ORIGINAL ARTICLE

Early regression of left ventricular hypertrophy after treatment with esmolol in an experimental rat model of primary hypertension

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Certain β -adrenergic blockers have proven useful in the regression of ventricular remodeling when administered as long-term treatment. However, early regression of left ventricular hypertrophy (LVH) has not been reported, following short-term administration of these drugs. We tested the hypothesis that short-term administration of the cardioselective β -blocker esmolol induces early regression of LVH in spontaneously hypertensive rats (SHR). Fourteen-month-old male SHRs were treated i.v. with vehicle (SHR) or esmolol (SHR-E) ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$). Age-matched vehicle-treated male Wistar-Kyoto (WKY) rats served as controls. After 48 h, left ventricular morphology and function were assessed using M-mode echocardiograms (left ventricular mass index (LVMI), ejection fraction and transmitral Doppler (early-to-atrial filling velocity ratio (*E/A*), E-wave deceleration time (Edec time)). The standardized uptake value (SUV) was applied to evaluate FDG (2-deoxy-2-[^{18}F]fluoro-D-glucose) uptake by the heart using PET/CT. Left ventricular subendocardial and subepicardial biopsies were taken to analyze changes in cross-sectional area (CSA) of left ventricular cardiomyocytes and the fibrosis was expressed as collagen volume fraction (CVF). LVMI was lower in SHR-E with respect to SHR ($P=0.009$). There were no significant differences in EF, *E/A* ratio or Edec time in SHR-E compared with SHR ($P=0.17$, 0.55 and $P=0.80$, respectively). PET acquisitions in SHR-E showed lower ^{18}F -FDG uptake than SHR ($P=0.003$). Interestingly, there were no significant differences in SUV in either SHR-E or WKY ($P=0.63$). CSA in subendocardial and subepicardial regions was minor in SHR-E with respect to SHR ($P<0.001$), and there were no significant differences in CVF between both groups. Esmolol reverses early LVH in the SHR model of stable compensated ventricular hypertrophy. This is the first study to associate early regression of LVH with administration of a short-term β -blocker.

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INTRODUCTION

Left ventricular hypertrophy (LVH) is a strong and independent predictor of cardiovascular morbidity and mortality in patients with hypertension.¹ Regression of LVH is one of the objectives of antihypertensive therapy and is associated with a reduction in the incidence of cardiovascular events.²

Esmolol is an ultrashort-acting cardioselective β -adrenergic blocker, with a half-life of ~ 2 min, a time to peak effect of about 6–10 min, and a washout time of 9 min.³ Therefore, esmolol is faster acting than other β -blockers. In addition, the most common adverse effects (hypotension and bradycardia) are quickly reversible.³

Treatment to achieve regression of LVH is a priority in clinical practice. The long-term effects of β -blockers have been shown in both human clinical practice^{4–6} and in experimental animal models.^{7,8}

However, early regression of LVH, following short-term use of these agents, has not been analyzed to date.

We tested the hypothesis that short-term administration (48 h) of esmolol could induce early regression of LVH in 14-month-old spontaneously hypertensive rats (SHRs), using a model of stable compensated LVH. The primary objective was to explore the effect of short-term administration of esmolol on left ventricular mass (LVM). Our secondary objective was to explore cardiac function and metabolism in SHRs.

METHODS

All procedures conformed to the Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised in 1996) and Spanish

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legislation (RD 1201/2005) and were approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Experimental design

The study animals—14-month-old male SHR (n = 33) and normotensive control Wistar-Kyoto (WKY) rats (n = 12)—were bred at the animal house of Universidad Autónoma de Madrid. All the rats were supplied with the standard rat chow and drinking water *ad libitum* and were maintained on a 12 h/12 h light/dark cycle. The animals were housed at a constant temperature of 24°C and relative humidity of 40%. SHRs were randomly divided into two groups (11 rats each): esmolol-treated rats (SHR-E) and vehicle-treated rats (SHR), which was the control group for the SHR-E. Another SHR group (eight rats) was treated with nitroglycerin (SHR-N). All the rats were anesthetized with an i.p. injection of diazepam 4 mg kg⁻¹ and ketamine 10 mg kg⁻¹, and a catheter was inserted into the right internal jugular vein under sterile conditions. The SHR-E received an i.v. infusion of esmolol at 300 µg kg⁻¹ min⁻¹ for 48 h, and the SHR-N an i.v. infusion of nitroglycerin at 0.5 mg h⁻¹ for 48 h. Control SHR and WKY received saline solution (vehicle). Infusion was stopped after 48 h of treatment and then transthoracic echocardiography was used to study morphology and cardiac function, and PET/CT was applied to study cardiac glucose metabolism. Thereafter, the rat was killed using carbon dioxide, and the hearts were investigated using histological methods.

