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RESEARCH PAPER

Cardiac dysfunction in adipose triglyceride lipase deficiency: treatment with a PPARα agonist

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BACKGROUND AND PURPOSE

Adipose triglyceride lipase (ATGL) has been identified as a rate-limiting enzyme of mammalian triglyceride catabolism. Deletion of the ATGL gene in mice results in severe lipid accumulation in a variety of tissues including the heart. In the present study we investigated cardiac function in ATGL-deficient mice and the potential therapeutic effects of the PPARα and γ agonists Wy14,643 and rosiglitazone, respectively.

EXPERIMENTAL APPROACH

Hearts isolated from wild-type (WT) mice and ATGL(-/-) mice treated with Wy14,643 (PPARα agonist), rosiglitazone (PPARγ agonist) or vehicle were perfused at a constant flow using the Langendorff technique. Left ventricular (LV) pressure–volume relationships were established, and the response to adrenergic stimulation was determined with noradrenaline (NA).

KEY RESULTS

Hearts from ATGL(-/-) mice generated higher LV end-diastolic pressure and lower LV developed pressure as a function of intracardiac balloon volume compared to those from WT mice. Likewise, passive wall stress was increased and active wall stress decreased in ATGL(-/-) hearts. Contractile and microvascular responses to NA were substantially reduced in ATGL(-/-) hearts. Cardiac contractility was improved by treating ATGL(-/-) mice with the PPARα agonist Wy14,643 but not with the PPARγ agonist rosiglitazone.

CONCLUSIONS AND IMPLICATIONS

Our results indicate that lipid accumulation in mouse hearts caused by ATGL gene deletion severely affects systolic and diastolic function, as well as the response to adrenergic stimulation. The beneficial effects of Wy14,643 suggest that the cardiac phenotype of these mice is partially due to impaired PPARα signalling.

Abbreviations

ATGL, adipose triglyceride lipase; CPP, coronary perfusion pressure; DEA/NO, 2,2-diethyl-1-nitroso-oxyhydrazine; FA, fatty acid; LVDevP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; LVSP, peak left ventricular systolic pressure; NA, noradrenaline; WT, wild-type; Wy14,643, [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid

Introduction

Cardiomyopathy is a frequent and significant complication of metabolic disorders such as obesity, insulin resistance and diabetes mellitus. At an early, preclinical stage, cardiomyopathy is characterized by left ventricular (LV) diastolic filling impairments (Schannwell *et al.*, 2002) and evolves over time to both systolic and diastolic dysfunction. Under physiological conditions, cardiomyocytes preferentially utilize long-chain fatty acids (FAs) for ATP generation

(Neely *et al.*, 1972). FAs are supplied to the heart via hydrolysis of triglyceride-rich lipoproteins and by uptake of serum albumin-bound FAs originating from adipose tissue stores. In situations of reduced oxygen availability, such as ischaemia or hypertrophy, cardiomyocyte metabolism is shifted towards the utilization of glucose (Wittels and Spann, 1968; Bishop and Altschuld, 1970). In the diabetic heart, the ability of cardiomyocytes to switch from free FA to glucose metabolism is disrupted. Due to the increased supply of circulating FAs and systemic insulin resistance, the diabetic cardiac phenotype is characterized by increased uptake of FAs and depressed glucose metabolism (Feuvray and Darmellah, 2008), resulting in markedly increased triglyceride storage in cardiomyocytes (Sharma *et al.*, 2004). There is convincing evidence that ectopic fat accumulation is toxic to cardiomyocytes and plays an important role in the progress of cardiomyopathy and heart failure (Borradaile and Schaffer, 2005).

Recently, genetic mouse models of cardiac-restricted steatosis have been engineered to unravel the molecular mechanisms of lipotoxicity. As shown by Schaffer and coworkers, myocyte-specific overexpression of the FA transporter protein 1 leads to the accumulation of FAs in the heart and results in a cardiac phenotype similar to that observed in early-stage metabolic cardiomyopathy (Chiu *et al.*, 2005). Murine hearts with an overexpression of glycosylphosphatidylinositol-anchored lipoprotein lipase (Yagyu *et al.*, 2003), PPARα (Finck *et al.*, 2002), or long-chain acyl-CoA synthetase A (Chiu *et al.*, 2001) show severe steatosis, systolic dysfunction and hypertrophy, resembling a phenotype observed in end-stage metabolic cardiomyopathies.

Adipose triglyceride lipase (ATGL) has been identified as the key enzyme of triglyceride catabolism, which functions as a monoacyl hydrolase and catalyzes the initial, rate-limiting step of the triacylglycerol lipolysis cascade (Zimmermann *et al.*, 2004). ATGL is predominantly expressed in adipose tissue but is also found to a lesser extent in cardiac muscle, skeletal muscle, testis and other tissues. Systemic deletion of the ATGL gene in mice resulted in massive accumulation of neutral lipids in most tissues (Haemmerle *et al.*, 2006). Similarly, humans with ATGL gene mutations develop neutral lipid storage disease with fat accumulation in multiple tissues (Fischer *et al.*, 2007; Campagna *et al.*, 2008; Hirano *et al.*, 2008). Given that ATGL-deficient mice exhibit increased glucose uptake and utilization, increased glucose tolerance and insulin sensitivity, the metabolic pattern of those animals is opposite to that of diabetic, obese individuals. In cardiac muscle, an age-dependent increase in the number and size of myocyte lipid droplets was observed. In parallel, as shown by two-dimensional echocardiography, cardiac dysfunction progressively developed with age, leading to severe impairments in, and premature death of, ATGL-deficient animals (Haemmerle *et al.*, 2006).

