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Tedisamil attenuates foetal transformation of myosin in the hypertrophied rat myocardium

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1 Reduction in repolarizing potassium currents has controversial effects on hypertrophic responses in cardiomyocytes of transgenic models and cultured cardiomyocytes. It remains thus unknown whether a blockade of potassium channels with tedisamil (*N*,*N*′dicyclopropylmethylene-9,9-tetramethylene-3,7-diazabicyclo(3.3.1)nonane dihydrochloride) has any effects on cardiac growth during postnatal development or pressure overload.

2 To test the hypothesis that a treatment with tedisamil affects cardiac growth or protein phenotype, sham-operated rats and rats with ascending aorta constriction were treated with tedisamil ($36\,mg\,kg\,day^{-1}$) for 7 weeks. Left ventricular mass and geometry, relative expression of myosin isoforms, hydroxyproline concentration and isovolumic ventricular function were assessed.

3 Rats with aortic constriction exhibited a marked increase in left ventricular weight and the diastolic pressure–volume relationship was shifted to smaller volumes. The hydroxyproline concentration remained unaltered. The proportion of α-myosin heavy chains was, however, reduced ($P<0.05$). Hypertrophied left ventricles manifested an enhanced overall performance but depressed myocardial contractility.

4 Administration of tedisamil was associated with decreased heart rate ($P<0.05$). In contrast, cardiac growth in sham-operated rats and concentric left ventricular hypertrophy of pressure-overloaded animals was not significantly altered. Hypertrophied hearts from rats treated with tedisamil expressed more α-myosin heavy chains (65 ± 4 *versus* $57\pm4\%$; $P<0.05$). Also, maximal rate of wall stress rise and decline was higher ($P<0.05$) in tedisamil-treated pressure-overloaded rats.

5 In the rat model of pressure-overloaded hypertrophy, tedisamil had no effect on cardiac growth but partially corrected myocardial dysfunction. Postulated mechanism of this effect is the phenotype modification of myosin filaments in hypertrophied myocardium.
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Abbreviations: ANCOVA, analysis of covariance; C_R, midwall circumference; I_f, hyperpolarization-activated current; I_K, delayed outward rectifying potassium currents; I_{to}, calcium-independent transient outward current; *P*, left intraventricular pressure; $\pm dP/dt_{max}$, maximal rate of intraventricular pressure rise and decline; *S*, mean wall stress; $\pm dS/dt_{max}$, maximal rate of wall stress rise and decline; *V*, left ventricular cavity volume; *W*, left ventricular wall volume

Introduction

The duration of action potentials is prolonged in hypertrophied cardiac myocytes (Gulch, 1980). Ionic currents responsible for this sustained depolarization appear to be diverse and depend on the underlying cause of cardiac hypertrophy as well as species examined. Attenuation of repolarizing potassium currents, particularly calcium-independent transient outward current (I_{to}), was identified as an underlying cause in most species, including man (reviewed in Swynghedauw *et al*., 2003). The depression of potassium currents results from a reduced expression of genes encoding corresponding potassium channel peptides, Kv4.2 and Kv4.3 (Kaprielian *et al*., 1999; Zhang *et al*., 2001) during cardiac hypertrophy, probably as a part of activation of the foetal gene programme. Among others, this foetal reprogramming also includes downregulation of expression of α-myosin heavy chains and upregulation of expression of β-myosin heavy chains, re-expression of foetal isoforms of calcium channels, hyperpolarization-activated channel (I_f), and Na^+K^+-ATPase (Swynghedauw *et al*., 2003).

Thus, the reduction in potassium currents seems to be part of cellular remodelling during hypertrophic growth and not the cause of cardiac hypertrophy. In favour of this view would be the observation that inherited as well as drug-induced long QT syndromes are not associated with cardiac hypertrophy (Nador *et al*., 1991). Similarly, action potential prolongation in mice due to I_{to} elimination caused by expression of nonconducting mutant of Kv4.2 subunit was not associated with cardiac hypertrophy (Barry *et al*., 1998). Knockout of potassium channel regulatory protein KCh1P minimized I_{to}, prolonged action potential and QT interval, but had no effect on myocytes size or contractility (Kuo *et al*., 2001).

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Yet, the relationship between potassium currents or channels and cardiac hypertrophy appears to be more complex (Sanguinetti, 2002). The reduced rate of repolarization could also be a mechanism, which initiates or contributes to the cardiac hypertrophy. A primary reduction in I_{to} due to expression of dominant-negative N-terminal fragment of the Kv4.2 pore-forming potassium channel subunit in transgenic mice was associated with cardiac hypertrophy and failure (Wickenden *et al*., 1999). Treatment with either cyclosporin A or verapamil prevented cardiac hypertrophy, interstitial fibrosis, foetal transformation of myosin molecules as well as impairment of contractility in animals expressing dominant-negative Kv4.2 (Sah *et al*., 2002). Overexpression of the dominant-negative N-terminal fragment of the Kv4.2 in cultured neonatal rat ventricular myocytes as well as blocking of Kv4.2 potassium channels with the heteropoda toxin-3 also induced hypertrophy that could be prevented by calcineurin inhibition and by blocking calcium entry with verapamil or high extracellular potassium (Kassiri *et al*., 2002). In the same model, overexpression of Kv4.2 potassium channels prevented the reduction in I_{to}, action potential prolongation and hypertrophic growth after application of phenylephrine (Zobel *et al*., 2002). In addition, a close relationship between reduced potassium current density, downregulated expression of potassium channels Kv1.5 and Kv2.1 and proliferation of vascular smooth muscle cells was identified in chronic hypoxic pulmonary hypertension (Michelakis *et al*., 2002).

Since the causal relationship between diminutions of potassium currents, action potential prolongation and growth stimulation remains controversial, the possibility exists that the pharmacologic blockade of potassium channels might significantly modify growth of cardiac myocytes. To test this hypothesis, we treated rats without and with left ventricular hypertrophy due to ascending aortic constriction with tedisamil (*N*,*N*′dicyclopropylmethylene-9,9-tetramethylene-3,7-diazabicyclo(3.3.1)nonane dihydrochloride) for 7 weeks. Tedisamil blocks I_{to}, delayed outward rectifying potassium currents (I_K), protein kinase A-activated chloride current and fast sodium current, the last one only at higher concentrations (reviewed in Doggrell, 2001). If the prolongation of the action potential is a factor contributing to cardiac growth, inhibition of I_{to} should stimulate the hypertrophy. Furthermore, tedisamil also inhibits ATP-sensitive potassium channels (Chi *et al*., 1996). This effect might have a significant impact on myocardial phenotype because blockade of ATP-sensitive potassium channels facilitates glucose utilization (Wasada *et al*., 2001), and glucose metabolism appears to be associated with the expression of certain myocardial genes, particularly genes encoding α- and β-myosin heavy chains (Zarain-Herzberg & Rupp, 2002). Thus, tedisamil could not only affect cardiac growth but also the molecular phenotype of the overloaded heart. In the present study we determined, therefore, the effect of tedisamil on cardiac size, ventricular geometry, expression of myosin isozymes and fibrosis as well as on ventricular and myocardial function during cardiac adaptation to the pressure overload.