Blood pressure and heart rate measurements

Systolic arterial pressure (SAP) and heart rate (HR) were measured (conscious animals prewarmed to 35°C in thermostatic cages) by the tail-cuff method with a photoelectric sensor (Niprem 546, Cibertec, Madrid, Spain). Several determinations were made at each session during the treatment (0, 12, 24, 36 and 48 h), and the findings were considered valid if 10 consecutive measurements were within 10 mm Hg of each other.

Echocardiographic studies

Transthoracic echocardiography was performed using the Acuson Sequoia C512 system (Acuson, Mountain View, CA, USA) equipped with a 13-MHz transducer. The images were acquired with the animals in left lateral decubitus. Transthoracic echocardiography was performed under anesthesia (i.p. diazepam 4 mg kg⁻¹ and ketamine 10 mg kg⁻¹) after 48 h of treatment. Values were determined by averaging the measurements of three consecutive cardiac cycles in accordance with the American Society of Echocardiography guidelines.⁹ M-mode imaging of the parasternal short axis (papillary level) enabled the measurement of LV end systolic and end diastolic internal dimensions (LVIDs and LVIDd, respectively), posterior wall diastolic thickness (PWD) and interventricular septal end diastolic thickness (IVSd). The above measurements were used for calculations of LVM on the basis of the following equation:

$$\text{LVM} = 0.8 [1.04(\text{IVSd} + \text{LVIDd} + \text{PWD})^3 - (\text{LVIDd})^3] + 0.6 \text{ g}$$

LVM was adjusted for body weight by calculating the left ventricular mass index (LVMI).⁸ LV ejection fraction and fractional shortening (FS) were calculated as measures of LV systolic function, as previously described.¹⁰ In addition, the pulsed-wave Doppler early-to-late transmitral peak diastolic flow velocity ratio (E/A ratio) was measured to assess the diastolic function (E, mitral peak early-filling velocity; and A, mitral peak flow velocity at atrial

contraction). The transmitral flow velocity profile was determined by positioning a sample volume at the tip of the mitral valve on the parasternal long-axis view. The E-wave deceleration time was measured as the time interval between peak E-wave velocity and the point where the E-wave descent (or its extrapolation) intercepted the zero line.

Transthoracic echocardiography also enabled the evaluation of relative wall thickness (RWT), calculated as follows: $\text{RWT} = \text{PWD} + \text{IVSd}/\text{LVIDd}$. RWT and LVM/BW are used to define the geometric patterns of the LV.^{11,12}

Cardiac PET/CT

PET/CT scans (SEDECAL, Madrid, Spain) were performed in nine rats: WKY (n = 3), SHR (n = 3), SHR-E (n = 3) and SHR-N (n = 3). The animals were deprived of food, but allowed free access to water for 8 h before study. They were anesthetized with isoflurane (3% induction and 1.5% for maintenance in 100% oxygen) using a nose cone. ¹⁸F-FDG (2-deoxy-2-[¹⁸F]fluoro-D-glucose) [$39.37 \pm 3.25 \text{ MBq}$ ($1.06 \pm 0.08 \text{ mCi}$)] was administered via the venous catheter in the tail vein, over a period of about 15 s, and the catheter was immediately flushed with 100 µl of saline. PET scans were performed for 54 min. After PET imaging, the CT scan was acquired for 8 min (350 µA, 45 kV, 200 µ, 8 shots and 360 projections).