PPARs are members of the nuclear receptor family of ligand-activated transcription factors. They are activated by FAs or FA derivatives and expressed in many cell types including adipocytes, cardiomyocytes, as well as endothelial and vascular smooth muscle cells. Given that lipolysis is necessary to provide FAs as ligands for PPARs, altered PPAR function might contribute to the development of cardiac dysfunction in ATGL-deficient animals. By modulating the expression of a wide variety of target genes, PPARs have pleiotropic effects, including the regulation of metabolism, inflammation, atherogenesis and thrombosis. The PPARα subtype appears to control the expression of genes involved in lipid metabolism, while the PPARγ subtype is a key regulator of glucose homeostasis (Barbier *et al.*, 2002; Marx *et al.*, 2004). In the last decade, genetic and pharmacological approaches have provided abundant evidence for a crucial role of PPARs in the pathogenesis of cardiovascular dysfunction (Schiffrin, 2005; Touyz and Schiffrin, 2006; Zahradka, 2007; Robinson and Grieve, 2009).

In the present study we applied Langendorff perfusion of isolated hearts to investigate the cardiac performance of 9- to 10-week-old ATGL-deficient mice in comparison with age-matched littermates *in vitro*. Special emphasis was placed on the isovolumic pressure–volume relationship and the cardiac responsiveness to adrenergic stimulation. The potential involvement of defective PPAR signalling was addressed by feeding the mice the PPARα and PPARγ agonists Wy14,643 and rosiglitazone, respectively.

Methods

Mice and experimental groups

Homozygous ATGL-deficient [ATGL(-/-)] mice of either sex and their corresponding wild-type (WT) littermates were used in this study. In our preliminary study (Haemmerle *et al.*, 2006), the first impairments in cardiac function and lipid accumulation in ATGL(-/-) mice became evident at the age of 6 weeks (comparable with an early diagnosis of neutral lipid storage disease in humans). Therefore, ATGL(-/-) and WT animals (6–7 weeks) were subjected to three different treatment protocols to influence the progression of cardiac dysfunction. The control group received standard mouse chow (4% fat, 20% protein), the PPARα agonist-treated group was fed control diet mixed with 0.1% Wy14,643 (Cayman Chemical, Ann Arbor, MI, USA) for 3 weeks (Crabb *et al.*, 2001), and the PPARγ agonist-treated group was fed control diet mixed with 0.013% rosiglitazone (Cayman Chemical) for 3 weeks (Kanda *et al.*, 2009). This protocol was chosen because ATGL(-/-) mice typically die from cardiac dysfunction at 9–10 weeks. All groups were provided with food and water *ad libitum*. Food intake was not different between the experimental groups. Animals were housed in approved cages and kept on a regular 12 h dark/light cycle. All animal care and experimental procedures complied with the Austrian law on experimentation with laboratory animals (last amendment, 2004), which is based on the US National Institutes of Health guidelines, and were approved by the local ethical committee[C3] of the Ministry of Science and Education in Austria.

Langendorff heart perfusion

Mice were injected with heparin 1000 $U \cdot kg^{-1}$ body weight (i.p.) and anaesthetized with urethane 1 $g \cdot kg^{-1}$. Hearts were rapidly excised and arrested in ice-cold Krebs–Henseleit buffer. After cannulation of the aorta with a 20 gauge needle, retrograde perfusion was established at a constant flow of 20 $mL \cdot min^{-1} \cdot g^{-1}$ wet weight with a modified Krebs–Henseleit bicarbonate buffer, pH 7.4 (composition in mM: NaCl 118, $NaHCO_3$ 25, KH_2PO4 1.2, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.25,

glucose 11) using the ISO-HEART perfusion system (Hugo Sachs Elektronik, March-Hugstetten, Germany) as previously described (Brunner *et al.*, 2001). The perfusate was filtered through a 5-μm filter before reaching the heart and continuously gassed with carbogen (95% O_2, 5% CO_2). Heart temperature, measured with a physitemp probe (Physitemp Instruments, Clifton, NJ, USA), was maintained at 37°C throughout the experiments. After removal of the left auricle, a tiny fluid-filled balloon made of a small square of polyethylene film was inserted into the left ventricle and connected to a pressure transducer via a 4F biluminal monitoring catheter (Vygon, Aachen, Germany) (Sutherland *et al.*, 2003). The following cardiac parameters were monitored using the PLUGSYS data acquisition and control set-up for circulatory studies (Hugo Sachs Elektronik) and recorded using a PowerLab system (ADInstruments Ltd, Hastings, UK): LV end-diastolic pressure (LVEDP), peak LV systolic pressure (LVSP), LV developed pressure (LVDevP), maximum rate of rise and fall of LV pressure ($+dP/dt_{max}$, $-dP/dt_{max}$), heart rate (obtained from the pressure signal using a differentiator and heart rate module, respectively) and coronary perfusion pressure (CPP; via a second pressure transducer attached to the aortic cannula).

