vessels, and pericardium. Samples were homogenized with a glass-teflon homogenizer (Potter Typ RN 24, 350 upm, IKA Labor Technik Stauffen) under constant 4 °C temperature. The homogenates were immediately shock-frozen in liquid nitrogen and stored at -80 °C in freezing buffer (in mmol/l: sucrose 400, NaH₂PO₄ 50, EDTA 10, piperazine-N,N-bis(2-ethane sulfonic acid) 5, 2-amino-2-(hydroxymethyl)-1,3-propanediol 5, pH 7.2). Protein content was measured according to Bradford (1976).

Western blot analysis

For detection of SERCA 2a, PLB, phosphorylated PLB-Ser16, phosphorylated PLB-Thr17, ryanodine receptor and Na⁺/Ca²⁺ exchanger (NCX) in WT and TKO myocardium, immunoblotting techniques were performed as described previously, with slight modifications (Schwinger *et al.* 1995). Equal amounts of membrane proteins (10 μ g) were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with 4 % stacking gel and 12 % acrylamide. The linear range of the antibodies used was determined in previous experiments (Schwinger *et al.* 1995). To control protein load, we performed Coomassie blue staining of the gel. After blotting, we controlled protein transfer using Ponceau red staining of the membrane. For the immunoreaction, the following commercially available primary antibodies were used: anti-SERCA 2a (mouse) from Affinity BioReagents (Golden, CO, USA); anti-ryanodine receptor (mouse) from Affinity BioReagents; anti-PLB phosphorylated at Ser16 and Thr17 (rabbit), antibodies against sequence RSAIRRASTIEY (residues 9-19 of PLB, +Y) in which Ser16 or Thr17 is phosphorylated, from Cyclacel (Dundee, UK); anti-PLB (mouse) from Upstate Biotechnology (Dundee, UK), clone A1, anti-Na⁺/Ca²⁺ exchanger (mouse) from Novus Biologicals (Littleton, CO, USA). For detection, after washing procedures with TBST and TBS, a secondary peroxidase-conjugated mouse or rabbit IgG (Sigma) was used where appropriate. To detect antibody binding, an enhanced chemoluminescence assay (Lumi-Light Western Blotting Substrate, Roche, Grenzach, Germany) was used. After exposure to film (Hyperfilm ECL, Amersham Biosciences, Buckinghamshire, UK) and scanning, the bands were quantified by densitometry.

Preparation of the SR

The SR was prepared according to the methods described earlier (Meissner and Henderson 1987,

Sitsapesan and Williams 1990). Myocardial tissue was ground in liquid nitrogen. The following preparation was made at 4 °C in the cold room. The tissue was chilled in ice-cold homogenization buffer (in mmol/l: sucrose 300, PMSF 1, PIPES 20, EDTA 10 and NaH₂PO₄ 50, pH 7.4), containing NaH₂PO₄ to inhibit phosphatases, thereby avoiding *in vitro* PLB dephosphorylation. PMSF irreversibly inhibits serine proteases by sulfonylation of the serine residue in the active site of the protease and thereby avoids protein decomposition. Connective tissue was carefully trimmed away, and myocardial tissue was homogenized with a motor-driven homogenizer (Braun, Melsungen, Germany). The preparations were stored at -80 °C in a buffer containing (in mmol/l) sucrose 400, HEPES 5, Tris 5, EDTA 10 and NaH₂PO₄ 50, pH 7.2.