Methods

Animal model

In all, 48 male 3-week-old Wistar/WU rats selected for experiment were randomized into four groups and housed at 21–23°C on 12 : 12-h light–dark cycle. The rats had free access to tap water and regular chow. Pressure overload was induced by constriction of the ascending aorta (Turcani & Rupp, 1997) in 24 rats under Hypnorm (fluanison, fentanyl) anaesthesia (1 ml kg^{-1}, i.p.). The 24-sham-operated animals underwent a right thoracotomy and the ascending aorta was isolated but not constricted. Tedisamil (36 mg kg^{-1} day^{-1}) was administered in the drinking water for 7 weeks to one sham-operated group and to one group with ascending aortic stenosis starting 24 h after surgery. The dose was maintained by monitoring the daily water consumption and body weight. In unpublished experiments (Dr D. Thormaehlen), 30 mg kg^{-1} day^{-1} tedisamil was administered by gavage for 4 weeks leading to a plasma concentration of 63 ng ml^{-1} as determined by HPLC with electrochemical detection (Friedrichs *et al*., 1996). Tedisamil was a gift from Dr D. Thormaehlen, Solvay Pharmaceuticals Research Laboratories, Hannover, Germany. During first 24 h after surgery, three rats died from the sham-operated tedisamil-treated group, one rat from the untreated sham-operated group as well as from the tedisamil-treated group with aortic stenosis.

Systolic arterial blood pressure and heart rate

Systolic arterial blood pressure and heart rate were measured in conscious animals by the tail-cuff method.

Measurement of left ventricular isovolumic contraction

The measurements were performed in open-chest rats under urethane anaesthesia (1.2 g kg^{-1}, i.p.) as described previously (Turcani & Rupp, 1997). A mediosternal thoracotomy was performed and the left ventricle was pierced at the apex with a steel cannula no. 1 connected to a Gould–Statham P23XL pressure transducer (Gould Electronics, Bilthoven, The Netherlands). The right carotid artery was cannulated with a polyethylene tubing (0.5 mm inner diameter) and forwarded to the aortic arch. The tubing was connected to a second Gould–Statham P23XL pressure transducer. Left ventricular pressure, left ventricular diastolic pressure (high amplification of left ventricular pressure), time derivative of ventricular pressure (dP/dt) and aortic pressure were recorded simultaneously on a Hellige Recomed recording system (Hellige, Freiburg, Germany).

For monitoring isovolumic contractions, the ascending aorta was clamped above the aortic valve for 6–8 s with a forceps as verified by the absence of pulsatile pressure in the aortic arch. A short tightening of a string around the inferior vena cava resulted in small end-diastolic volumes, that is, low preload values. The preload increased gradually after relieving the inferior vena cava flow while clamping the aorta. This procedure was repeated four to six times. The functional analysis was based on the recording exhibiting the highest systolic pressure development. Haemodynamic measurements could not be completed in one rat of the tedisamil-treated sham-operated group and in one tedisamil-treated rat with ascending aortic constriction due to premature death.

Left ventricular passive pressure–volume relations were assessed after recording isovolumic contractions. The atrioventricular groove was ligated with a silk string and the right ventricle was emptied by incision. The left ventricle was filled with a defined volume of saline and emptied in 50 μl steps

while recording the passive left ventricular pressure. Three reproducible pressure–volume curves were generated within 3–4 min after the ligation. No effects of anoxia on the pressure–volume relation could be detected within this period of time. Using the passive pressure–volume relation, end-diastolic cavity volumes required for further analysis were derived from the measured end-diastolic pressures.

Data analysis

The approach permitted the construction of complete left ventricular pressure–volume and stress–length diagrams. Left ventricular and myocardial function was thus assessed independent of left ventricular mass, geometry and loading conditions. Systolic peak pressures that are equivalent to end-systolic pressures under isovolumic conditions were plotted against end-diastolic volumes resulting in end-systolic pressure–volume curves. All auxotonic pressure–volume values had to reside within this isovolumically determined end-systolic pressure–volume relation. The area between end-systolic and end-diastolic pressure–volume curves up to maximum end-systolic pressure was used as an index of left ventricular working capacity. Transformation of the left ventricular pressure–volume diagram to the stress–length relation permitted the evaluation of myocardial performance when left ventricular mass and geometry are altered. Myocardial contractility was evaluated on the basis of normalized stress–length area, that is, the area between the end-systolic and end-diastolic mean wall stress *versus* normalized midwall circumference (length) curves. In analogy with papillary muscle, the normalized midwall circumference was calculated as the ratio between a given midwall circumference and the midwall circumference associated with peak developed wall stress. Pressure–volume data were transformed into stress–length data using a thick-walled spherical shell. Since calculations assumed that the specific density of myocardium was $1\,g\,cm^{-3}$, left ventricular weight in grams equalled the ventricular wall volume in cm^3.

The mean (systolic or diastolic) wall stress (S) was derived from the following formula (Sandler & Dodge, 1963): $S = P/\{[(V+W)/V]^{2/3} - 1\}$, where P is left intraventricular pressure, V is left ventricular cavity volume and W is left ventricular wall volume. Since the contraction was isovolumic, end-diastolic volume derived from the passive pressure–volume curve was identical with the left ventricular cavity volume (V) during the respective beat. Midwall circumference (C_R) was calculated according to: $C_R = \pi\{(\frac{3}{4}/\pi)^{1/3}[(V^{1/3} + (V+W)^{1/3}]\}$ (Mirsky & Parmley, 1973). To evaluate the velocity of contraction and relaxation at the myocardial level, rate of mean wall stress rise ($+dS/dt$) or decline ($-dS/dt$) was calculated using the recorded $\pm dP/dt$ values by: $\pm dS/dt = \pm(dP/dt)/\{[(V+W)/V]^{2/3} - 1\}$.

Myosin isozymes

For determination of myosin isozymes of the same cardiac region, a portion of the left ventricular free wall (about 100 mg) was cut from the apex to the base and stored in liquid nitrogen. The myosin isozymes were separated by nondissociating gel electrophoresis in the presence of pyrophosphate (Rupp *et al*., 1992). The electrophoresis buffer was 20 mM $Na_4P_2O_7$ containing 10% ($v\,v^{-1}$) glycerol (pH 8.8). The gel contained 3.8% acrylamide and 0.12% *N,N′*-methylene-*bis*-acrylamide in electrophoresis buffer. Polymerization was achieved by adding 0.07 ml TEMED and 0.27 ml persulphate solution (13% ($w\,v^{-1}$)) to 24 ml of gel solution. Pre-electrophoresis was at $10\,V\,cm^{-1}$ for 60 min. Electrophoresis was carried out at 2–3°C and $10\,V\,cm^{-1}$ for 24 h. Myosin was extracted at 0°C from muscle specimens by agitation with 3 vol ($v\,w^{-1}$) of 40 mM $Na_4P_2O_7$, 1 mM 1,4-dithioerythritol and 5 mM EGTA (pH 8.8). Gels were stained with Coomassie brilliant blue R250 and gels were scanned using a Quick Scan densitometer (Helena Laboratories, Beaumont, TX, U.S.A.). The isozymes V_1, V_2, V_3 were quantitated by measuring peak heights. This approach is justified, since the constituent components V_1, V_2 and V_3 do not overlap at the peak positions as shown by simulation of the tracings using Gaussian curves (Rupp *et al*., 1992). The proportion of α-myosin heavy chains was calculated using the equation: $\alpha\text{-MHC} = V_1 + V_2/2$.