Experimental protocols

Hearts were perfused for 30 min to establish stable baseline conditions. Thereafter, baseline pressure–volume relationships were determined for each heart. The balloon-volume (V_b) was set to the lowest possible volume at which minimal LV pressure tracings (<1 mmHg) could just be recorded. This volume was defined as zero volume. The balloon was then inflated in 5 μL increments using an airtight glass syringe (Hamilton Co., Whittier, CA, USA). LVEDP and LVSP were obtained 1 min after each increment when a new steady state was reached. LV volume was increased up to a value at which maximal LVDevP was reached and a further increase led to a decrease of LVDevP (optimal filling volume; V_{max}). LVEDP was then readjusted to 5 mmHg and baseline conditions re-established. Subsequently, noradrenaline (NA; 1 nM–3 μM; non-cumulative dosing) was added via a sideline. At the end of each experiment, hearts were dissected and the different compartments weighed.

Calculation of wall stress

LV wall thickness and radius of the left ventricle were derived from the weight of the LV wall plus septum and the balloon volume, respectively, as described previously for rat hearts (Strömer *et al.*, 1997; Wölkart *et al.*, 2000). LV wall stress was then derived from Laplace's law using the relation described by Mirsky (1979).

Statistical analysis

All data are presented as mean values ± SEM of five experiments per experimental group. In WT hearts, the potency (EC_{50}) of agonists was calculated by fitting individual dose-response curves to a Hill-type model. Concentration-response curves recorded with ATGL(-/-) hearts could not be fitted due to very minor effects of the agonists. ANOVA with *post hoc* Bonferroni–Dunn test was used for comparison between groups using StatView (Version 5.0) software (SAS Institute Inc., Cary, NC, USA). Significance was assumed at $P < 0.05$.

Results

Body and heart weights

At ~10 weeks of age, the body weight of ATGL(-/-) mice was slightly higher than that of WT, but the difference was not statistically significant (20.4 ± 0.9 g and 22.0 ± 0.3 g for untreated WT and ATGL(-/-), respectively). The body weight of ATGL(-/-) mice was significantly decreased after feeding the animals for 3 weeks with the PPARα agonist Wy14,643 (18.7 ± 0.2 g). In contrast, the PPARγ agonist rosiglitazone increased body weight to 24.8 ± 0.3 g. In WT animals, WY14,643 treatment also considerably decreased body weight (14.7 ± 0.3 g), while rosiglitazone was without effect. Triglyceride accumulation in cardiac muscle was already substantially more in untreated ATGL(-/-) mice, demonstrated by substantially higher heart wet weights (183 ± 6 mg) as compared with untreated WT (96 ± 2 mg; $P < 0.05$). Similar to body weights, heart weights were decreased in Wy14,643-treated (131 ± 4 mg) and increased in rosiglitazone-treated (215 ± 10 mg) ATGL(-/-) animals [$P < 0.05$ vs. untreated ATGL(-/-)]. A similar but less pronounced pattern was observed in WT animals. Thus, heart-to-body weight ratios were significantly increased in untreated ATGL(-/-) mice as compared with untreated WT (8.3 ± 0.4 and 4.7 ± 0.1, respectively). The decrease in body weight due to PPARα activation was more pronounced in WT (–28%) than in ATGL(-/-) (–18%) animals, while the decrease in heart weight was more pronounced in ATGL(-/-) (–28%) than in WT (–12%) mice. Hence, Wy14,643 treatment decreased the heart-to-body weight ratio in ATGL(-/-) but increased it in WT mice. Rosiglitazone treatment did not affect this parameter in WT or ATGL(-/-) animals (Table 1). Dissection of the hearts revealed that the twofold increase in relative weight was not due to proliferation of specific compartments, indicating whole heart hypertrophy (not shown).

LV pressure–volume relationship and wall stress

Untreated ATGL(-/-) hearts showed a steeper increase in LVEDP with increasing V_B. This pronounced diastolic dysfunction was ameliorated by treating ATGL(-/-) mice with Wy14,643, whereas rosiglitazone had no effect. In contrast, the LVEDP pressure–volume curve was shifted leftwards in WT mice after Wy14,643 treatment (Figure 1A). LVSP was not different between experimental groups at any balloon volume (Figure 1B), but LVDevP was used as indicator of systolic dysfunction rather than LVSP, because evaluating systolic pressure without considering diastolic preload does not reflect the *in vivo* situation. As shown in Figure 1C, ATGL(-/-) hearts generally exhibited a lower LVDevP than WT. Again, contractility of ATGL(-/-) hearts was improved by Wy14,643 but not by rosiglitazone. Optimal filling volume (V_{max}) and maximal LVDevP were significantly higher in WT (42 ± 1 μL, 114 ± 11 mmHg) than in ATGL(-/-) hearts (19 ± 2 μL, 82 ± 9 mmHg). The latter two parameters were improved by Wy14,643 treatment [29 ± 2 μL, 101 ± 5 mmHg; $P < 0.05$ vs.