Measurement of SERCA 2a activity

Analysis of SERCA 2a activity was carried out according to the method described by Chu *et al.* (1988), which is based on coupled enzymatic reactions as follows: i) ATP \rightarrow ADP + P_i (this reaction is catalyzed by SERCA 2a), ii) ADP + phosphoenolpyruvate \rightarrow ATP + pyruvate (catalyzed by pyruvate kinase), and iii) pyruvate + NADH \rightarrow lactate + NAD⁺ (catalyzed by lactate dehydrogenase). The oxidation of NADH was continuously monitored by the decrease in absorbance at 340 nm with a spectrophotometer (Beckman DU 640). The reaction was carried out in 1 ml at 37 °C. 20 μ l of the SR preparation (final protein concentration, 50 μ g/ml) was suspended in 1000 μ l of the reaction mixture. The reaction mixture had the following composition (in mmol/l: MOPS 21, NaN₃ 4.9, EGTA 0.06, KCl 100, MgCl₂ 3, phosphoenolpyruvate 1, NADH 0.2 and pyruvate-lactate dehydrogenase LDH enzyme mixture (8.4/12 U)). This led to the following concentrations in the cuvette (in mmol/l): MOPS 21, NaN₃ 4.9, EGTA 0.06, KCl 100, MgCl₂ 3, phosphoenolpyruvate 1, NADH 0.2, sucrose 7, HEPES 0.05, Tris 0.05, EDTA 0.2, PMSF 0.01, PIPES 0.2 and NaH₂PO₄ 1, pH 7.2. CaCl₂ was added to the reaction mixture to yield the desired free Ca²⁺ concentrations, calculated according to the method of Fabiato and Fabiato (1979). Calculations performed by this method are an approximation. We did not measure Ca²⁺ concentrations directly. The reaction was started with ATP (1 mmol/l) and was constant over at least 5 min. The basal activity was measured in the absence of Ca²⁺ and in the presence of EGTA (4 mmol/l) simultaneously. All experiments were carried out in duplicate. Experiments were performed as described before (Schwinger *et al.* 1995).

Table 1. Morphometric analysis and echocardiography.

Parameter	WT	TKO	p-value
Body weight (g)	30.81 ± 3.08 (n=5)	31.00 ± 0.94 (n=5)	0.900
Heart weight (mg)	121 ± 3 (n=5)	94 ± 7 (n=5)	< 0.001
Heart to body weight ratio (mg/g)	3.96 ± 0.49 (n=5)	3.02 ± 0.18 (n=5)	0.004
Cross-sectional area (% of WT)	100 ± 26.3 (n=4)	67.0 ± 31.6 (n=5)	< 0.001
Heart rate (beats/min)	403 ± 24 (n=4)	267 ± 69 (n=7)	0.005
Left ventricular end-systolic volume (μ l)	7.4 ± 4.9 (n=4)	15.1 ± 5.0 (n=7)	0.036
Left ventricular end-diastolic volume (μ l)	37.2 ± 6.1 (n=4)	39.0 ± 6.2 (n=7)	0.649
Ejection fraction (%)	81 ± 11 (n=4)	62 ± 9 (n=7)	0.013

Triton X-skinned fibres measurements

Left ventricular muscle fibres were prepared as previously described (Brixius and Schwinger 2000). Briefly, the fibre bundles (diameter <0.2 mm) were dissected from the left ventricular papillary muscles and permeabilized at 4 °C for 20 h in a solution containing 50 % (v/v) glycerol, 1 % Triton X, and (in mmol/l) NaN₃ 10, ATP 5, MgCl₂ 5, EGTA 4, 1,4-dithioerythritol (DTE) 2, and imidazole 20 (pH 7.0). Afterwards, the fibres were stored in a similar solution but without Triton X at -20 °C. For the experiments, the Triton X-skinned fibre bundles were prepared under the microscope and then mounted isometrically and connected to a force transducer in a perfusion cuvette (Scientific Instruments, Heidelberg, Germany) in relaxation solution (composition in mmol/l: imidazole 20, Na₂ATP 10, NaN₃ 5, EGTA 5, MgCl₂ 12.5, dithiothreitol 1, creatine phosphate 10, creatine kinase 1 mg/ml, free Ca²⁺ 10 nM, pH 7.0). Fibre length was adjusted so that resting tension was just threshold (slack position). The fibres were then incubated in contraction solution (composition as above, but with increasing concentrations of free Ca²⁺ estimated according to the method of Fabiato and Fabiato (1979)). The experiments were performed at room temperature. To normalize force, the absolute force values obtained were divided by the cross-sectional area of the skinned fibres (CSA = 2 π r²). Experiments were performed as described previously (Brixius and Schwinger 2000).

