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Distinct load dependence of relaxation rate and diastolic function in *Oryctolagus cuniculus* and *Ratus norvegicus*

Abstract This study investigated potential differences on load dependence of relaxation rate and diastolic function between *Oryctolagus cuniculus* and *Ratus norvegicus*, which have constitutive differences in the mechanisms involved in myocardial inactivation. Load dependence of relaxation rate and diastolic function were evaluated with the response of left ventricular time constant τ and diastolic pressure-dimension relation to beat-to-beat aortic constrictions in open-chest rabbits and rats. Afterload levels were normalized, being expressed as a percentage of peak isovolumetric pressure (relative load). In control heartbeats, relaxation rate and diastolic function were similar in the two animal species. They presented, however, distinct responses to afterload elevations. In rabbits, time constant decreased ∼7% and diastolic pressure-dimension relation remained unchanged when afterload was elevated to a relative load of 73–76%. Above this afterload level, a significant deceleration of relaxation rate (increase of time constant) and an upward shift of diastolic pressure-dimension relation were observed. In rats, afterload elevations accelerated pressure fall up to a relative load of 97–100% and no afterload-induced shift of the diastolic pressure-dimension relation was observed. This study provides, therefore, evidence that *Oryctolagus cuniculus* has lower afterload reserve of myocardial relaxation and diastolic function than *Ratus norvegicus*.

Keywords End-diastolic pressure-volume relation · Rabbits · Rats · Time constant τ

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Abbreviations *ED* End-diastolic · *LV* Left ventricular · *LVP* Left ventricular pressure · *NCX* Na^+/Ca^{2+} exchanger · *PV* Pressure-volume · *SERCA2* Sarcoplasmic reticulum Ca^{2+}-ATPase

Introduction

Myocardial relaxation is an important determinant of both early (Shintani and Glantz 1994) and late (Leite-Moreira et al. 1999a; Leite-Moreira and Correia-Pinto 2001) left ventricular (LV) diastolic filling. It is modulated by non-uniformity, inactivation and load (Gillebert et al. 2000). Non-uniformity refers to the temporal and spatial asynchronous distribution of load and inactivation during myocardial relaxation (Brutsaert 1987; Leite-Moreira and Gillebert 1996). Inactivation refers to the processes whereby Ca^{2+} is transported out of the cytosol (Bers 2002), in order to achieve its diastolic levels, and cross-bridge detachment occurs. The four pathways involved in the first are phospholamban (PLB)-modulated uptake of Ca^{2+} by the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), Ca^{2+} extrusion via the Na^+/Ca^{2+} exchanger (NCX), mitochondrial Ca^{2+}-uniport and sarcolemmal Ca^{2+}-ATPase, with the two latter being responsible for only about 1% of total (Bers 2002). The quantitative importance of the two first major routes varies between species (Negretti et al. 1993; Bassani et al. 1994; Hove-Madsen and Bers 1993; Lewartowski et al. 1992). In rabbit ventricular myocytes, SERCA2a removes 70% of the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), while NCX removes 28%. On the other hand, SERCA2a activity is higher in rat ventricular myocytes, being responsible for the uptake of 92% of $[Ca^{2+}]_i$, whilst NCX contributes to the removal of only 7% (Bers 2002). In addition, rat hearts express predominantly the faster myosin heavy chain (MHC)-α isoform (Meehan et al. 1999), while rabbit hearts express predominantly the slower MHC-β isoform, which has a higher affinity for Ca^{2+} (Reiser and Kline 1998).

Load changes influence calcium regulatory mechanisms and myofilament properties. In rabbits, it was previously demonstrated that afterload elevations have a biphasic effect on relaxation rate and end-diastolic (ED) pressure-volume (PV) relation (Leite-Moreira et al. 1999a). In this regard, afterload elevations up to a certain level accelerate LV relaxation and do not affect the ED-PV relation, reflecting a compensatory response and the presence of afterload reserve. Greater elevations of afterload slow LV relaxation and upward shift the ED-PV relation, resulting in diastolic dysfunction because afterload reserve has been exhausted. The level of afterload above which a decompensatory response occurs may be shifted by pharmacological agents such as caffeine (Leite-Moreira et al. 1999b) and β-adrenergic stimulation (Leite-Moreira et al. 2001). Caffeine, which decreases $[Ca^{2+}]_i$ uptake by SERCA2a and increases myofilament Ca^{2+} sensibility (Wendt and Stephenson 1983), shifts the transition to a decompensatory response towards smaller afterload levels. On the other hand, β-adrenergic stimulation, which enhances SERCA2a activity and decreases myofilament Ca^{2+} sensibility, thereby shifts the transition to a decompensatory response toward higher afterload levels. Diastolic disturbances induced by afterload are therefore attenuated. These results are highly suggestive of a relation between SERCA2a activity and the occurrence of afterload-induced disturbances of relaxation and diastolic function.