Collagen content

Collagen content of ventricular tissue was assessed by determining the hydroxyproline concentration. A 70–100 mg portion of the left ventricle was freeze-dried and processed essentially as given by Stegemann & Stalder (1967).

Measurement of R–αT *intervals of the ECG*

ECG measurements were performed in a separate experiment in conscious male Sprague–Dawley rats (320–380 g body wt.). Subcutaneous ECG electrodes were implanted and exteriorized at the neck 4–11 days before the experiment. A quasi-continuous on-line evaluation (30 s intervals) by computerized biosignal processing was used (Buschmann *et al*., 1980) to measure the R–αT intervals (i.e. from the onset of QRS to the apex of T, Figure 1) over a 6-h period. Tedisamil dissolved in carboxymethyl cellulose (Tylose®; $5\,mg\,kg^{-1}$) was given orally by gavage at single doses of 1.7, 3.6, 7.8, 17 and $36\,mg\,kg^{-1}$ and its effect on R–αT was compared with a control group treated with Tylose®.

Statistical analysis

Normality of distribution was checked by the Kolmogoroff–Smirnoff test, and equality of variances according to Cochran. T-test (two-tailed) for independent samples was used to compare the effect of tedisamil at different oral doses on the R–αT interval with the time-matched values of the vehicle group. Multiple comparisons were made by one- and two-way analysis of variance and the *post hoc* Spjotvoll/Stoline test (Statistica/w, Statsoft, Tulsa, OK, U.S.A.). Analysis of covariance (ANCOVA) was used to analyse tedisamil effect on myosin isoforms independent of changes in ventricular weight. Statistical significance was assumed at $P < 0.05$.

Results

R–αT *interval*

Orally applied tedisamil prolongs R–αT interval in a dose- and time-dependent manner (Table 1, Figure 1). Low doses of

tedisamil (1.7 mg kg^{-1}, 3.6 mg kg^{-1}) were without significant effect on R–αT duration. Prolonged R–αT interval was detected 30 min after a single oral application of 17 or 36 mg kg^{-1} of tedisamil and this effect lasted for 4 h after the dose of 36 mg kg^{-1}.

Heart rate and systolic arterial blood pressure

Heart rate was reduced in tedisamil-treated rats during the 6th and 7th week of experiment. A blood pressure raising effect of tedisamil was not observed unequivocally. Systolic arterial blood pressure was, however, significantly higher in tedisamil-treated sham-operated rats than in the untreated rats with ascending aortic constriction (Table 2).

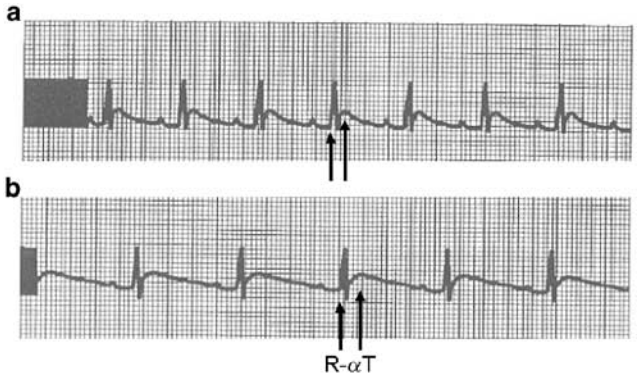

Figure 1 Representative ECG tracings of a rat (a) before and (b) after 1 mg kg^{-1} i.v. administration of tedisamil. Heart rate was reduced from 424 to 284 beats min^{-1}. The R–αT intervals were calculated from the onset of QRS to the apex of T.

Body and organs growth

Neither ascending aortic constriction nor tedisamil affected body weight. Lung weight that was measured as an indicator of pulmonary oedema due to left ventricular failure was also not different. In rats with ascending aortic constriction, heart weight was significantly increased (Table 2). However, attenuation in gain of cardiac mass is suggested in tedisamil-treated rats with aortic constriction by the significant interaction (Table 2, F-values) between the pressure overload and tedisamil treatment. In other words, pressure overload and tedisamil treatment interact in their effects on the relative cardiac mass, that is, the effect of pressure overload was significantly modified by tedisamil.

Heart adaptation to ascending aortic constriction

An increase in left ventricular weight and atrial weight was characteristic of rats with ascending aortic constriction (Table 3). Tedisamil significantly reduced the increase in atrial weight while left ventricular hypertrophy was not reduced significantly. Tedisamil significantly modified the effect of pressure overload on the left ventricular and atrial weight (Table 3) also. Left ventricular growth resulted in concentric

Table 1 Effect of tedisamil on the R–αT interval at different oral doses

Time after drug administration (min)	R–αT *interval* (ms) *Tedisamil* (mg kg^{-1})					
	Vehicle (n = *8*)	*1.7* (n = *7*)	*3.6* (n = *8*)	*7.8* (n = *9*)	*17.0* (n = *7*)	*36.0* (n = *9*)
Predrug	30±2.8	31±3.0	32±5.3	29±2.0	30±2.4	29±3.0
30	31±4.2	31±5.4	32±5.4	30±2.3	48±10.4*	52±9.5*
60	31±4.2	31±5.0	32±4.9	33±6.2	46±6.0*	46±10.2*
120	29±3.1	31±2.3	33±5.1	38±4.5*	48±11.0*	56±8.9*
180	31±5.4	32±3.4	34±4.7	32±2.8	35±4.2	43±11.8*
240	32±3.7	30±3.6	32±4.0	33±7.1	33±5.4	40±7.8*
300	31±3.0	30±3.6	33±4.6	32±2.1	33±5.4	36±5.2
360	31±3.3	31±4.6	33±5.0	32±2.7	33±5.4	36±5.6

Values are means±s.d. *$P<0.05$ compared with the time-matched values of the vehicle group; t-test (two-tailed) for independent samples.

Table 2 Heart rate and systolic arterial blood pressure, body, heart and lung weight

	Sham		*Aortic constriction*		*F(1,39)*
	Untreated (n = *11*)	*Tedisamil* (n = *9*)	*Untreated* (n = *12*)	*Tedisamil* (n = *11*)	
Heart rate (beats min^{-1})	401±21	355±9*†	388±16	360±12*†	3.80
Systolic pressure (mmHg)	133±6	141±12†	127±10	128±12	1.15
Body weight (g)	321±25	304±34	304±29	297±26	0.27
Heart weight (mg)	942±69	936±72	1334±160*	1227±84*	2.36
Heart weight/body weight (mg g^{-1})	2.940±0.140	3.091±0.236	4.381±0.303*	4.143±0.230*	7.20#
Lung weight (g)	1.568±0.189	1.445±0.128	1.507±0.235	1.397±0.187	0.01
Lung weight/body weight (mg g^{-1})	4.893±0.492	4.77±0.380	4.958±0.679	4.714±0.519	0.14

Values are means±s.d. *$P<0.05$ compared with sham-operated untreated rats; †$P<0.05$ compared with untreated rats with aortic constriction; one-way ANOVA followed by the *post hoc* Spjotvoll/Stoline test. F, test values for the interaction between pressure overload due to aortic constriction and tedisamil treatment by two-way ANOVA; within parentheses degrees of freedom; #$P<0.05$ for the interaction between pressure overload and tedisamil. Data for the heart rate and systolic arterial blood pressure are the means of four measurements (tail-cuff method in unanaesthetized rats) during last 2 weeks of the experiment.

hypertrophy as indicated by the reduction in left ventricular cavity volume (Figure 2a) and substantial reduction in left ventricular cavity volume referred to wall volume (Table 3). Hypertrophy was not observed in right ventricles (Table 3).