Measurement of force development

Force development was measured as described before (Brixius and Schwinger 2000, Guth and Wojciechowski 1986) (experimental setup, Scientific Instruments, Heidelberg, Germany). The relaxation solution contained (in mmol/l) imidazole 20, Na₂ATP 10, NaN₃ 5, EGTA 5, MgCl₂ 12.5, phospho(enol)-pyruvate 5,

NADH 0.6, P₁,P₅-di(adenosine 5') pentaphosphate 0.2 (myokinase inhibitor), and cyclopiazonic acid 25, together with pyruvate kinase 100 U/ml and lactate dehydrogenase 125 U/ml. The contraction solution contained calcium EGTA 5 mmol/l instead of EGTA. Both solutions were mixed by a gradient mixer so that Ca²⁺ was successively increased every 30 s. Free Ca²⁺ concentration was determined by calculator programs designed for experiments in skinned muscle cells (Fabiato and Fabiato 1979). Measurement of developed tension started 3 s after the solution was exchanged. Developed tension had reached a stable plateau at that time.

Running wheel tests

Running wheel tests have been shown to be suitable to assess cardiovascular function of transgene mouse models of human cardiovascular diseases (Bernstein 2003). Mice were held in cages for 4 days in quiet surroundings at room temperature of 20-22 °C having the possibility to exercise on running wheels with free access to water and standard laboratory chow diet. The results were measured by a tachometer attached to the running wheel.

Materials

All chemicals were of analytical grade or the best grade commercially available. All compounds were dissolved in twice-distilled water and did not change the pH of the medium.

Statistical analysis

Data are presented as mean ± S.D. Data analysis was performed using Student's t-test for paired and unpaired data, where appropriate. Significance was considered at p<0.05 value.