After images were obtained, the standardized uptake value was used to evaluate FDG uptake by the heart. The standardized uptake value was calculated and normalized according to body weight and the injected dose of FDG.¹³

Morphometric analysis of LV

After performing the echocardiogram, the heart was excised, weighed and fixed in 4% paraformaldehyde. Equatorial cross-section of LV was paraffin-embedded. Cross-sectional area (CSA) of cardiomyocytes was measured in the subepicardium and subendocardium (~25 cardiomyocytes measured in each picture and three pictures per region, for a total of ~150 cardiomyocytes measured in each LV), as described previously.¹⁴ Collagen volume fraction was determined as the ratio of collagen surface area with respect to myocardial surface area.¹⁵

Statistical analysis

The primary end point was LVM in SHRs after short-term administration of esmolol, which is compared between groups. The variable was expressed as mean ± s.d. We used the Kolmogorov–Smirnov test to analyze the distribution of quantitative variables. The parameters were compared using repeated measures analysis of variance (physiological parameters) or single-factor (rat) analysis of variance (physiological, echocardiographic and histological parameters). A *post hoc* Bonferroni's correction was applied. Statistical significance was set at a *P*-value < 0.05. Statistical analysis was performed using SPSS 17.0 for windows (SPSS, Chicago, IL, USA) and S-PLUS 6.1.

RESULTS

Physiological parameters

Values of body weight and blood glucose in WKY, SHR, SHR-E and SHR-N are shown in Table 1. Esmolol lowered SAP in SHR-E with respect to control SHR, and the SAP values during the first 36 h of treatment with esmolol were comparable to those of the WKY

Table 1 Physiological variables and organ weight of WKY and SHR groups

	WKY (n = 9)	SHR (n = 8)	SHR-E (n = 8)	SHR-N (n = 8)	<i>P</i> -value ANOVA
BW (g)	436.81 ± 37.34	400.49 ± 25.84*	400.29 ± 20.74*	417.26 ± 30.30	0.050
HW (g)	1.51 ± 0.13	2.07 ± 0.48*	1.58 ± 0.14§	1.93 ± 0.05††	0.001
Blood glucose (mg dl ⁻¹)	97 ± 4.24	90 ± 16.97	93 ± 4.24	111 ± 1.15	0.241

Abbreviations: ANOVA, analysis of variance; BW, body weight; HW, heart weight; SHR, spontaneously hypertensive rats treated with vehicle; SHR-E, spontaneously hypertensive rats treated with esmolol; SHR-N, spontaneously hypertensive rats treated with nitroglycerin; WKY, Wistar-Kyoto rats treated with vehicle. Statistically significant differences between WKY, SHR, SHR-E and SHR-N are shown (**P* < 0.05 vs. WKY; †*P* < 0.001 vs. WKY; §*P* < 0.05 vs. SHR; ††*P* < 0.01 vs. SHR-E). Values are given as mean ± s.d. ANOVA, BW, HW.

(Figure 1a). Nitroglycerin showed the same level of SAP reduction as the esmolol group. HR remained unchanged in SHR, SHR-N and WKY, but decreased after treatment in SHR-E compared with SHR ($P<0.001$), SHR-N ($P<0.001$) and WKY ($P<0.001$) (Figure 1b).

Echocardiographic parameters

Left ventricular geometry. The left ventricular geometry indices obtained from M-mode echocardiography are shown in Table 2. Left

Figure 1a and b Changes in systolic arterial pressure (a) and heart rate (b) for Wistar-Kyoto rats treated with vehicle (WKY), spontaneously hypertensive rats treated with vehicle (SHR), spontaneously hypertensive rats treated with nitroglycerin (SHR-N) and spontaneously hypertensive rats treated with esmolol (SHR-E). Data were obtained at 0, 12, 24, 36 and 48 h of treatment. Values are given as mean $\pm$ s.d. Statistically significant differences are shown: * $P<0.05$ vs. WKY; † $P<0.01$ vs. WKY; ‡ $P<0.001$ vs. WKY; ‖ $P<0.01$ vs. SHR; # $P<0.001$ vs. SHR; ‡‡ $P<0.001$ vs. SHR-E, corrected for multiple comparisons-Bonferroni.