Additionally, it was also documented that afterload-induced diastolic disturbances are related not only with relaxation rate but also with the available time to relax (Leite-Moreira and Correia-Pinto 2001). The time available to relax is altered by heart rate, which is significantly higher in rats. Taking into account these molecular and heart rate differences between rabbits and rats, it would be interesting to investigate how rats handle all these factors without compromising normal cardiac diastolic physiology.

As *Oryctolagus cuniculus* and *Ratus norvegicus* have different heart rate and constitutive differences in gene expression and activity of SERCA2a and NCX, we hypothesized that load dependence of relaxation and diastolic function would also be significantly different.

Materials and methods

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study was carried out in 36 male adult healthy animals: 19 New Zealand white rabbits (*O. cuniculus*, 12 weeks old) and 17 Wistar rats (*R. norvegicus*, 7 weeks old).

Experimental preparation

The rabbits were premedicated with ketamine hydrochloride (50 mg kg^{-1}, i.m.) and xylazine hydrochloride (5 mg kg^{-1}, i.m.), while rats were anaesthetized with pentobarbital (6 mg/100 g, i.p.). A tracheostomy was performed and mechanical ventilation

initiated (Harvard Small Animal Ventilator, Model 683), delivering oxygen-enriched air. Respiratory rate and tidal volume were adjusted, according to species, in order to keep arterial blood gases and pH within physiological limits. Anaesthesia was maintained with ketamine hydrochloride (33 ml kg^{-1} h^{-1} i.m.), pentobarbital sodium (12.5 mg kg^{-1} i.v. before opening the chest and then 2.5 mg kg^{-1} i.v. as needed), and vecuronium bromide (0.5 mg h^{-1} i.v.) for rabbits and with an additional bolus of pentobarbital (2 mg/100 g) as needed for rats. A central vein was cannulated and a pre-warmed solution 20 mEq KCl and 40 mEq NaHCO$_3$ in 500 ml 0.9% NaCl was then administrated to compensate for perioperative fluid losses.

In both animal species, the heart was exposed through a median sternotomy and the pericardium widely opened. The ascending aorta was dissected and a silk suture (1-0 in rabbits and 3-0 in rats) was placed around it to allow its external occlusion during the experimental protocol. Left ventricular pressure (LVP) was measured with a high-fidelity micromanometer (3-F, SPR-524, Millar Instruments, Houston, Tex., USA) inserted through an apical puncture wound into the LV cavity. The manometers were calibrated against a mercury column and zeroed after stabilization for 30 min in a water bath at body temperature. LV dimensions were measured with miniaturized ultrasonic dimension gauges using a sonomicrometer amplifier (Triton Electronics, San Diego, Calif., USA). In rabbits, one pair of crystals (3 mm) was sutured in place onto the LV anterior and posterior epicardial surfaces to measure LV external anterior-posterior diameter and a third crystal (1 mm) was tunnelled at a 30–45º angle into the subendocardial facing the LV anterior epicardial crystal. The anterior epicardial crystal and the subendocardial crystal were combined to measure wall thickness. In rats, one crystal was placed in the interventricular septum (1 mm) and another one on the epicardial surface of the LV free wall (2 mm), allowing the direct measurement of LV septal-free wall dimension. In all animals, a limb ECG (II) was recorded throughout. At the end of the experiment, the animals were sacrificed with an overdose of anaesthetics and the position of crystals and manometers verified at necropsy.

Experimental protocol

After complete instrumentation, the animal preparation was allowed to stabilize for 30 min before the beginning of the experimental protocol, which consisted in randomly performing multiple graded left ventricular pressure elevations, by abruptly narrowing or occluding the ascending aorta during the diastole separating two heartbeats. The preceding beat is control and the following beat is test heartbeat. The analysed intervention, therefore, was a selective alteration of afterload without changes in preload or long-term load history (Gillebert et al. 1997). Systolic LVP of the first heartbeat following the intervention varied as a function of the extent of aortic constrictions. The animal was stabilized for several beats before another intervention was performed. The animals were not paced, but heart rate did not vary significantly during the experimental protocol (214 ± 9 and 295 ± 16 beats min^{-1} for rabbits and rats, respectively).