Table 1

Effects of the PPAR agonists Wy14,643 and rosiglitazone on body and heart weights of WT and ATGL(-/-) mice

Genotype Treatment	WT Untreated	Wy14,643	Rosiglitazone	ATGL(-/-) Untreated	Wy14,643	Rosiglitazone
Body weight (BW; g)	20.4 ± 0.9	14.7 ± 0.3*	21.0 ± 0.4	22.0 ± 0.3	18.7 ± 0.2†	24.8 ± 0.3*†
Heart weight (HW; mg)	96 ± 2	84 ± 2*	105 ± 2*	183 ± 6*	131 ± 4*†	215 ± 10*†
HW/BW ($mg \cdot g^{-1}$)	4.7 ± 0.1	5.7 ± 0.1*	5.0 ± 0.2	8.3 ± 0.4*	7.0 ± 0.2*†	8.7 ± 0.5*

Data are mean values ± SEM of five hearts.
*$P < 0.05$ versus untreated WT; †$P < 0.05$ versus untreated ATGL(-/-) (ANOVA).

untreated ATGL(-/-)], but unaffected by rosiglitazone treatment (23 ± 5 μL, 84 ± 5 mmHg). Wy14,643 treatment induced opposite effects in WT animals, that is, it reduced maximal LVDevP (98 ± 6 mmHg) and V_{max} (30 ± 4 μL). Importantly, evaluation of absolute pressure values negates differences in heart size and muscle mass. To compensate for differences in heart weights (and consequential wall thickness), wall stress was calculated for each filling volume V_B. As shown in Figure 2A, diastolic wall stress was increased in untreated ATGL(-/-) hearts over the whole V_B range, suggesting that reduced compliance of cardiac muscle caused by triglyceride accumulation was not effectively compensated for. Diastolic wall stress of ATGL-deficient hearts was not decreased by either of the two PPAR agonists, indicating that these drugs did not significantly affect myocardial compliance. In WT animals, diastolic wall stress was seemingly increased upon Wy14,643 treatment. However, when differences in the size of the ventricular cavities were taken into account by plotting diastolic wall stress versus V_B normalized by V_{max}, this effect in WT hearts was no longer evident and an improved diastolic function was revealed in Wy14,643-treated ATGL(-/-) hearts (Figure 2B).

Finally, significantly decreased systolic wall stress in ATGL(-/-) hearts at all filling volumes suggests the development of cardiomyopathy in ATGL-deficient mice (Figure 2C). Wy14,643 increased systolic wall stress in WT and ATGL(-/-) hearts at $V_B > 25$ μL, while no effect was observed with rosiglitazone. Again, after normalization of wall stress to differences in the size of the ventricular cavity, the effect of Wy14,643 was not apparent in WT and more pronounced in ATGL-deficient hearts (Figure 2D).

Effects of NA

Cardiomyopathic impairments were further analysed by assessing pump function and heart rate in response to adrenergic stimulation. Diastolic pressure was adjusted to 5 mmHg to mimic cardiac preload under physiological conditions. In line with the data shown in Figure 1C, ATGL-deficient hearts showed significantly reduced LVDevP under basal conditions but also exhibited markedly impaired response to NA stimulation as compared with WT littermates (EC_{50}: 13 ± 7 nM; Figure 3A). This loss of the β-adrenoceptor response was not simply confined to reduced inotropy (reduced increase in LVDevP and $+dp/dt_{max}$; Figure 3A and B, respectively), but also apparent as reduced lusitropy (reduced increase in $-dp/dt_{max}$; Figure 3C), indicating severe systolic and diastolic dysfunction. Importantly, the contractile response to adrenergic stimulation was not improved by treating ATGL(-/-) mice with PPARα or PPARγ agonists. While treating WT mice with the PPARα agonist reduced basal LVDevP (see above), the inotropic response to β-adrenoceptor stimulation was not altered (Δ increase of 79 ± 10 and 70 ± 5 mmHg in untreated and Wy14,643-treated WT animals, respectively). Rosiglitazone had no effect in WT animals.

Basal heart rate was not changed in ATGL deficiency, but the chronotropic response to NA was diminished in untreated ATGL(-/-) hearts (Figure 4A). The response to NA was fully restored by Wy14,643, whereas rosiglitazone had no effect. In WT animals, neither Wy14,643 nor rosiglitazone treatment had any effects on heart rate. Microvascular function of ATGL(-/-) hearts was assessed as NA-induced reduction in CPP, reflecting coronary vasodilatation. As shown in Figure 4B, NA caused pronounced coronary relaxation that was largely abolished in ATGL-deficient hearts. The response to NA was partially restored in hearts obtained from Wy14,643-treated ATGL(-/-) mice, whereas feeding the animals rosiglitazone had no effect (Figure 4B). In WT mice, NA-induced vasodilatation was not affected by either of the two drugs.

Discussion

Previous work has shown that progressing lipid accumulation and myocardial fibrosis in hearts of ATGL-deficient mice was accompanied by LV hypertrophy and impaired LV systolic function *in vivo* (Haemmerle *et al.*, 2006). In the present study, we confirmed and extended these observations using an *in vitro* model of cardiac function that is independent of potential interference by circulating humoural and/or vegetative signals. In addition, we obtained a complete picture of the isovolumic pressure–volume relationship by assessing cardiac function on several points of the Frank–Starling mechanism and showed that PPARα activation improved cardiac contractility.