Changes in expression of myosin heavy chains and concentration of hydroxyproline

Expression of isomyosin V1, which is a homodimer of two α-myosin heavy chains, was markedly reduced in pressure-overloaded left ventricles. The proportion of heterodimer V2 (consisting of one α- and one β-myosin heavy chain) and isomyosin V3 (a homodimer of two β-myosin heavy chains) was increased (Table 4). Consequently, the proportion of α-myosin heavy chains was reduced and the proportion of β-myosin heavy chains was enhanced in left ventricles of animals with ascending aortic constriction. This effect of pressure overload was modified by tedisamil (Table 4), that is, repression of the expression of α-myosin heavy chains was significantly smaller in tedisamil-treated pressure-overloaded rats than in untreated rats with ascending aortic constriction. Expression of α-myosin heavy chains was reduced proportionally to the increase in left ventricular weight in untreated as well as in tedisamil-treated animals. After controlling for the effect of left ventricular weight on α-myosin heavy chains, the stimulating effect on α-myosin heavy chain synthesis by tedisamil appears to be independent of changes in the size of the left ventricle (Figure 3). The hydroxyproline concentration that was used as a biochemical marker of interstitial fibrosis was not affected by 7 weeks of pressure overload and tedisamil treatment.

Performance of isovolumically beating left ventricles

While the pressure-overloaded ventricles developed higher maximal intraventricular pressure (Figure 2b), peak developed mean wall stress was significantly reduced only in untreated animals (Figure 2c). Similarly, overall ventricular performance as judged by the maximal pressure–volume area was augmented in rats with ascending aortic constriction but the normalized stress–length area, an index of myocardial performance, was reduced (Figure 2b and c, Table 5). Although the

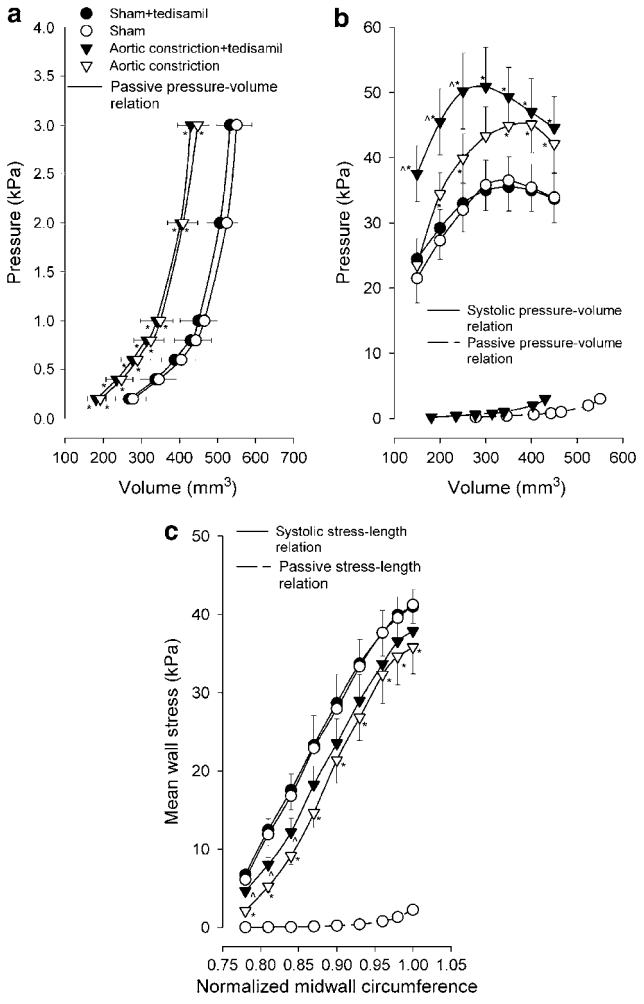

Figure 2 Effects of aortic constriction and tedisamil treatment on left ventricular function. Passive (diastolic) pressure–volume relation (a), end-systolic pressure–end-diastolic volume relation (b) and stress–length relation (c) during isovolumic contractions. Symbols represent means, error bars represent standard deviations. $^{*}P<0.05$ compared with sham-operated rats. $^{\wedge}P<0.05$ compared with untreated rats with aortic constriction. Ascending aortic constriction lasted for 7 weeks. Tedisamil was given in drinking water in a dose 36 mg kg^{-1} day^{-1} for 7 weeks.

Table 3 Dimensions of cardiac chambers

	Sham		*Aortic constriction*		*F(1,39)*
	Untreated (n = *11*)	*Tedisamil* (n = *9*)	*Untreated* (n = *12*)	*Tedisamil* (*n* = *11*)	
Left ventricular weight (mg)	685±54	675±53	1029±124*	954±66*	1.70
Left ventricular weight/body weight (mg g^{-1})	2.14±0.10	2.23±0.18	3.38±0.21*	3.22±0.19*	5.44$^{\#}$
Right ventricular weight (mg)	185±22	187±24	190±22	187±23	0.12
Right ventricular weight/body weight (mg g^{-1})	0.58±0.07	0.62±0.08	0.63±0.06	0.63±0.05	0.8
Left and right atrial weight (mg)	73±11	73±13	115±40*	85±9†	4.46$^{\#}$
Left and right atrial weight/body weight (mg g^{-1})	0.23±0.03	0.24±0.04	0.38±0.13*	0.29±0.04†	5.07$^{\#}$
Left ventricular cavity volume/wall volume	0.60±0.07	0.61±0.06	0.30±0.04	0.32±0.04	0.06

Values are means±s.d. $^{*}P<0.05$ compared with sham-operated untreated rats; $^{\dagger}P<0.05$ compared with untreated rats with aortic constriction; one-way ANOVA followed by *post hoc* Spjotvoll/Stoline test. *F*, test values for the interaction between pressure overload due to aortic constriction and tedisamil treatment by two-way ANOVA; within parentheses degrees of freedom; $^{\#}P<0.05$ for the interaction between pressure overload and tedisamil. Data for the left ventricular cavity volume/wall volume ratio are given for the intraventricular pressure 6 mmHg.