Data acquisition and analysis

Recordings were made with respiration suspended at end expiration and parameters were converted on-line to digital data with a frequency of 500 Hz. To distinguish between ED at the beginning and at the end of the analysed cardiac cycle, ED at the beginning was referred to as ED(pre), while ED at the end was referred to as ED(post). Peak rates of LVP rise (dP/dt_{max}) and fall (dP/dt_{min}) were measured. LVP was measured at the beginning of the cardiac cycle (LVP$_{ED(pre)}$), at peak systole (LVP$_{max}$), at its protodiastolic nadir (LVP$_{min}$), and at the end of the cardiac cycle (LVP$_{ED(post)}$). Afterload levels were presented as relative load, which consists in peak systolic LVP of a given heartbeat

expressed as percentage of peak pressure of the corresponding isovolumetric beat (Leite-Moreira and Gillebert 1994). Time intervals were measured from ED(pre) to dP/dt_{min} and from dP/dt_{min} to ED(post). Rate of pressure fall was evaluated with dP/dt_{min} and the time constant τ. For calculating τ, the portion of the LVP tracing between dP/dt_{min} and a pressure equal or below the value of ED(post) was selected. The curve was fitted (SigmaPlot 5.0 SPSS) to a monoexponential model with a non-zero asymptote, given by the following equation:

$$P(t) = P_{0*}e^{-t/\tau} + P_{\infty}$$

where P_{∞} is a non-zero asymptote (mmHg), P_0 is an amplitude constant (mmHg), t is time (ms), and τ is the time-constant of the exponent (ms). The correlation coefficient (r^2) yielded values > 0.97. According to this formula, relaxation will be 97% complete after a time interval of 3.5τ (ms) starting at the onset of LVP fall (Weisfeldt et al. 1978; Leite-Moreira et al. 1999a). LV dimension was measured at the beginning of the cardiac cycle (LVP$_{ED(pre)}$), at its minimal value (LVD$_{min}$) and at the end of the cardiac cycle (LVP$_{ED(post)}$).

Statistical analysis

Group data are presented as mean $\pm$ SEM. To compare the multiple afterload levels in rats and rabbits, we performed two-way repeated-measures ANOVA. When treatments were significantly different, the Student-Newman-Keuls test was selected to perform pairwise multiple comparisons. Statistical significance was set at $P < 0.05$.

Results

For both species and from multiple available interventions we selected for further analysis, in addition to control heartbeats, cardiac cycles whose relative loads were closer to 70% (rabbits, $69 \pm 1\%$; rats, $70 \pm 1\%$), 80% (rabbits, $81 \pm 1\%$; rats, $82 \pm 1\%$), 90% (rabbits, $90 \pm 1\%$; rats, $91 \pm 1\%$) and 100% (isovolumetric heartbeats). In both species dP/dt_{max} did not change significantly with afterload interventions. Relative load of the control beats was significantly higher in rabbits ($56 \pm 1\%$) than in rats ($49 \pm 2\%$).

Effects of LV afterload elevations on LVP fall and diastolic function

Effects of LV afterload elevations on LVP fall and diastolic function are illustrated in Figs. 1 and 2 and summarized in Tables 1 and 2. At baseline, both species presented similar relaxation rates as measured by dP/dt_{min} and time constant τ. As illustrated in Fig. 1, effects of afterload elevations on relaxation rate were assessed with the fractional changes in the time constant τ. Although in both species, τ presented biphasic response to afterload elevations, they were not identical. In rats, $\tau_{test}/\tau_{control}$ decreased ($\tau_{test}/\tau_{control} < 1$, acceleration of pressure fall) over almost the entire range of afterload elevations from control to isovolumetric beats. This acceleration was maximal at a relative load of 80%. Above this afterload level acceleration of pressure fall became smaller and was no more observed in isovolumetric beats (relative load of 100%). On the other hand, in rabbits, relaxation rate accelerated up to a relative load of 70%, while afterload elevations reaching or exceeding a relative load of 80% resulted in a progressive and significant increase of $\tau_{test}/\tau_{control}$ ($\tau_{test}/\tau_{control} > 1$, deceleration of pressure fall). The transition from acceleration to deceleration occurred at relative loads of 73–76% and 97–100% for rabbits and rats, respectively.

ED LV pressures and dimensions showed distinct responses to afterload elevations. Whereas LVP$_{ED(pre)}$, ID$_{ED(pre)}$ and ID$_{ED(post)}$ were not affected, LVP$_{ED(post)}$ increased significantly with afterload elevations in rabbits, but only marginally in rats. As ID$_{ED(post)}$ did not change with afterload in rabbits and rats that means that in the first diastole after an elevation of the afterload there was no significant filling beyond ID$_{ED(pre)}$. Therefore, the difference between ED LVP at the end and beginning of the cardiac cycle (LVP$_{ED(post)}$−LVP$_{ED(pre)}$) reflects the magnitude of the upward shift of the ED LVP-ID relation and the occurrence of diastolic dysfunction, which was observed in rabbits but not in rats (Fig. 2).