Diastolic dysfunction was apparent as reduced compliance of the left chamber, rendering ATGL(-/-) hearts more susceptible to even small changes in preload. Importantly, despite a considerably increased wall thickness, calculated passive wall stress was higher in ATGL(-/-) hearts, a condition that is observed in the transition from compensated to

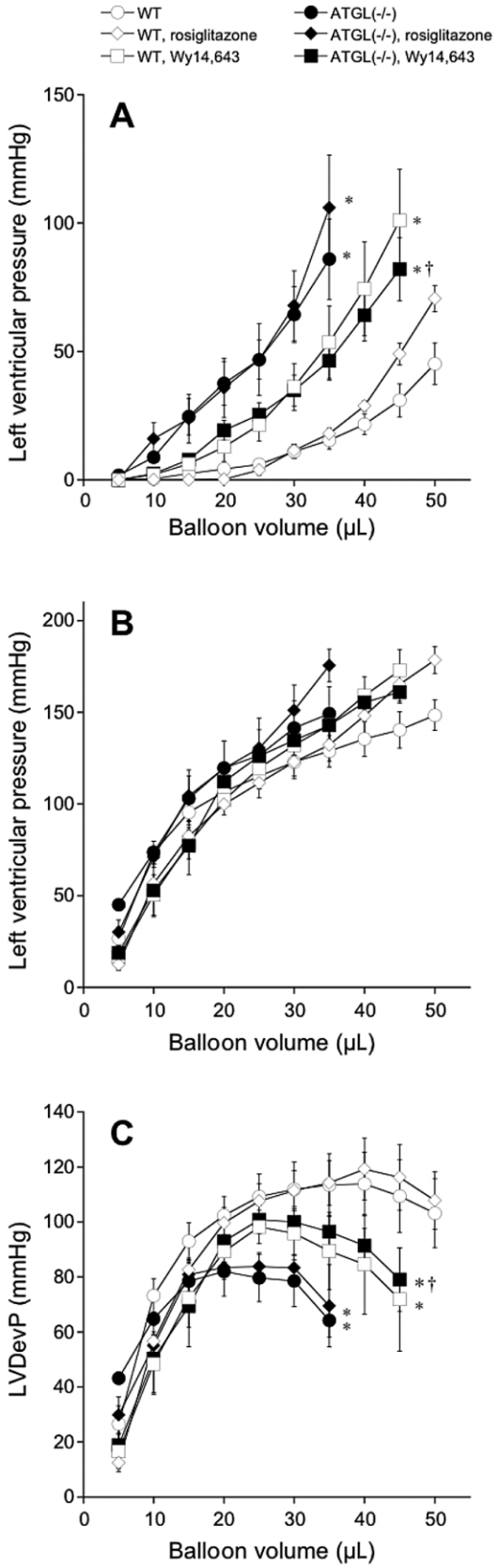

Figure 1

Effect of increasing balloon volume on LV end-diastolic (A), systolic (B) and corresponding developed (C) pressure of hearts isolated from WT and ATGL(-/-) mice treated with rosiglitazone, Wy14,643 or standard mouse chow. Data are mean values ± SEM of five hearts. *$P < 0.05$ versus untreated WT; †$P < 0.05$ versus untreated ATGL(-/-) (ANOVA).

decompensated cardiac hypertrophy (Veliotes *et al.*, 2005). The sustained elevation of diastolic wall stress most likely triggered further fibrosis of cardiac muscle, eventually leading to congestive heart failure. Cardiomyopathy of ATGL(-/-) hearts was evident as compromised LV pressure development in response to varying LV filling volumes. The lower developed pressure in ATGL(-/-) hearts was associated with a lower active stress, showing that contractile dysfunction is due to impaired force development of the ventricular muscle, rather than increased muscle mass. Massive fibrosis and apoptosis may partially contribute to this pathology (Haemmerle *et al.*, 2006). Interestingly, the response to inotropic stimulation by NA was also impaired in ATGL-deficient hearts, presumably due to reduced contractile reserve (cf. Figure 1C). Our observations showing a markedly decreased velocity of contraction and relaxation at both baseline condition and after NA stimulation (cf. Figure 3B,C) indicate increased stiffness of the cardiac muscle, most likely reflecting advanced fibrosis. Reduced muscle compliance might also, to some extent, account for the significantly less pronounced increase in heart rate in response to NA, possibly through increased occurrence of arrhythmias. Finally, decreased β-adrenoceptor density and/or receptor-effector coupling might have affected the response to NA in ATGL(-/-) hearts. However, the contractile response to NA was more severely affected by ATGL deficiency than the chronotropic effect (cf. Figures 3A and 4A), suggesting that impaired receptor-effector coupling may account for the reduced chronotropic response, whereas additional mechanisms, for example, fibrosis and/or mitochondrial dysfunction, appear to cause impaired contractility.