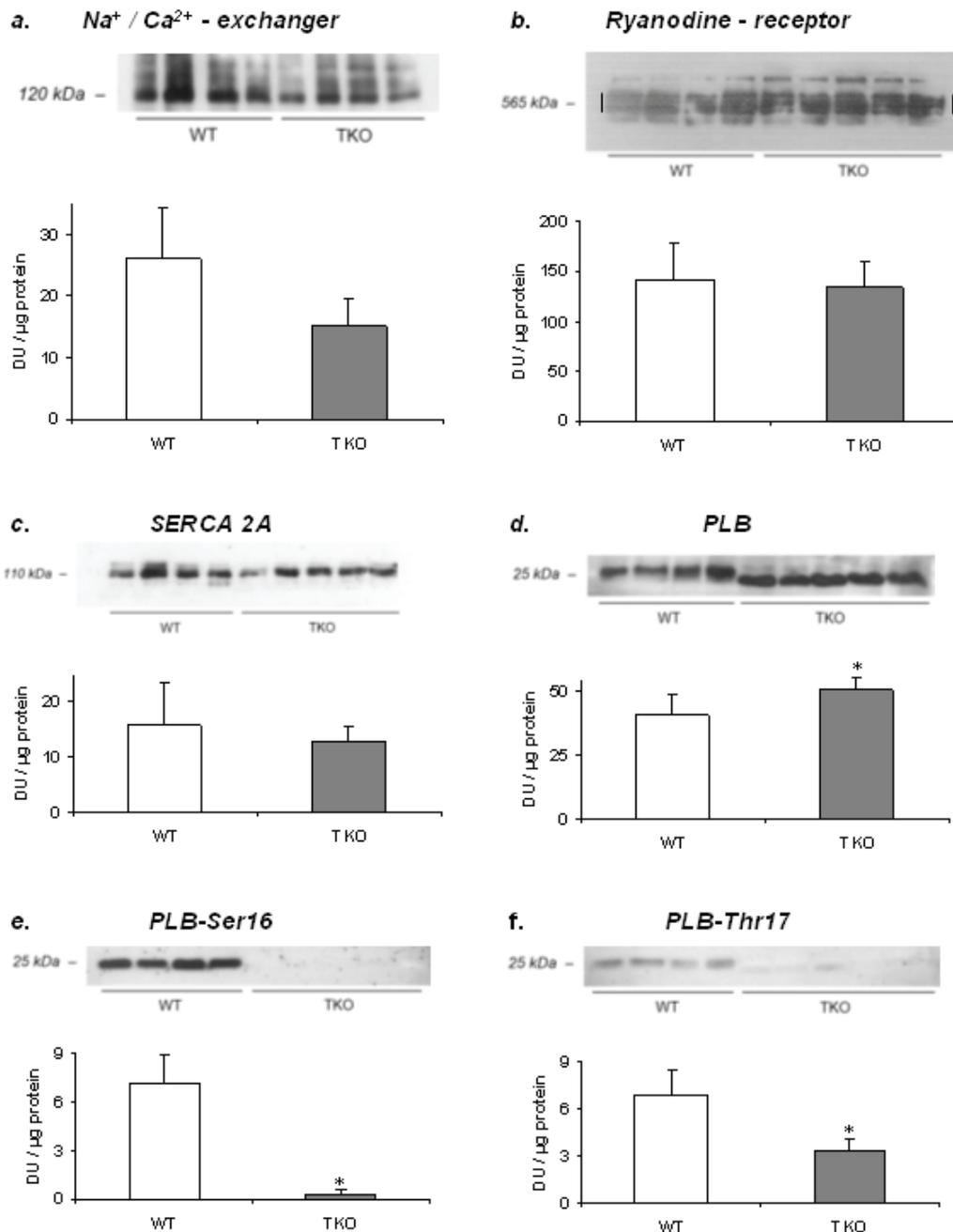


Fig. 1. The protein expression of NCX, ryanodine receptor and SERCA 2a was not significantly changed, whereas PLB (* $p < 0.02$ vs. WT) expression was significantly increased and PLB-Ser16 (* $p < 0.0001$ vs. WT) and PLB-Ser17 (* $p < 0.005$ vs. WT) expression was decreased in TKO-mice. Data was normalized to calsequestrin expression.

Results

Morphometric analysis and echocardiography

Body weight was similar in TKO and WT mice, whereas heart weight and heart-to-body-weight ratio were significantly decreased in TKO mice. In line with these findings, myocyte cross-sectional area was also significantly decreased. Echocardiographic analysis provided evidence that heart rate and ejection fraction were significantly diminished in TKO mice as compared

to WT mice. Thus, chronic β -AR deficiency is accompanied by cardiac hypotrophy as well as negative inotropy and chronotropy (Table 1).

Ca^{2+} regulatory proteins

To investigate whether the negative inotropy may be due to alterations of the Ca^{2+} regulatory proteins, we performed Western blot experiments comparing TKO ($n=5$) and WT mice ($n=4$). The expression of the ryanodine receptor, the Na^+/Ca^{2+} exchanger and of