In rabbits diastolic dysfunction became apparent when relaxation rate decelerated ($\tau_{test}/\tau_{control} > 1$). In rats, this never occurred since afterload elevations elicited almost always acceleration of myocardial relaxation ($\tau_{test}/\tau_{control} < 1$).

Evaluation of LV completeness of myocardial relaxation

In Fig. 3, afterload-induced upward shift of the ED LVP-ID relation (diastolic dysfunction) is plotted against the difference between available and predicted times for the LV to relax. Available time for the ventricle to relax corresponds to the measured time interval from onset of left ventricular pressure fall till the next end-diastole. On the other hand, the predicted time was computed as 3.5τ (Leite-Moreira et al. 1999a; Leite-Moreira and Correia-Pinto 2001), which estimates 97% completion of relaxation. When the difference between available and predicted times was negative, this means that there was a deficit in time for the ventricle to relax. In rats, such deficit only occurred in isovolumetric beats, while in rabbits an significantly bigger deficit was present not only in isovolumetric beats but also for the relative load of 90%. Interestingly, when such a deficit in time for the ventricle to relax occurred a significant afterload-induced diastolic dysfunction was observed.

Discussion

In the current study, we tested the hypothesis that load dependence of relaxation and diastolic function in *O. cuniculus* and *R. norvegicus*, which have different

heart rate and constitutive gene expression of sarco-plasmic reticulum Ca^{2+}-ATPase (SERCA2a) and Ca^{2+} extrusion via the NCX (Negretti et al. 1993; Bassani et al.

1994; Hove-Madsen and Bers 1993; Lewartowski et al. 1992), would be distinct. We could demonstrate that, although myocardial relaxation rate at baseline was similar in both animal species, they showed a distinct response to afterload elevations, with rats having a higher afterload reserve of diastolic function than rabbits. This could have a biological meaning since heart rate is significantly higher in rats than in rabbits. In fact, with the pronounced afterload-induced acceleration of relaxation, rats could compensate the shortest time available that its LV has to relax.

Investigation of load dependence of relaxation rate and diastolic function, in the in situ intact heart, must be performed with beat-to-beat load manipulations in the presence of a widely opened pericardium, as we did in the present study, in order to exclude several confounding factors, such as neurohumoral activation, pericardial constraint, preload changes and long-term load history (Gillebert et al. 2000).