Table 4 Left ventricular myosin isozymes, myosin heavy chains isoforms and hydroxyproline concentration

	Sham		*Aortic constriction*		*F(1,39)*
	Untreated (n = *11*)	*Tedisamil* (n = *9*)	*Untreated* (n = *12*)	*Tedisamil* (n = *11*)	
Myosin V1 (%)	61±5	62±10	42±3*	52±6*†	4.22#
Myosin V2 (%)	24±3	22±5	29±2*	25±2†	0.32
Myosin V3 (%)	15±3	16±5	29±3*	23±4*†	6.86#
α-myosin heavy chain (%)	73±4	74±8	57±3*	65±5*†	4.71#
β-myosin heavy chain (%)	27±4	26±8	43±3*	35±5*†	4.69#
Hydroxyproline ($\mu g\,mg^{-1}$ dry weight)	2.69±0.25	2.67±0.19	2.77±0.36	2.68±0.21	0.21

Values are means±s.d. *$P<0.05$ compared with sham-operated untreated rats; †$P<0.05$ compared with untreated rats with aortic constriction; one-way ANOVA followed by *post hoc* Spjotvoll/Stoline test. *F*, test values for the interaction between pressure overload due to aortic constriction and tedisamil treatment by two-way ANOVA; within parentheses degrees of freedom; #$P<0.05$ for the interaction between pressure overload and tedisamil.

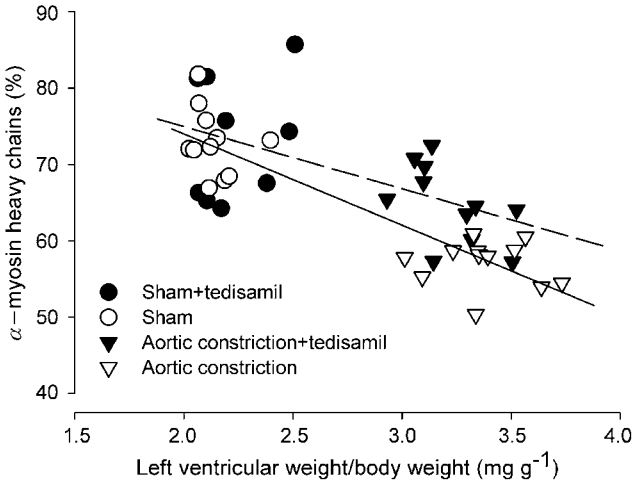

Figure 3 Correlation between the left ventricular weight/body weight ratio and proportion of α-myosin heavy chains. Correlation involving untreated sham-operated rats and untreated rats with ascending aortic constriction (solid line): $y = 98.31–12.09x$, $r = -0.896$, $P > 0.05$. Correlation involving tedisamil-treated rats, sham-operated as well as rats with ascending aortic constriction (dashed line): $y = 91.5–8.21x$, $r = -0.564$, $P = 0.05$. Expression of α-myosin heavy chains is significantly different in untreated and tedisamil-treated rats after controlling for the relative left ventricular weight by ANCOVA; $F(1,40) = 5.727$; $P < 0.05$. Ascending aortic constriction lasted for 7 weeks. Tedisamil was given in drinking water in a dose $36\,mg\,kg^{-1}\,day^{-1}$ for 7 weeks.

improvement of this variable in tedisamil-treated rats with ascending aortic constriction remained below statistical significance, a greater proportion of α-myosin heavy chains was associated with an enhanced myocardial performance (Figure 4a) and tedisamil significantly modified the attenuating effect of pressure overload on the normalized stress–length area (Table 5). When compared with untreated pressure-overloaded rats, tedisamil-treated animals with aortic constriction developed higher intraventricular systolic pressures at lower filling volumes (150–250 mm^3) and pressures (Figure 2b). This can be interpreted as a sign of a positive inotropic effect of tedisamil in pressure-overloaded ventricles, as there was no difference in the left ventricular weight and geometry in rats with ascending aortic constriction. Figures 5 and 6 show original recordings of isovolumic contractions of one untreated and one tedisamil-treated rat with aortic constriction. Left ventricular weight and volume was very similar; however, tedisamil-treated rat exhibited higher values of isovolumically developed pressures at comparable end-diastolic pressures. Readings of maximum and minimum dP/dt values were also higher in the tedisamil-treated rat.

Tedisamil-treated animals with ascending aortic constriction exhibited increased values of maximum rate of intraventricular pressure rise and decline, as well as for maximum rate of ventricular wall stress rise and decline (Table 5). The depressant effect of the pressure overload on the velocity of contraction and relaxation was significantly modified by the

Table 5 Characteristics of isovolumically beating left ventricles

	Sham		*Aortic constriction*		*F(1,39)*
	Untreated (n = *11*)	*Tedisamil* (n = *8*)	*Untreated* (n = *12*)	*Tedisamil* (n = *10*)	
Heart rate ($beats\,min^{-1}$)	309±28	304±24	314±29	299±31	0.01
Maximal ventricular pressure–volume area (mJ)	5.5±0.9	5.6±0.5	9.0±2.5*	9.9±1.4*	0.642
Ventricular normalized stress–length area (kPa)	5.4±0.8	5.1±0.6	3.8±0.6*	4.4±0.4*	5.33#
Peak+dP/dt_{max} ($kPa\,s^{-1}$)	1004±227	991±201	1066±156	1313±234*†	7.56#
Peak−dP/dt_{max} ($kPa\,s^{-1}$)	454±116	478±104	455±97	577±82	2.00
Peak+dS/dt_{max} ($kPa\,s^{-1}$)	1183±191	1060±158	723±124*	986±171*†	14.10#
Peak−dS/dt_{max} ($kPa\,s^{-1}$)	417±79	415±74	281±31*	350±30†	4.473#

Values are means±s.d. *$P<0.05$ compared with sham-operated untreated rats; †$P<0.05$ compared with untreated rats with aortic constriction; one-way ANOVA followed by *post hoc* Spjotvoll/Stoline test. *F*, test values for the interaction between pressure overload due to aortic constriction and tedisamil treatment by two-way ANOVA; within parentheses degrees of freedom; #$P<0.05$ for the interaction between pressure overload and tedisamil. Highest observed values for maximum rate of intraventricular pressure rise (+dP/dt_{max}), decline (−dP/dt_{max}), as well as maximum rate of ventricular wall stress rise (+dS/dt_{max}) and decline (−dS/dt_{max}) are given.