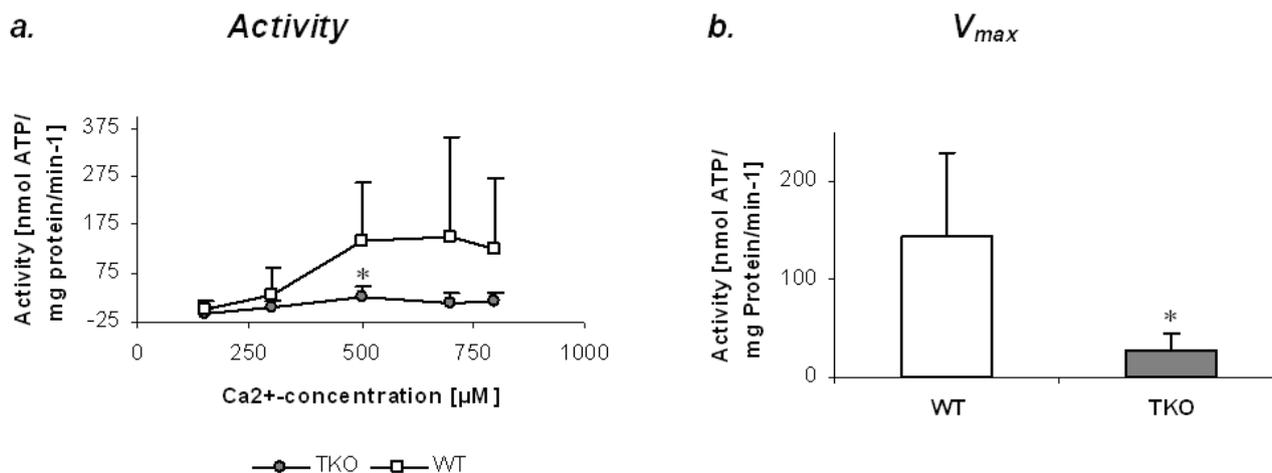


Fig. 2. The maximal activity (V_{max}) of SERCA 2a in TKO mice was 28.1 ± 16.3 nmol ATP/mg protein/min⁻¹ ($n=6$) compared to 144.4 ± 84.0 nmol ATP/mg protein/min⁻¹ ($n=4$) in WT mice. The activity of SERCA 2a was significantly decreased in TKO mice (* $p < 0.01$ vs. WT).

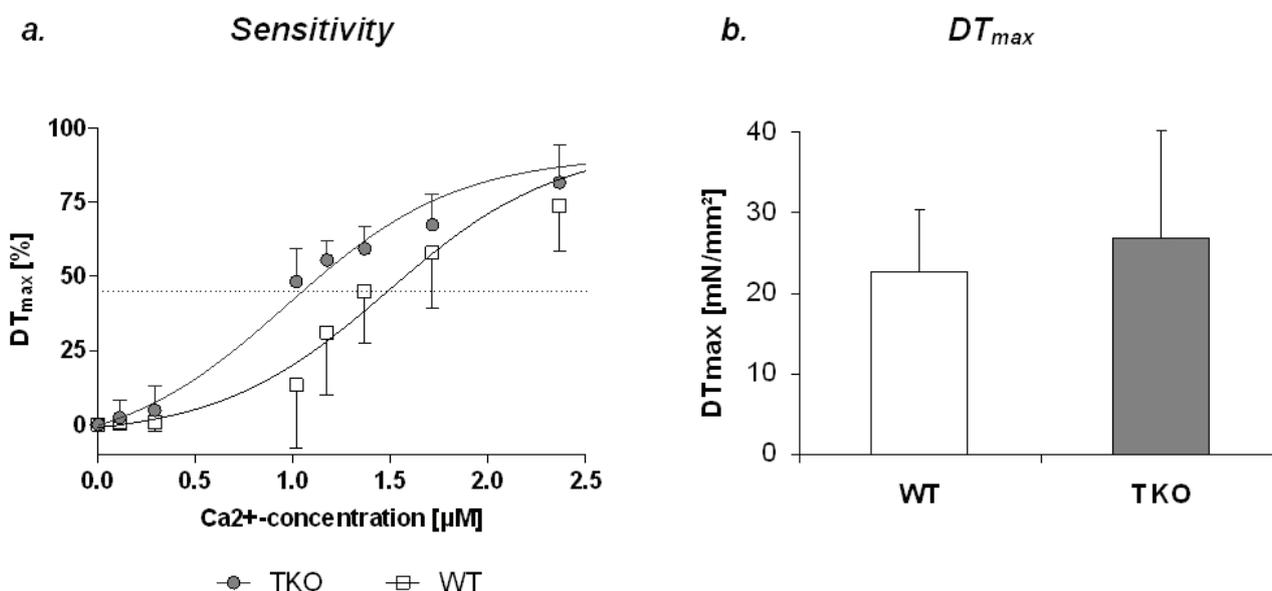


Fig. 3. The maximal force (DT_{max}) of skinned fibers in TKO mice was similar to WT mice, calcium-sensitivity was significantly increased in TKO mice vs. WT mice.