An obvious possibility is that heart function in ATGL(-/-) mice is affected by the disordered balance between carbohydrate and FA utilization for energy production. Typically, during development of cardiac hypertrophy and heart failure, myocardial metabolism switches from utilization of long-chain FAs to glucose because of the higher efficiency of glycolysis in terms of ATP generated mol^{-1} O_2 consumed (Bishop and Altschuld, 1970). The effect of catecholamine stimulation on cardiac energy metabolism is less clear. An early study reported about twofold to threefold and threefold to fourfold increases, respectively, in the rates of glucose and triglyceride utilization during adrenergic stimulation *in vitro* (Williamson, 1964). Others have reported a reduced cardiac glucose uptake after adrenergic stimulation *in vivo* (Capaldo *et al.*, 1992; Huang *et al.*, 1997). Glucose uptake and utilization are increased in ATGL(-/-) cardiac muscle together with attenuated isoprenaline-stimulated FA mobilization in white adipose tissue (Haemmerle *et al.*, 2006), even though gene expression studies suggest concerted down-regulation of oxidative pathways required for energy production from glucose in ATGL-deficient mice (Pinent *et al.*, 2008). Since our

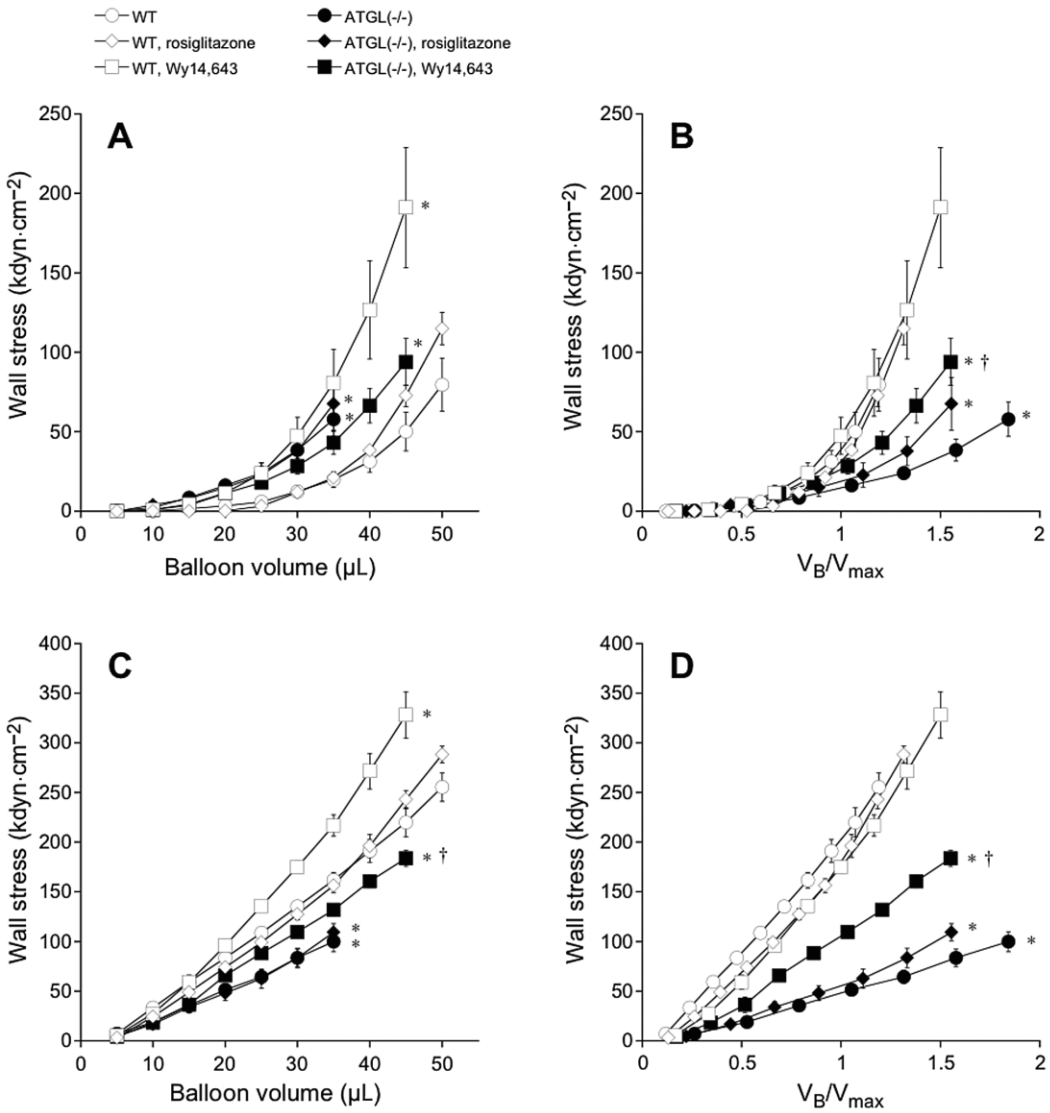

Figure 2

Dependence of LV end-diastolic (A,B) and systolic (C,D) wall stress on intracardiac balloon volume (V_B, left) and balloon volume normalized by optimal filling volume (V_{max}, right) of hearts isolated from WT and ATGL(-/-) mice treated with rosiglitazone, Wy14,643 or standard mouse chow. Data are mean values ± SEM of five hearts. *$P < 0.05$ versus untreated WT; †$P < 0.05$ versus untreated ATGL(-/-) (ANOVA).

observations of impaired mitochondrial respiration in ATGL-deficient hearts (submitted) indicate that the same applies to cardiac muscle, it is likely that glycolysis alone is not sufficient to provide enough primary energy for an adequate response to adrenergic stimulation.