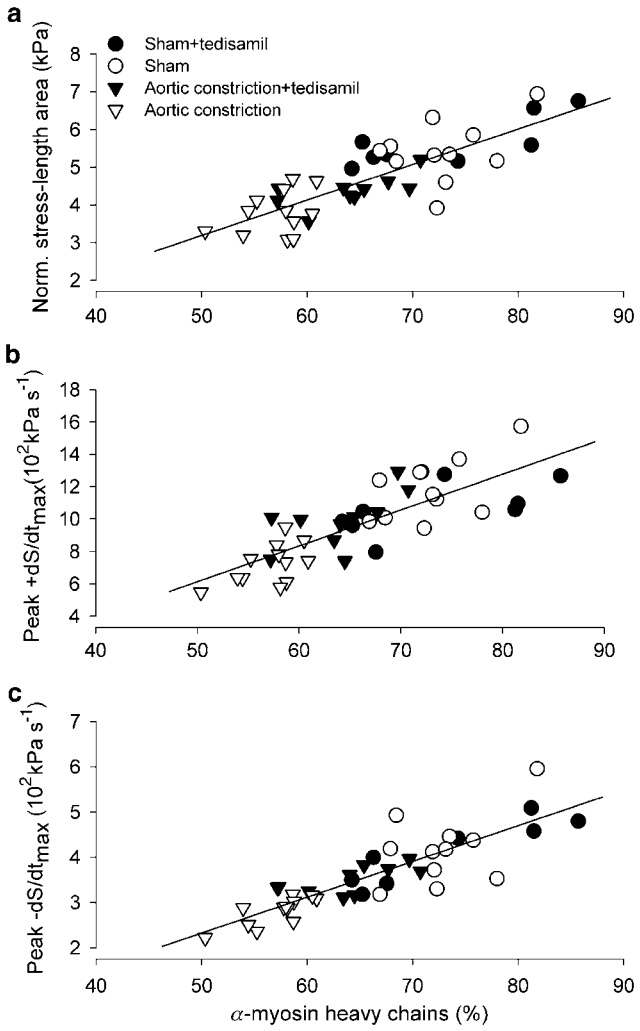

Figure 4 Correlation between the proportion of α-myosin heavy chains and indices of myocardial function: (a) normalized stress–length area, (b) maximum rate of ventricular wall stress rise (peak $+\mathrm{d}S/\mathrm{d}t_{max}$) and (c) maximum rate of ventricular wall stress decline (peak $-\mathrm{d}S/\mathrm{d}t_{max}$). Ascending aortic constriction lasted for 7 weeks. Tedisamil was given in drinking water in a dose $36\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ for 7 weeks. (a) Correlation involving rats from all experimental groups: $y=-0.801+0.082x$, $r=0.82$, $P<0.05$; (b) correlation involving rats from all experimental groups: $y=-493.4+22.2x$, $r=0.799$, $P<0.05$; (c) correlation involving rats from all experimental groups: $y=-163.8+79.25x$, $r=0.855$, $P<0.05$.

tedisamil treatment and was partially removed (Table 5). Rates of ventricular wall stress rise (Figure 4b) and decline (Figure 4c) improved proportionally to the increased percentage of α-myosin heavy chains in the tedisamil-treated pressure-overloaded rats.

Discussion

Effects of tedisamil on R–αT *interval, heart rate and systolic blood pressure*

Most of the described effects of tedisamil on ECG, heart rate and blood pressure are short-term observations following an i.v. application. After 14 days of oral administration, tedisamil caused bradycardia and prolongation of the QT interval in patients with stable angina (Fox *et al*., 2000). In the current experiment, dose-dependent prolongation of the R–αT interval was documented after a single oral application of tedisamil. Longer action potentials and changes in ventricular transmural voltage gradients may be responsible for this R–αT interval prolongation. A marked effect of tedisamil on R–αT interval can be attributed to a very well-developed I_{to} in rat ventricular myocytes and is in accordance with the importance of this outward current in the repolarization of rat ventricles (Beatch *et al*., 1991).

As a consequence of I_{to} inhibition in pacemaker cells of the sinus node, tedisamil is expected to reduce heart rate. A 7–11% reduction of the heart rate was recorded in our experiment after a long-term p.o. administration of tedisamil. This reduction in heart beat frequency is comparable with data reported after an acute i.v. application in dogs (Wallace *et al*., 1995) or after a 2-week p.o. treatment of patients with angina (Fox *et al*., 2000). However, tail-cuff blood pressure measurements represent a significant stress to conscious animals and may therefore underestimate the bradycardic effect of tedisamil. Actually, during maximum exercise, tedisamil did not decrease heart rate in healthy men (Demolis *et al*., 1997).

Reduction of heart rate to a similar extent due to vagal stimulation was associated with markedly improved long-term survival of rats after myocardial infarction through prevention of cardiac remodelling and the worsening of the pumping function (Li *et al*., 2004). Nevertheless, bradycardia in the present study did apparently not reach values that might be expected to affect cardiac growth. Reduction in heart rate, such as in antrioventricular blockade, leads to a volume overload and an increase in ventricular cavity volume (Verduyn *et al*., 2001). There were no signs of such a remodelling in tedisamil-treated rats (Figure 2a, Table 3).

Although stimulation of contractile activity by tedisamil was confirmed in isolated vascular preparations (Doggrell & Nand, 2001), tedisamil has no consistent effect on blood pressure (reviewed in Doggrell, 2001). Our plethysmographic tail-cuff blood pressure measurements of only systolic arterial blood pressure cannot confirm or exclude the hypertensive effect of tedisamil. Even so, there was a tendency towards higher systolic arterial blood pressure readings in sham-operated tedisamil-treated rats. In pressure-overloaded rats, this effect might have been blunted by the constriction of the ascending aorta.

Heart hypertrophy related to the reduction in potassium currents

Hypertrophy induced by the primary reduction in repolarizing potassium currents can be prevented by the inhibition of calcineurin with cyclosporin A or by the inhibition of L-type calcium channels (Kassiri *et al*., 2002; Sah *et al*., 2002). This strongly suggests a calcium–calcineurin-mediated mechanism of hypertrophy induction (Molkentin *et al*., 1998). The long-term administration of tedisamil did not provide evidence in favour of activation of the calcium–calcineurin–hypertrophy pathway and rather supports the notion that reduction in I_{to} is not causally associated with cardiac hypertrophy (Barry *et al*., 1998; Kuo *et al*., 2001). There was no increment in the mass of

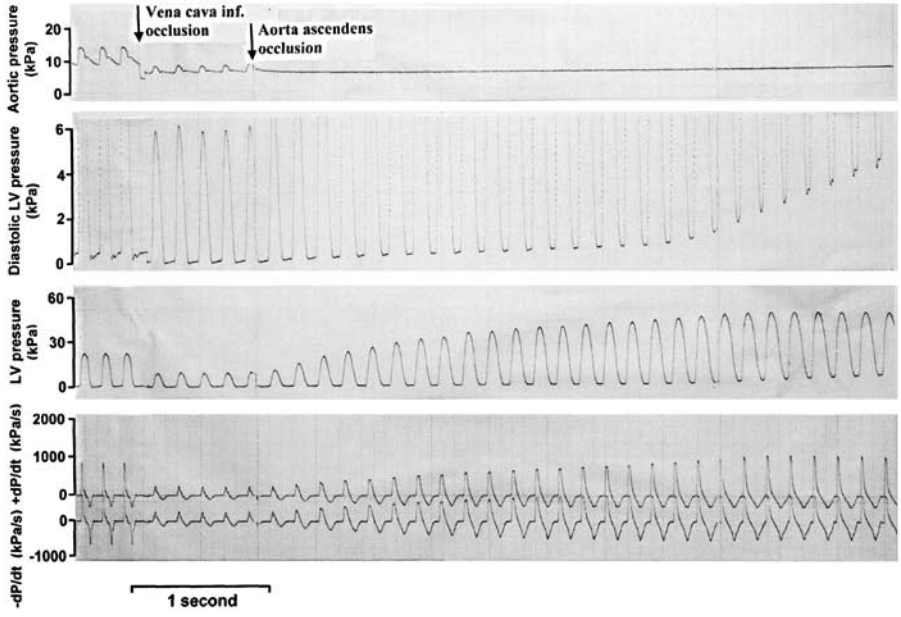

Figure 5 Original recording of isovolumic contractions of an untreated rat with ascending aortic constriction. Left ventricular weight was 1045 mg; left ventricular volume was 338 mm^3 at the intraventricular pressure of 0.8 kPa. Arrows indicate clamping of the vena cava inferior and ascending aorta, respectively.