SERCA 2a were unchanged in the TKO compared to WT mice. The expression of phospholamban was significantly increased, whereas phosphorylation of phospholamban at serine 16 and threonine 17 revealed a marked decrease (Fig. 1). In line with these findings, maximal SERCA 2a activity was significantly decreased in TKO compared to the WT mice (Fig. 2).

Myofibrillar function

To assess myofibrillar function, we measured Ca²⁺ sensitivity of skinned fibres in TKO and WT mice by performing measurements of force development of Triton X-skinned fibres within increasing Ca²⁺

concentrations (Fig. 3). A similar maximal calcium-dependent tension (WT 22.6 ± 7.7 mN/mm², $n=6$; TKO 26.9 ± 13.4 mN/mm², $n=6$; n.s.), but an increased myofibrillar calcium-sensitivity was found in TKO as compared to WT mice (EC_{50} : WT 1.49, 95 % confidence interval 1.36-1.62 µM, $n=6$; TKO 0.97, 95 % confidence interval 0.83-1.11 µM, $n=6$).

Running wheel tests

To investigate the influence of the cardiac alterations observed in TKO mice on the exercise capacity, TKO and WT mice performed a voluntary running wheel experiment. Mean velocity (WT 0.34

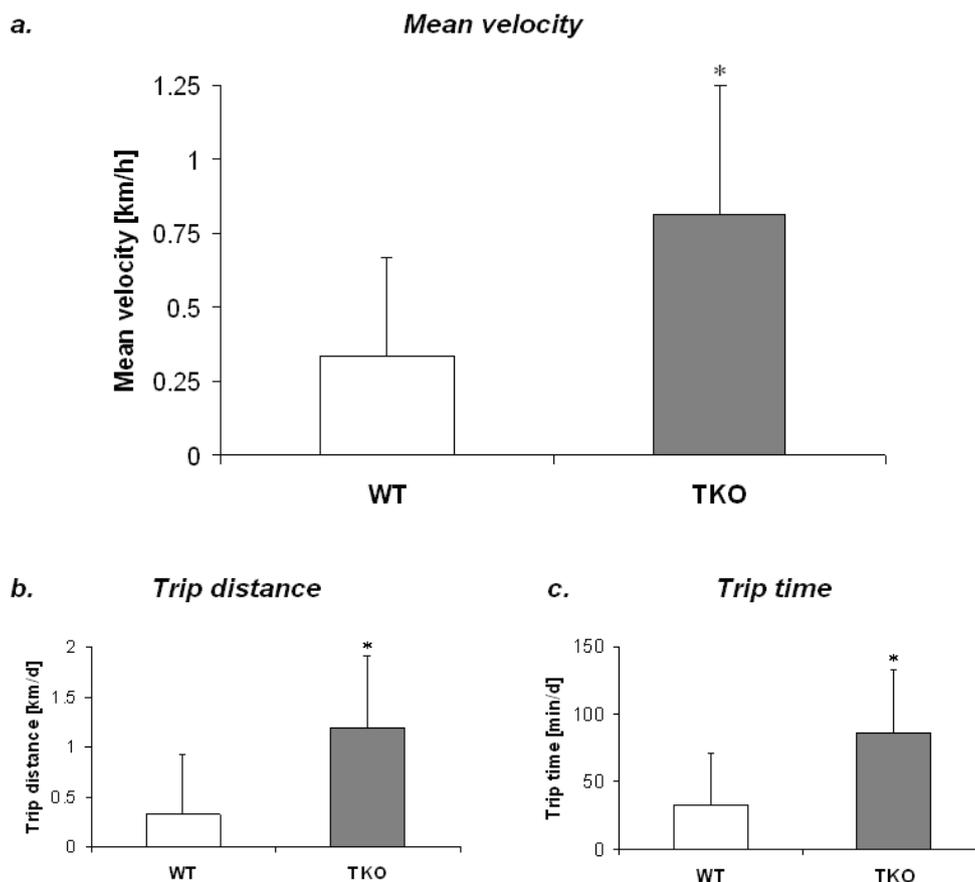


Fig. 4. In running wheel tests there could not be shown any diminished exercise capacity of TKO mice compared to WT mice. An increased willingness to run could be observed in TKO mice which reached statistical significance for mean velocity (* $p < 0.04$ vs. WT), trip distance (* $p < 0.03$ vs. WT) and trip time (* $p < 0.04$ vs. WT).

± 0.33 km/h, $n=6$; TKO 0.81 ± 0.44 , $n=6$), trip distance (WT 0.33 ± 0.60 km/d, $n=6$; TKO 1.19 ± 0.72 km/d, $n=6$) and trip time (WT 29.0 ± 37.7 min/d, $n=6$; TKO 86.6 ± 46.5 min/d, $n=6$) were significantly increased in TKO as compared to WT mice (Fig. 4).

Discussion

The present study investigated the influence of β -adrenoceptor deficiency on cardiac function using a knockout (KO) model. It was shown that the $\beta_{1/2/3}$ -KO leads to a significantly decreased heart weight, heart rate and ejection fraction accompanied by altered expression and activity of important Ca^{2+} regulating proteins. Chronic $\beta_{1/2/3}$ -AR deficiency reduced SERCA 2a activity by an increase in the expression level of PLB and a diminished PLB-Ser16 and PLB-Thr17 phosphorylation. In spite of these findings, we did not observe relevant functional impairments in running wheel tests but rather an increased willingness to run. The decreased SERCA 2a activity following β -adrenoceptor deficiency may be,

at least partly, compensated by an increased myofibrillar calcium-sensitivity.

The regulation of cardiomyocyte growth under physiological and pathophysiological conditions is very complex, but one of its controlling signaling pathways is the β -adrenergic system (MacLellan and Schneider 2000). In β_1 -overexpression (OE)-mice, an increased heart-to-body weight ratio was described which led to a substantial hypertrophy of cardiomyocytes and a concomitant fibrosis (Engelhardt *et al.* 1999). While in early studies β_2 -OE-animals did not seem to have any morphological differences compared to their WT, it was shown in later studies that overexpression of the β_2 -adrenoceptor was linked with cardiac hypertrophy associated with a cumulative mortality of 81 % within 15 months compared to 4 % in WT-mice (Du *et al.* 2000). β_3 -OE-mice featured a decreased heart-to-body weight ratio compared to their WT (Kohout *et al.* 2001). Whereas overexpression of the various β -adrenoceptor subtypes alters cardiac morphology, neither the β_1 - nor the β_3 -KO-mice had an altered heart-to-body weight ratio

(Rohrer *et al.* 1996, Varghese *et al.* 2000). Recently it could be shown, however, that a $\beta_{1/2}$ -knockout leads to a decreased heart-to-body weight ratio (Kiriazis *et al.* 2008). Since the present study also found a decrease in the heart-to-body weight ratio in TKO mice, whereas the β_1 - and β_3 -knockouts did not effect the heart-to-body weight ratio at all, it may be concluded that in maintaining a certain myocyte volume there has to be a functional redundancy among the β -adrenoceptors, so that only a blockade of at least the β_1 - and the β_2 -adrenoceptor entails significant changes in the heart-to-body-weight ratio.