We recently found that ATGL deficiency leads to severely impaired activation of PPARα and PPARδ target genes in cardiac and skeletal muscle, suggesting that ATGL-catalyzed triglyceride hydrolysis is essential for the supply of FA-derived PPAR ligands in these tissues (unpublished observations). The role of PPARs in cardiac function is not well understood (Zahradka, 2007; Robinson and Grieve, 2009). Activation of PPARγ was reported to inhibit hypertrophy of cardiac myocytes in response to mechanical strain (Yamamoto *et al.*, 2001; Asakawa *et al.*, 2002), whereas activation of PPARα, but not PPARγ, was shown to prevent apoptosis of cardiac myocytes after ischaemia/reperfusion injury (Yeh *et al.*, 2006) and to regulate cardiomyogenesis from embryonic stem cells (Sharifpanah *et al.*, 2008). In a rat coronary artery ligation model, a PPARα agonist attenuated the progression of heart failure by reducing fibrosis and heart weight, while PPARγ activation even exacerbated cardiac dysfunction (Linz *et al.*, 2009). In support of the protective role of PPARα signalling, PPARα-deficient mice were reported to exhibit cardiac contractile dysfunction due to oxidative damage of myosin (Guellich *et al.*, 2007).

In line with previous observations, we observed that PPARα activation of WT animals led to reduced body weight and substantial alterations in heart weight and function (Zungu *et al.*, 2009). In our set-up, reduced contractility after

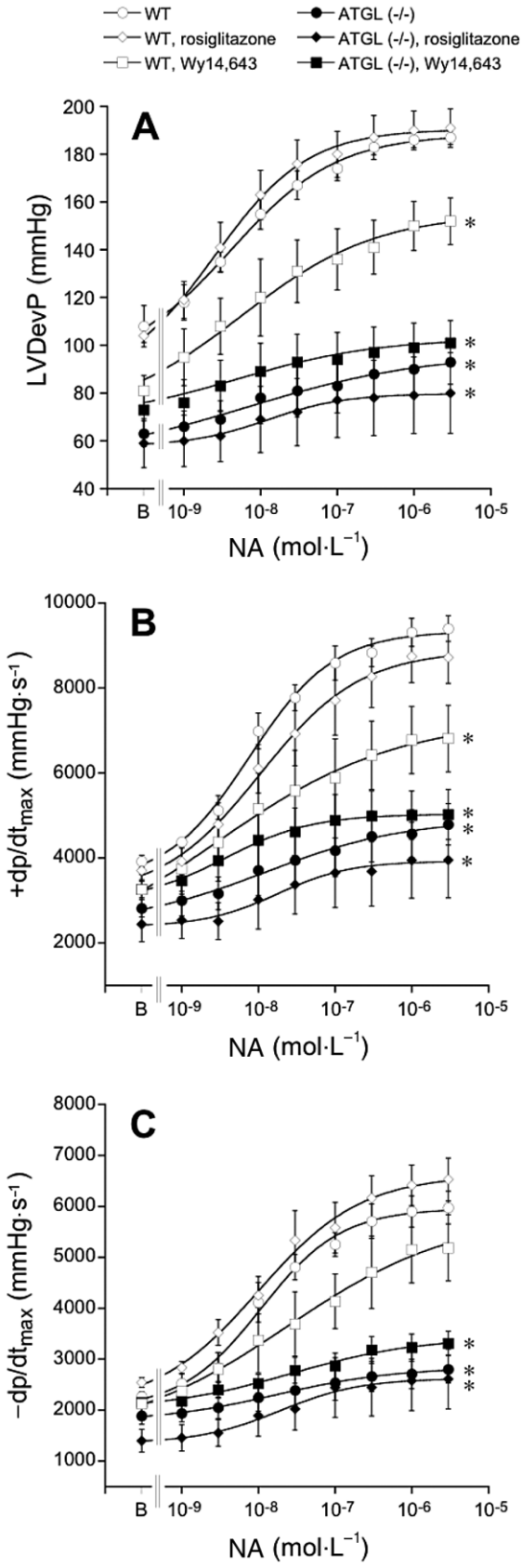

Figure 3

Effects of NA on LVDevP (A), maximum rate of LV pressure increase ($+dP/dt_{max}$; B) and decrease ($-dP/dt_{max}$; C) of hearts isolated from WT and ATGL(-/-) mice treated with rosiglitazone, Wy14,643 or standard mouse chow. Data are mean values ± SEM of five hearts. $P < 0.05$ versus untreated WT (ANOVA); B indicates basal function.