Myocardial relaxation is an important determinant of diastolic function. It is essentially modulated by the interaction of afterload with the underlying mechanisms involved in the decline of $[Ca^{2+}]_i$ to its diastolic levels (Leite-Moreira and Gillebert 1996; Leite-Moreira et al. 1999b). Quantitative importance of the various mechanisms involved in this process varies amongst animal species (Hove-Madsen and Bers 1993; Bassani et al. 1994), namely rabbits and rats, as outlined in the Introduction. Relaxation rate, as assessed by dP/dt_{min} and τ, seems to be highly dependent of SERCA2a activity as previously demonstrated either in a transgenic mouse line overexpressing SERCA2a (He et al. 1997) and NCX knockout mice (Yao et al. 1997, 1998). As rat hearts have significantly higher SERCA2a activity than rabbit hearts (Negretti et al. 1993; Bassani et al. 1994; Hove-Madsen and Bers 1993; Lewartowski et al. 1992) we expected myocardial relaxation to be faster in rats than in rabbits. Interestingly, the present study showed that, at baseline, relaxation rate was similar in the two species and that the
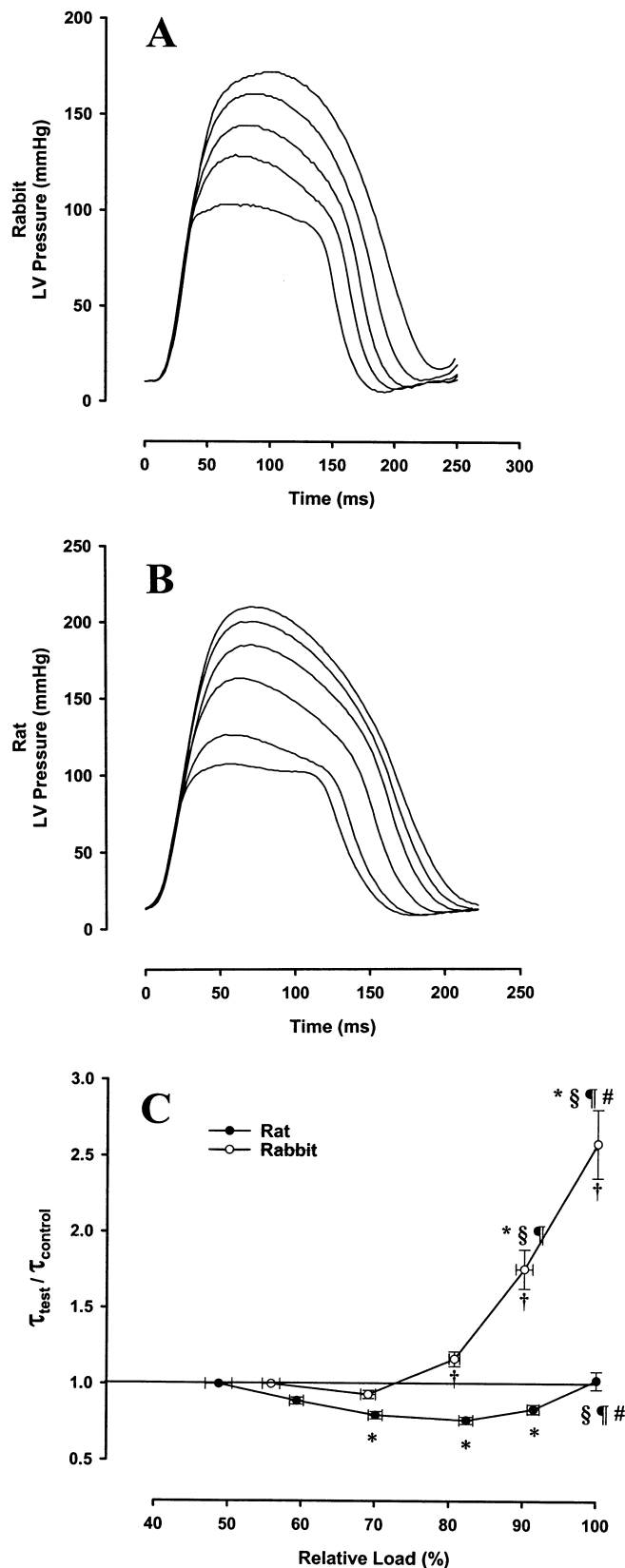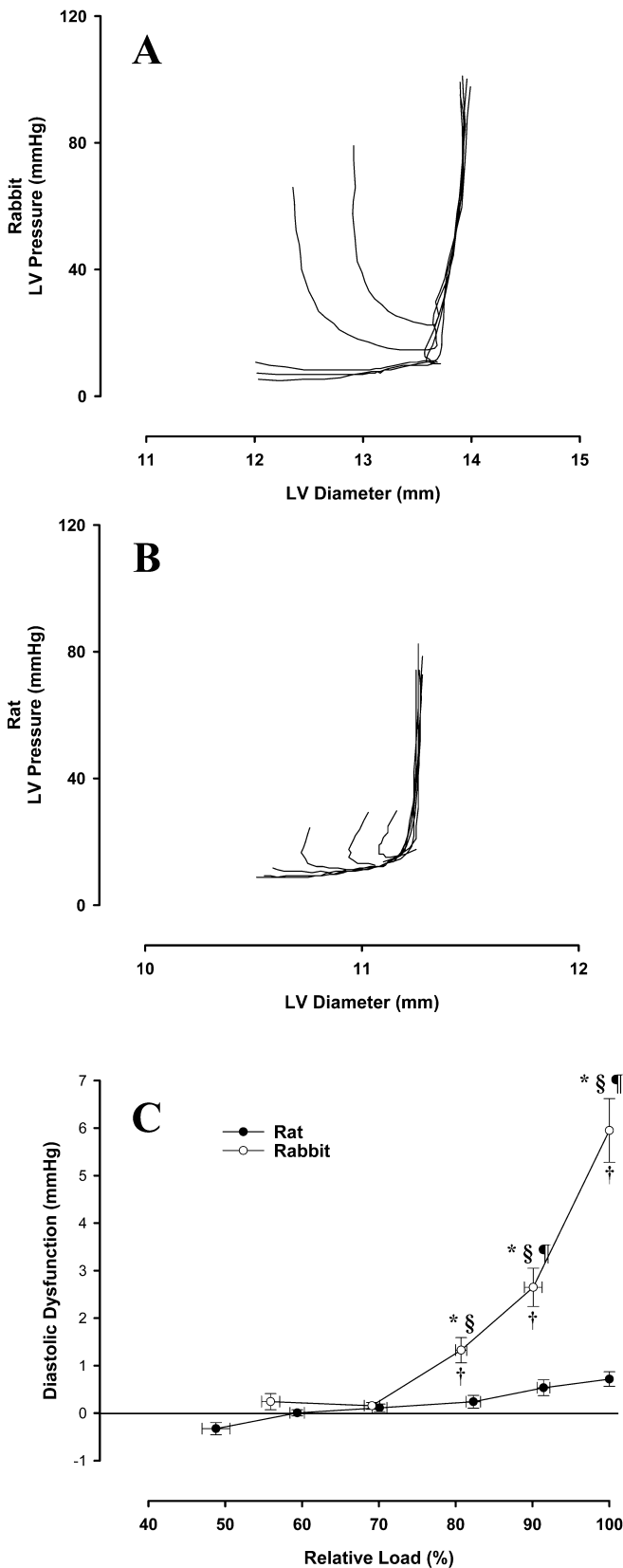
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Fig. 1A–C Effects of selective afterload elevations on left ventricular (LV) pressure time-courses (A, B) and relaxation rate (C) in both species. In the A (rabbit) and B (rat), a representative example of five superposed heartbeats with increasing afterloads are displayed. In contrast to rat, in rabbits diastolic LV pressures at the end of highly afterloaded heartbeats were significantly higher than end-diastolic (ED) LV pressures at the beginning of the cardiac cycles. In C, relaxation rate was assessed with the fractional changes in the time constant τ ($\tau_{test}/\tau_{control}$). In rats, afterload elevations elicited acceleration of myocardial relaxation with transition from acceleration to deceleration occurring at a relative load of 97–100%. In rabbits, acceleration of myocardial relaxation was evident only from control to 70% relative load ($\tau_{test}/\tau_{control}$ <1), whereas afterload elevations reaching or exceeding a relative load of 80% progressively decreased relaxation rate ($\tau_{test}/\tau_{control}$ >1). In rabbits, transition from acceleration to deceleration occurred at a relative load of 73–76%. In C results are mean ± SEM. Significant differences between animal species, $P<0.05$: † rabbit versus rat. Significant differences between afterload levels, $P<0.05$: * versus control; § versus 70%; ¶ versus 80%; # versus 90%