ventricular mass (LVM) was significantly higher in control SHR than in WKY ($P<0.01$). Interestingly, LVM was significantly lower after 48 h of treatment in SHR-E than in SHR (SHR-E vs. SHR $P<0.01$), and it showed no differences with respect to WKY. LVMI was significantly higher in SHR than in WKY ($P<0.001$). In addition, this indicator of LVH was significantly lower in SHR-E than in SHR (SHR-E vs. SHR $P<0.01$). LVMI in SHR-E remained elevated at the limit of statistical significance with respect to WKY ($P=0.05$). There were no significant differences in LVM and LVMI in SHR-N compared with SHR ($P=0.37$ and $P=0.73$, respectively); however, either parameters were significantly higher in SHR-N compared with SHR-E (Table 2). SHR presented concentric LVH associated with a significantly higher LVMI and RWT than the WKY ($P<0.001$ and $P<0.001$, respectively). Following treatment with esmolol, both LVMI and RWT were significantly lower in SHR-E compared with SHR ($P<0.01$ and $P<0.001$, respectively). RWT showed no significant differences in SHR-E in comparison with the WKY. Compared with WKY, SHR exhibited a significantly larger IVSd and PW, and a reduced LVIDd and LVIDs. SHR-E showed significantly higher LVIDd and lower IVSd and PW than the SHR treated with vehicle (Figure 2).

Cardiac function. Two indicators of systolic function, FS and ejection fraction, were significantly higher in control SHR than in WKY ($P<0.01$ and $P<0.01$, respectively) (Table 2), thus reflecting the supernormal systolic function of SHR at 14 months of age. Furthermore, there were no significant differences in either functional parameters in SHR-E compared with SHR ($P=0.19$ and $P=0.17$, respectively), neither SHR-E nor SHR compared with SHR-N (Table 2). The data for diastolic function are shown in Table 3. There were no significant differences in the E/A ratio or E-wave deceleration time in SHR and WKY. Although SHR-E showed no significant differences in E-wave deceleration time compared with SHR and WKY, the E/A ratio tended to be higher in SHR-E than in SHR ($P=0.55$); however, the difference was not statistically significant. There were no significant differences in the E/A ratio or E-wave deceleration time in SHR-N, SHR-E and SHR.

Quantitative analysis of FDG uptake

The standardized uptake value obtained from the PET study is shown in Figure 3a and b. SHR showed higher ^{18}F -FDG uptake than WKY ($P<0.01$). Interestingly, PET acquisitions of the SHR showed lower ^{18}F -FDG uptake after 48 h of treatment with esmolol than SHR treated with vehicle ($P<0.01$) and SHR treated with nitroglycerin

Table 2 Echocardiographic parameters with M-mode measurements for the WKY and SHR groups

	WKY (n = 9)	SHR (n = 8)	SHR-E (n = 8)	SHR-N (n = 8)	P-values ANOVA
LVIDd (cm)	0.50 $\pm$ 0.05	0.40 $\pm$ 0.06 [†]	0.57 $\pm$ 0.15 [‖]	0.63 $\pm$ 0.13 ^{*,‖}	0.002
LVIDs (cm)	0.22 $\pm$ 0.06	0.10 $\pm$ 0.04 [‡]	0.22 $\pm$ 0.14	0.16 $\pm$ 0.04 ^{*,§}	0.023
IVSd (cm)	0.27 $\pm$ 0.05	0.40 $\pm$ 0.05 [‡]	0.26 $\pm$ 0.04 [#]	0.30 $\pm$ 0.01 ^{‖,**}	<0.001
PWd (cm)	0.28 $\pm$ 0.06	0.37 $\pm$ 0.05 [†]	0.28 $\pm$ 0.07 [§]	0.31 $\pm$ 0.02 [§]	0.006
LVM (g)	1.48 $\pm$ 0.25	1.87 $\pm$ 0.20 [†]	1.57 $\pm$ 0.10 [‖]	2.02 $\pm$ 0.34 ^{†,**,‡}	<0.001
LVMI (mg g ⁻¹)	3.41 $\pm$ 0.65	4.71 $\pm$ 0.62 [‡]	3.95 $\pm$ 0.33 ^{*,‖}	4.82 $\pm$ 0.51 ^{†,‡,‡‡}	<0.001
RWT	1.13 $\pm$ 0.23	2.00 $\pm$ 0.43 [‡]	1.06 $\pm$ 0.5 [#]	1.00 $\pm$ 0.20 [#]	<0.001
EF (%)	78.64 $\pm$ 9.03	93.08 $\pm$ 4.69 [†]	85.52 $\pm$ 10.93	92.10 $\pm$ 4.86 [†]	0.005
FS (%)	55.01 $\pm$ 11.22	74.99 $\pm$ 8.80 [†]	64.51 $\pm$ 14.81	73.31 $\pm$ 9.63 [†]	0.007