Human heart failure is characterized by profound dysregulation of intracellular Ca^{2+} homeostasis. Thus, structural and functional alterations in the Ca^{2+} regulatory proteins present in the sarcoplasmic reticulum (SR) have been shown to be strongly involved in the pathogenesis of heart failure (Yano *et al.* 2005). Although it is a matter of an ongoing discussion whether SERCA 2a and PLB expression is altered, it can be stated that SERCA 2a activity is depressed in the setting of human heart failure due to an attenuated PLB-Ser16- and PLB-Thr17 phosphorylation (Dash *et al.* 2001, Schwinger *et al.* 1995). PLB phosphorylation causes a disinhibition of SERCA 2a resulting in an enhanced diastolic Ca^{2+} sequestration into the SR (Frank *et al.* 2003).

The diminished SERCA 2a activity we found in TKO-mice was due to an increased PLB expression and a decreased PLB-Ser16 and PLB-Thr17 phosphorylation. It should be pointed out that PLB-Thr17 phosphorylation was decreased, but not totally absent in $\beta_{1/2/3}$ -deficient mice. For some time it has been discussed whether Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII)-dependent phosphorylation of PLB-Thr17 is reliant on the presence of β -adrenergic stimulation or not. Whereas some studies came to the conclusion that phosphorylation of PLB-Ser16 and β -adrenergic stimulation are prerequisites for PLB-Thr17 phosphorylation, other studies reported PLB-Ser16-independent phosphorylation of PLB-Thr17 (for review see Mattiazzi *et al.* 2005). Finally, the experiments with a phosphatase inhibitor ('okadaic acid') showed, that PLB-Thr17 can indeed be phosphorylated in the absence of significantly elevated cAMP-levels or concomitant PLB-Ser16 phosphorylation (Mundina-Weilenmann *et al.* 1996). Regarding the β -adrenoceptor-independent phosphorylation of phospholamban, the important role of type 1 phosphatase (PP1) has to be mentioned. PP1 represents the main SR phosphatase which among others dephosphorylates

CaMKII. This facilitates CaMKII to perpetuate its activity independently of the intracellular Ca^{2+} concentration (Bradshaw *et al.* 2003). Thus this mechanism may be responsible for the slightly sustained PLB-Thr17 phosphorylation in TKO mice. Further studies have to verify this assumption.

It is somewhat surprising that such a complex organism, that a mouse represents, can survive without any β -adrenoceptors. Obviously the organism has other mechanisms besides the β -adrenergic system by which cardiovascular function can be sustained. One of these mechanisms might be the increased myofibrillar calcium-sensitivity which could be found in TKO-mice. Furthermore, studies with β_1 -KO-mice could show, that heart rate in these animals is regulated by the vagal tone, therefore by the parasympathetic nervous system (Rohrer *et al.* 1998). In addition, it has been shown that TKO mice have a reduced metabolic rate compared to WT mice (Bachman *et al.* 2002). This can be seen as a contributing factor to the reduced cardiac output found in these animals. So despite of a complete knockout of the β -adrenoceptors the TKO mice still performed even better in the running wheel tests than their WT littermates. Whereas both β_1 - and $\beta_{1/2}$ -KO-mice showed no differences in exercise capacity in treadmill experiments (Rohrer *et al.* 1998, 1999), β_2 -KO-mice also has an increased exercise capacity (Chruscinski *et al.* 1999). In these mice during exercise fat burning was increased which led to a prolonged availability of glycogen reserves in the musculature while the body fat content was significantly lower in β_2 -KO-mice compared to WT mice. So it seems that the greater exercise capacity of TKO mice may be, at least partly, attributed to the lack of the β_2 -adrenoceptors. A limitation of the present study is that the animals were running voluntarily. Therefore, it is possible that our data do not necessarily represent maximal exercise capacity of these animals.

Conclusions

Despite β -adrenoceptor deficiency is going along with cardiac hypotrophy and a decrease in cardiac function, willingness to run is not impaired. These results indicate that compensatory mechanisms may be activated to maintain cardiac function in increased cardiac stress situations. These mechanisms have to be focused in future studies.

Conflict of Interest

There is no conflict of interest.

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