differences only became manifest in response to an afterload challenge. Indeed, rabbit hearts revealed lower afterload reserve of diastolic function than rats.

Afterload reserve of diastolic function was previously defined on the basis of the response of rate of pressure fall and position of the diastolic pressure-volume relation to acute beat-to-beat afterload elevations. As already described in previous studies, this response was biphasic in dogs (Leite-Moreira and Gillebert 1994) and rabbits (Leite-Moreira et al. 1999a; Leite-Moreira and Correia-Pinto 2001). In both these species, smaller afterload elevations, up to a relative load of 81–84% in dogs (Leite-Moreira and Gillebert 1994), and 73–76% in rabbits (Leite-Moreira et al. 1999a, Leite-Moreira and Correia-Pinto 2001), accelerated LV relaxation rate and did not affect the LV end-diastolic pressure-volume relation, indicating a compensatory response and the presence of afterload reserve of diastolic function. On the contrary, afterload elevations exceeding those relative loads markedly slowed LV relaxation rate and shifted the end-diastolic pressure-volume relation upwards. This traduces a decompensatory response, indicating that afterload reserve of diastolic function has exhausted. The present study confirmed the results previously observed in rabbits but showed that rat hearts respond to afterload elevations in a compensatory way almost during the entire range of afterloads between control and isovolumetric beats.

Decompensatory response was either not observed, or present only in isovolumetric and beats very close to isovolumetric. Transition from compensation to decompensation could be estimated in rats at a relative load of 97-100%. These findings nicely fit our hypothesis of a relation between this transition that determines afterload reserve of diastolic function and SERCA2a activity, which is significantly higher in rats than in rabbits. Another potential mechanism for this finding is the distinct isoform of MHC predominantly expressed by each of these animal species. In fact, while rat hearts express predominantly the faster MHC-α isoform (Meehan et al. 1999), rabbit hearts predominantly express the slower MHC-β isoform, which has a higher affinity for Ca^{2+} (Reiser and Kline 1998). It should be remembered, however, that changes in MHC isoforms have a bigger impact on contraction than on relaxation (Perez et al. 1999). This could explain why indices of contractility, such as dP/dt_{max} and peak isovolumetric pressure, were significantly higher in rats than in rabbits,