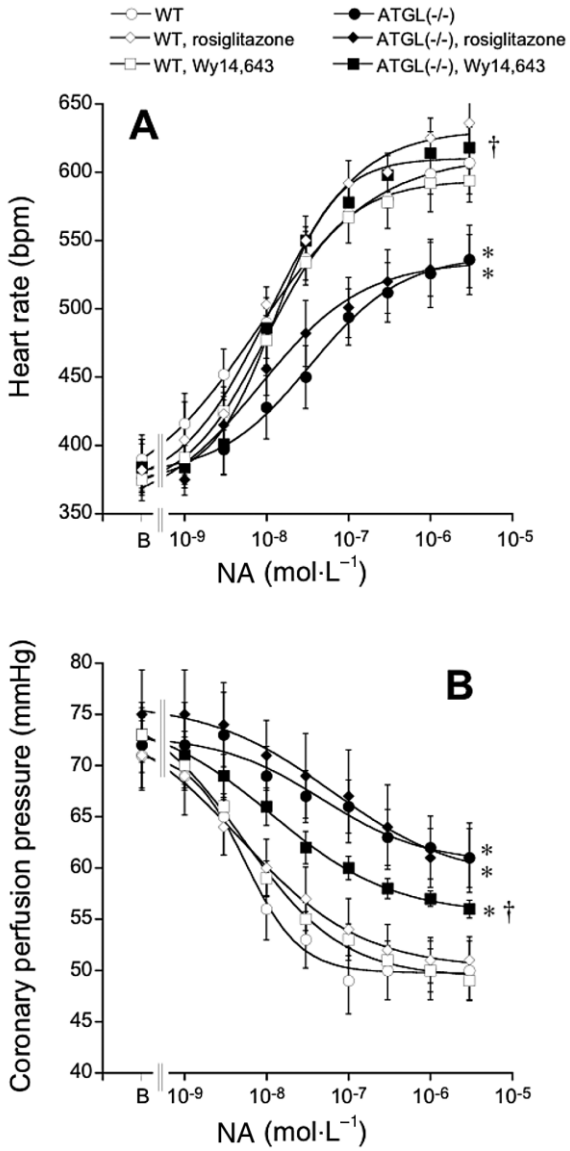

Figure 4

Effects of NA on heart rate (A) and coronary perfusion pressure (B) of WT and ATGL(-/-) mice treated with rosiglitazone, Wy14,643 or standard mouse chow. Data are mean values ± SEM of five hearts. $P < 0.05$ versus untreated WT; †$P < 0.05$ versus untreated ATGL(-/-) (ANOVA); B indicates basal function.

Wy14,643 treatment of WT mice was mainly due to reduced cardiac muscle mass (cf. Figure 2B,D), although additional mechanisms like impaired mitochondrial function (Keller *et al.*, 1993) cannot be excluded. Despite this unfavourable

effect in WT animals, Wy14,643 reduced cardiac hypertrophy and improved diastolic and systolic function in ATGL-deficient mice, indicating that PPARα target genes are essential for maintaining normal cardiac function. Our data clearly indicate that this improvement was not simply caused by altered cardiac growth. As stimulation of PPARα triggers the expression of genes involved in lipid metabolism (Marx *et al.*, 2004), treating the mice with Wy14,643 may have led to improved energy supply to the heart through increased rates of FA β-oxidation. Another possible explanation is improved mitochondrial function. Further experiments are needed to address this issue.

The inotropic and chronotropic effects of NA were markedly decreased in ATGL-deficient hearts. Based on the observation that targeted gene deletion of PPARα causes down-regulation of β-adrenoceptors (Loichot *et al.*, 2006), Wy14,643 may have restored the chronotropic response by increasing NA receptor density. However, the explanation for this finding is probably more complex because β-adrenoceptor-mediated contractility was not improved by Wy14,643 in ATGL(-/-) mice and unaltered in WT mice (in terms of Δ increase). The reduced contractile response to NA may reflect attenuated FA mobilization in ATGL-deficient cardiac muscle, as observed previously in white adipose tissue of ATGL(-/-) mice (Haemmerle *et al.*, 2006). Thus, even though the PPARα agonist improved mitochondrial function, glycolysis is apparently not sufficient to supply enough primary energy for a NA-triggered contraction. The observation that the increase in coronary flow in response to NA was reduced in ATGL(-/-) hearts suggests that ATGL deficiency may affect microvascular function. This possibility is currently being investigated in our laboratory.

Rosiglitazone had no cardioprotective effects at all. As the survival rate of PPARγ-deficient mice is extremely low (Duan *et al.*, 2007), the role of PPARγ signalling in the heart is only poorly understood and highly controversial. Animal *in vitro* studies suggest a cardioprotective role of glitazones in ischaemia/reperfusion injury (Shimabukuro *et al.*, 1996; Khandoudi *et al.*, 2002; Molavi *et al.*, 2006), while recent clinical surveys indicate that the risk of heart failure is even increased by rosiglitazone (Nissen and Wolski, 2007; Home *et al.*, 2009). Notably, in mice overexpressing lipoprotein lipase, another model of lipotoxic cardiomyopathy, PPARγ (but not PPARα) agonists were shown to reduce cardiac lipid levels and markers of cardiomyopathy (Vikramadithyan *et al.*, 2005). However, in that model, the protective effects of PPARγ activation may have been simply due to re-allocation of triglycerides and FAs from the heart to adipose tissue.

In summary, the present study shows that severe cardiac dysfunction due to lipid accumulation in ATGL-deficient mice is improved by activation of PPARα, suggesting that treatment with fibrates may be beneficial in human neutral lipid storage disease.

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Conflict of interest

None declared.

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