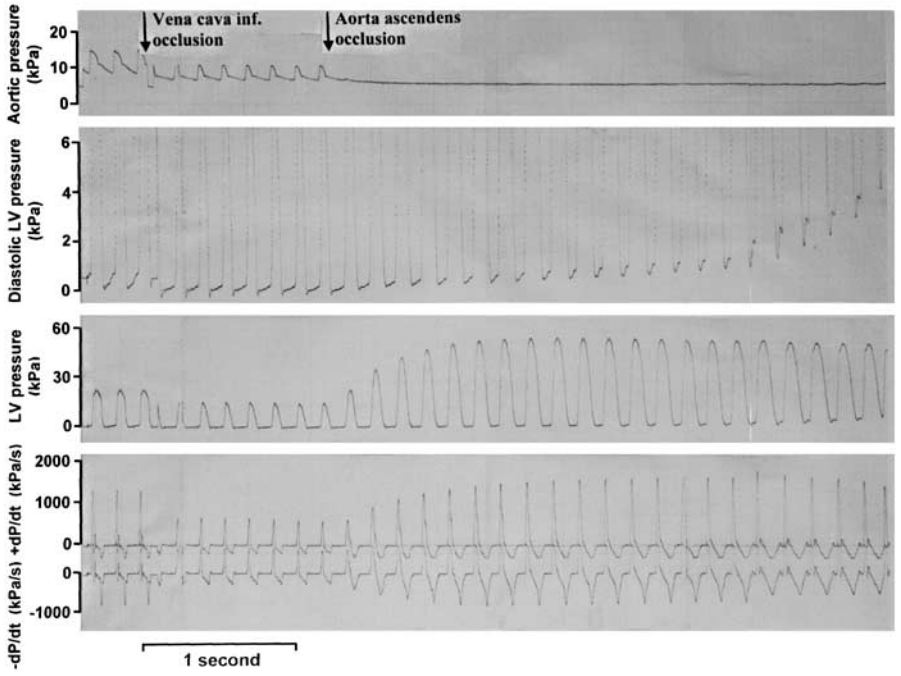

Figure 6 Original recording of isovolumic contractions of a tedisamil-treated rat with ascending aortic constriction. Left ventricular weight was 998 mg; left ventricular volume was 310 mm^3 at the intraventricular pressure of 0.8 kPa. Arrows indicate clamping of the vena cava inferior and ascending aorta, respectively.

cardiac chambers in tedisamil-treated rats, neither in pressure-overloaded nor in sham-operated animals.

Tedisamil blocks I_{to} and I_K and prolongs action potential duration (Dukes *et al*., 1990). In rodents, reduction of I_{to} is associated with the elevation of intracellular calcium concentration *via* L-type calcium channels and Na–Ca exchangers (reviewed in Sah *et al*., 2003). In the present study, blocking of the potassium channels can be inferred from the prolongation of the R–αT interval as well as the bradycardic effect in tedisamil-treated animals. However, absence of augmented growth argues against increased calcium transients in cardiac myocytes. On the other hand, action potential prolongation seems not to be a sufficient condition for the growth induction. For example, cardiac hypertrophy is absent, if action potential

is prolonged consequently to the inherited or drug-induced reduction in I_K (Swynghedauw *et al*., 2003) or some mutations in transient outward potassium channel (Barry *et al*., 1998; Kuo *et al*., 2001).

Changes of myocardial contractility associated with tedisamil application

Currently, no direct information is available on acute and chronic effects of tedisamil on intracellular calcium transients. Augmentation of cardiac contractile function by tedisamil observed in some experiments (Doggrell & Nand, 2001) would be consistent with a higher influx of extracellular calcium through L-type channels. However, positive inotropic effects were not always reported after tedisamil administration (Thormann *et al*., 1993). This could be explained by depressed calcium transient after I_{to} reduction in larger animals (Sah *et al*., 2003) or by inhibition of fast sodium channels at higher concentrations of tedisamil (Dukes *et al*., 1990). Chronic effects of tedisamil on calcium transients may also significantly differ from acute ones. If the action potential prolongation is associated with reduced heart rate, the lower number of action potentials per minute may counteract the increased calcium influx during one action potential. In addition, tedisamil could not only increase calcium influx but also stimulate decline in intracellular calcium levels (Seki *et al*., 2003).

In this study, augmentation of myocardial contractility correlated with the increased proportion of α-myosin heavy chains of cardiac myocytes (Figure 4a–c). A putative additional positive inotropic effect of tedisamil due to increased intracellular calcium would be expected to result in two separate correlation lines in plots of α-myosin heavy chain percentage *versus* indices of myocardial contractility, that is, one line for untreated rats and a separate line shifted to higher contractility values arising from tedisamil-treated animals. As no such shift was detected, positive inotropic effect of chronic tedisamil administration can fully be explained by the increment in α-myosin heavy chain proportion.

Isomyosin composition remained unchanged in tedisamil-treated sham-operated animals. Consistently, no changes in myocardial contractility were detected. Thus, this finding also supports the notion that there is either no significant augmentation in calcium transients during chronic tedisamil application or increased cytoplasmic calcium is effectively buffered.

Effect of tedisamil on myosin heavy chain isoforms expression

The relative expression of α- and β-myosin heavy chains isoforms is regulated developmentally, hormonally and metabolically (Rupp & Jacob, 1992). In pressure-overloaded rat ventricles, expression of α-myosin heavy chains is down-regulated and expression of β-myosin heavy chains is upregulated (Schwartz *et al*., 1978). The higher the overload, the greater the hypertrophy and the concomitant switch in the relative expression of myosin heavy chains isoforms (Turcani & Rupp, 2000). In the current study, the effect of tedisamil on relative myosin isozyme expression seems, however, not to be mediated by changes in the degree of hypertrophy. Two-way ANOVA proved that tedisamil modified the effect of pressure overload on α-myosin heavy chains expression, although cardiac mass remained unaltered (Table 4). In addition, α-myosin heavy chain expression was significantly different in tedisamil-treated and -untreated animals after any confounding effect of relative left ventricular weight was removed (Figure 3).

The major finding of this study is the tedisamil-induced enhancement of α-myosin heavy chain expression in the hypertrophied myocardium that was accompanied by improved myocardial contractile properties. As this effect was rather unexpected, we can only postulate that tedisamil might affect myosin isozymes expression through metabolic signals. The relative expression of myosin isozymes appears to be influenced by fuel metabolism, whereby a reduced glucose oxidation is associated with depressed α-myosin heavy chain expression (Zarain-Herzberg & Rupp, 2002). Although in the hypertrophied pressure-overloaded myocardium, glucose oxidation appears to be increased due to peroxisome proliferator-activated receptor-alpha deactivation (Barger *et al*., 2000; Young *et al*., 2001), it seems that the elevation of glucose oxidation is not sufficiently high to match the accelerated glycolysis. Thus, glucose oxidation becomes less 'coupled' in hypertrophied hearts than in nonhypertrophied hearts (Sambandam *et al*., 2002). In patients with aortic stenosis, insulin resistance occurred and myocardial glucose uptake was reduced (Paternostro *et al*., 1999). As tedisamil blocks ATP-sensitive potassium channels (Chi *et al*., 1996) and blockers of ATP-sensitive potassium channels correct the basal and insulin-stimulated glucose transport in skeletal muscle (Wasada *et al*., 2001), such a mechanism could enhance glucose utilization and thereby upregulate the expression of α-myosin heavy chains in pressure-overloaded ventricles. In sham-operated rats, with normal glucose oxidation, blocking of ATP-sensitive potassium channels could probably not further upregulate α-myosin heavy chain expression (Table 4).