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Fig. 2A–C Effects of afterload elevations on the position of the diastolic pressure-dimension relation. In **A** (rabbit) and **B** (rat), a representative example of five superposed heartbeats with increasing afterloads are displayed. In contrast to the rat, in the rabbit the diastolic portion of the pressure-dimension loops was upward shifted in highly afterloaded heartbeats. In **C** the upward shift of the diastolic pressure-dimension relation (diastolic dysfunction) is presented as a function of the relative load either in rats and rabbits. Significant diastolic dysfunction was observed when afterload exceeded a relative load of 73–76% in rabbits and 97–100% in rats. In **C** results are mean ± SEM. Significant differences between animal species, $P < 0.05$: † rabbit versus Rat. Significant differences between afterload levels, $p < 0.05$: * versus Control; § versus 70%; versus 80%; # versus 90%

Table 1 Effects of afterload on parameters of left ventricular contraction and relaxation

Parameter	Species	Afterload elevations				
		Control	70%	80%	90%	100%
LVP_{max}	Rabbit	85.6 ± 2.8	$102.0 \pm 3.1^{*}$	$116.5 \pm 3.8^{*, \S}$	$132.8 \pm 3.5^{*, \S}$	$145.9 \pm 4.6^{*, \S, \#, \P}$
(mmHg)	Rat	96.2 ± 4.3	$137.6 \pm 2.9^{a,*}$	$161.8 \pm 3.7^{a,*, \S}$	$179.7 \pm 3.9^{a,*, \S}$	$196.9 \pm 4.8^{a,*, \S, \#, \P}$
dP/dt_{max}	Rabbit	3381 ± 135	3234 ± 134	3124 ± 151	3139 ± 134	3306 ± 169
(mmHg/s)	Rat	5068 ± 343	5104 ± 303^{a}	5131 ± 290^{a}	5184 ± 275^{a}	$5247 \pm 277^{a,\#, \P}$
dP/dt_{min}	Rabbit	-2790 ± 136	$-2897 \pm 110^{*}$	$-2604 \pm 107^{*}$	$-2181 \pm 93^{*}$	-1830 ± 95
(mmHg/s)	Rat	-3403 ± 343	-3735 ± 235^{a}	-3972 ± 412^{a}	$-3886 \pm 353^{a,*, \S}$	$-3585 \pm 529^{a,*, \S, \P}$
Time to $dP/$	Rabbit	160.3 ± 3.8	161.9 ± 4.7	175.2 ± 5.3	181.2 ± 6.4	187.5 ± 7.2
dt_{min} (ms)	Rat	108.9 ± 6.7^{a}	124.4 ± 8.6^{a}	132.5 ± 8.5^{a}	140.6 ± 8.6^{a}	$143.6 \pm 9.3^{a,\P}$
Time constant	Rabbit	18.2 ± 9.8	16.0 ± 1.1	20.2 ± 1.5	$30.1 \pm 2.7^{*, \S}$	$44.2 \pm 3.3^{*, \S, \#, \P}$
τ (ms)	Rat	24.8 ± 2.3	19.3 ± 1.4	$18.7 \pm 1.6^{*}$	20.3 ± 1.8^{a}	24.9 ± 2.2^{a}

Results presented as mean $\pm$ SEM; rabbits, $n=19$; rats, $n=17$ (LVP_{max} peak systolic left ventricular pressure; dP/dt_{max}, dP/dt_{min} peak rates of LVP rise and fall, respectively)
Significant differences between animal species: $P<0.05$: [a]versus rabbit

Significant differences between afterload levels $P<0.05$: *versus control; §versus 70%; ¶versus 80%; #versus 90%

Table 2 Effects of afterload changes on LV diastolic parameters

Parameter	Species	Afterload elevations				
		Control	70%	80%	90%	100%
$LVP_{ED(pre)}$ (mmHg)	Rabbit	5.6 ± 0.5	5.7 ± 0.6	5.9 ± 0.6	6.3 ± 0.7	6.1 ± 0.7
	Rat	7.0 ± 1.3	6.9 ± 1.3	6.9 ± 1.2	7.6 ± 1.2	7.4 ± 1.1
$ID_{ED(pre)}$ (mm)	Rabbit	12.5 ± 0.7	12.1 ± 0.7	12.0 ± 0.7	12.2 ± 0.7	11.9 ± 0.7
	Rat	9.2 ± 0.6^{a}	9.2 ± 0.6^{a}	9.3 ± 0.6^{a}	9.3 ± 0.6^{a}	10.0 ± 0.6^{a}
$LVP_{ED(post)} - LVP_{ED(pre)}$ (mmHg)	Rabbit	0.24 ± 0.17	0.16 ± 0.07	$1.33 \pm 0.27^{*, \S}$	$2.65 \pm 0.4^{*, \S, \P}$	$5.95 \pm 0.67^{*, \S, \P, \#}$
	Rat	-0.32 ± 0.13	0.11 ± 0.08	0.241 ± 0.13^{a}	0.54 ± 0.17^{a}	0.72 ± 0.15^{a}
$ID_{ED(post)}$ (mm)	Rabbit	12.4 ± 0.7	12.1 ± 0.7	12.1 ± 7.3	12.4 ± 0.7	12.2 ± 0.6
	Rat	9.3 ± 0.7^{a}	9.2 ± 0.6^{a}	9.2 ± 0.6^{a}	9.3 ± 0.6^{a}	9.3 ± 0.6^{a}

Results presented as mean $\pm$ SEM; rabbits, $n=19$; rats, $n=17$ ($LVP_{ED(pre)}$ and $LVP_{ED(post)}$ end-diastolic left ventricular pressures at the beginning and at the end of the heartbeat, respectively; $ID_{ED(pre)}$ and $ID_{ED(post)}$ end-diastolic internal diameters at the beginning and the end of the heartbeat, respectively; LVP_{min}, minimal left ventricular pressure; ID at LVP_{min} internal diameter at minimal left ventricular pressure)

Significant differences between animal species, $P<0.05$: [a]versus rabbit
Significant differences between afterload levels, $P<0.05$: *versus control; §versus 70%; ¶versus 80%; #versus 90%

while relaxation rate in control beats was similar in the two animal species.

The observations reported in this study reflect, therefore, the different expression of regulatory calcium proteins between studied species. It should be emphasised that calcium regulatory proteins (Tate et al. 1990, 1996) and myosin isoforms (Farrar et al. 1988) are to some degree dependent on age and previous endurance performance. In our study this aspect can be excluded since all animals were instrumented at matched developmental ages and all animals had similar husbandry conditions.

Distinct load dependence of relaxation expressed by rats and rabbits might have a biological relevance. In fact, if the rat presented a relaxation behaviour similar to the rabbit, rats would develop serious diastolic intolerance to increased afterload, because the higher heart rate of rats means that they have significantly shorter time available for the ventricle to relax than do rabbits. This does not happen because the rat's molecular machinery allows myocardial relaxation to respond to afterload elevations essentially with acceleration and not with deceleration as observed in larger animal species such as rabbits and dogs. This issue might be particularly relevant to physiological adaptation to physical exercise. In fact, we can speculate that given their elevated physiological heart rate, rats will presumably respond to exercise using preferentially preload reserve rather than contractility and heart rate augmentation.

This study, therefore, provided evidence that _O. cuniculus_ has a lower afterload reserve of myocardial relaxation and diastolic function than _R. norvegicus_. These data could have a biological significance since heart rate in rats is considerably higher than in rabbits. Additionally, as these are two of the most commonly used animal species in cardiovascular physiology, the differences described in the present study should be taken in account when drawing conclusions about physiological and/or
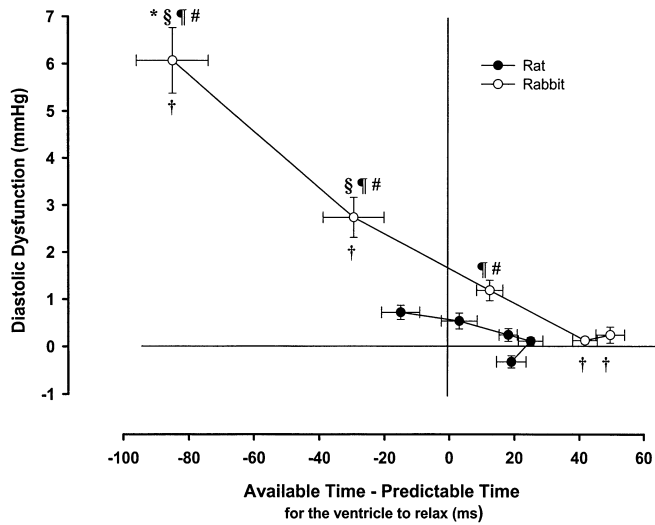
Fig. 3 The upward shift of the diastolic pressure-dimension relation (diastolic dysfunction) was plotted as function of the difference between the available and the predicted time for the ventricle to relax (time deficit) in both species. Results are presented as mean $\pm$ SEM. Significant differences between species, $P < 0.05$: † rabbit versus rat. Significant differences between afterload levels, $P < 0.05$: * versus control; § versus 70%; ¶ versus 80%; # versus 90%

pathophysiological observations on relaxation and diastolic function.

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