As regards the mild bradycardic action of tedisamil, it should be mentioned that β-blockers are well-established drugs for reducing heart rate. This is associated with lower myocardial oxygen requirements, which is useful in ischaemic heart disease and heart failure (Kobinger, 1985). Since the use of β-blockers as bradycardic agents is associated with some undesirable aspects, such as negative inotropism, bronchial constriction, impotence and adverse effects on glucose and lipid metabolism, tedismil may represent a more suitable drug with negative chronotropic effects. Yet, to explain the results of the present study, we postulated a new potentially useful pharmacological effect, that is, tedisamil-induced limitation of foetal transformation of the overloaded heart by inhibiting ATP-sensitive potassium channels with subsequent stimulation of glucose oxidation.

Effect of tedisamil on ventricular performance

Assessment of isovolumic ventricular contraction allows estimation not only of ventricular performance but also of myocardial contractility and myocardial velocity of contraction and relaxation. This was important in the current study as the tedisamil effects on ventricular hypertrophy and myocardial contractility might alter the overall ventricular performance. As expected, pressure overload was compensated partially by the increased ventricular mass and partially by changes in ventricular geometry, that is, reduction in the left ventricular cavity volume as well as thickening of the

ventricular wall. There were no morphological signs of left ventricular decompensation, that is, increase in left ventricular cavity volume, right ventricular weight or lung weight. However, myocardial contractility as well as velocity of contraction and relaxation was depressed in pressure-overloaded ventricles. This represents a mismatch between the normal or increased heart rate and the slowed contraction that adversely affects also diastolic filling of the ventricle (Hansen & Rupp, 1994). An impaired ventricular filling is a characteristic feature of rats with ascending aortic stenosis (Litwin *et al*., 1995) and is probably responsible for the increased filling pressure and large atrial hypertrophy in this model (Turcani & Rupp, 1997). The bradycardic effect of tedisamil as well as the upregulation of α-myosin heavy chain expression with consequently enhanced rate of relaxation and contraction is expected to improve the filling of an overloaded ventricle and to reduce elevated atrial pressures. Indeed, atrial hypertrophy was significantly reduced in tedisamil-treated rats with aortic constriction. Tedisamil also significantly modified the effect of pressure overload on ventricular growth (Table 3). The tendency toward smaller left ventricular hypertrophy in tedisamil-treated rats could be associated with an improved myocardial contractility due to increased α-myosin heavy chain expression rather than enhanced calcium cycling (Figure 4). Positive inotropic interventions that increased intracellular calcium have typically deleterious effects on cardiac adaptation to the overload (Katz, 1989). This seems valid not only for Na^+K^+-ATPase blockers or phosphodiesterase inhibitors but also for increased intracellular calcium and hypercontractility due to reduction in I_{to} (Sah *et al*., 2002).

The correlation between the type of myosin heavy chains and the contractile performance of cardiac myocytes was very close. The type of myosin heavy chain determined the shape of force–velocity relationships, velocity of loaded shortening and overall power output-generating capacity of cardiac myocytes (Herron *et al*., 2001). Furthermore, a small increase in α-myosin heavy chain expression (+15%) significantly (+52%) augmented myocyte power output (Herron & McDonald, 2002). In animal heart failure models, prolonged survival after converting enzyme inhibition (Brooks *et al*., 1997) or mechanical unloading (Sabbah *et al*., 2003) was accompanied by augmented α-myosin heavy chains expression. Even in humans who express predominantly β-myosin heavy chains, an improvement in function of failing hearts after β-adrenergic blockade (Lowes *et al*., 2002) was associated with an increase in α-myosin heavy chain mRNA. Nonetheless, all these interventions are based on reduction in hemodynamic load imposed on the failing heart which, however, has physiological limits. Thus, the effectiveness of these therapeutic approaches is limited. The present study demonstrates that blunting of foetal transformation of myosin molecules by tedisamil occurred in the absence of a significant reduction of cardiac pressure overload.

Limitations

Owing to the long-term character of this study and well-known significant stress effects of handling of animals, we have applied tedisamil in drinking water. A great care was taken to maintain the constancy of the applied amount of tedisamil to each rat by the measuring the consumed water and body weight of the animals every second day. Our data show that the tedisamil dose of 36 mg kg^{-1} p.o. has a marked effect on R–αT interval. This interval was taken as a substitute for QT because in the rat ECG the end of the repolarization phase cannot be determined with sufficient reliability. Although the administration in the drinking water has its drawbacks, R–αT interval prolongation after an oral application of tedisamil as well bradycardia at the end of experiment suggests that the tedisamil effect was present throughout the experiment. The adaptive character of the baroreflex response to an increased peripheral resistance makes it highly unlikely that the observed bradycardia results from any putative vasoconstricting effect of tedisamil.

Acute changes in ventricular and myocardial function could be assessed by several methods with adequate precision. Such an approach is much more complicated when, in addition to loading conditions, heart rate, autonomic influences and myocardial contractility also the ventricular size and geometry are altered. To detect possible modifications in myocardial contractility under these conditions, we have analysed isovolumic contractions of the left ventricle in an open-chest model. The derived indices of ventricular and myocardial function are independent of loading conditions as well as ventricular size and geometry (Turcani & Rupp, 1997). The peak isovolumic left ventricular pressure is rather insensitive to changes in heart rate (Seipel & Hoffmeister, 1989); moreover, the heart rate during isovolumic contractions was not significantly different (Table 5). Crossclamping of the ascending aorta and the urethane anaesthesia probably led to the maximum available sympathetic support for the contracting ventricle. It cannot be excluded that indices derived from the maximum pressure developing capacity of the ventricle and the use of several model-dependent transformations may differ from the analysis of an ejecting ventricle in the closed thorax. However, this restriction does not invalidate the results of the present comparative study.

The study leaves a number of intriguing questions, which cannot be addressed in whole animals. Thus, it remains to be shown whether tedisamil affects calcium transients and to what extent inhibition of ATP-sensitive potassium channels is associated with an increased glucose oxidation. Despite these limitations, the present study provides some unexpected data that can plausibly be explained by the postulated new pharmacological effect of tedisamil.

In conclusion

Long-term administration of tedisamil (36 mg $kg\,day^{-1}$ in drinking water) did not affect significantly cardiac growth in rats with ascending aortic constriction and sham-operated rats. However, tedisamil treatment was accompanied by the greater expression of α-myosin heavy chains in pressure-overloaded left ventricles. This shift in relative expression of myosin isoforms was associated with an improved myocardial and ventricular performance. The reduced heart rate together with the enhanced myocardial contractility may support the cardiac adaptation to hemodynamic overload and thus represents a potentially useful pharmacological action of tedisamil.

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