Abbreviations: ANOVA, analysis of variance; EF, left ventricular ejection fraction; FS, left ventricular fractional shortening; IVSd, interventricular septal end diastolic thickness; LVIDd, left ventricular end diastolic diameter; LVIDs, left ventricular end systolic diameter; LVM, left ventricular mass; LVMI, left ventricular mass index; PWd, left ventricular posterior wall diastolic thickness; RWT, relative wall thickness; SHR, spontaneously hypertensive rats treated with vehicle; SHR-E, spontaneously hypertensive rats treated with esmolol; SHR-N, spontaneously hypertensive rats treated with nitroglycerin.

Statistically significant differences between WKY, SHR, SHR-E and SHR-N are shown (* $P<0.05$ vs. WKY; † $P<0.01$ vs. WKY; ‡ $P<0.001$ vs. WKY; § $P<0.05$ vs. SHR; ‖ $P<0.01$ vs. SHR; # $P<0.001$ vs. SHR; ** $P<0.05$ vs. SHR-E; ‡‡ $P<0.01$ vs. SHR-E). Values are given as mean $\pm$ s.d. ANOVA, LVIDd, LVIDs, IVSd, PWd, LVM, LVMI, RWT, EF, FS.

Figure 2 Examples of M-mode echocardiograms from a Wistar-Kyoto rat treated with vehicle (WKY), a spontaneously hypertensive rat treated with vehicle (SHR), a spontaneously hypertensive rats treated with nitroglycerin (SHR-N) and a spontaneously hypertensive rat treated with esmolol (SHR-E) at 14 months of age. IVSd indicates interventricular septal end diastolic thickness and PWd left ventricular posterior wall diastolic thickness. Note that SHR-E showed lower IVSd and PW than SHR.

Table 3 Transmitral inflow measurements for the WKY and SHR groups

	WKY (n = 9)	SHR (n = 8)	SHR-E (n = 8)	SHR-N (n = 8)	P-value ANOVA
E-wave (cm s^{-1})	50.43 $\pm$ 16.90	37.25 $\pm$ 14.86	59.17 $\pm$ 15	44.67 $\pm$ 4.50	0.112
A-wave (cm s^{-1})	29.29 $\pm$ 15.94	33.25 $\pm$ 28.65	39.50 $\pm$ 15.95	30.67 $\pm$ 8.01	0.731
E/A ratio	1.94 $\pm$ 0.72	1.52 $\pm$ 0.78	1.65 $\pm$ 0.62	1.50 $\pm$ 0.24	0.580
Edec time (ms^{-1})	42.14 $\pm$ 8.03	43.50 $\pm$ 5.32	42.60 $\pm$ 13.15	35.00 $\pm$ 9.80	0.441

Abbreviations: A-wave, mitral peak filling velocity at atrial contraction; E/A, early-to-atrial filling velocity ratio; Edec time, E-wave deceleration time; SHR, spontaneously hypertensive rats treated with vehicle; SHR-E, spontaneously hypertensive rats treated with esmolol; SHR-N, spontaneously hypertensive rats treated with nitroglycerin; WKY, Wistar-Kyoto rats treated with vehicle. No significant differences between WKY, SHR, SHR-E and SHR-N. Values are given as mean $\pm$ s.d.

($P < 0.01$). There were no significant differences in standardized uptake value in either SHR-E or WKY.

Histological parameters

Value of heart weight (HW) in WKY, SHR, SHR-E and SHR-N is shown in Table 1. CSA of cardiomyocytes in subendocardial and subepicardial regions was minor in SHR-E with respect to SHR ($P < 0.001$), and there were no significant differences in either SHR or SHR-N (Table 4 and Figure 4). Interestingly, SHR-E showed no significant differences in CSA in subendocardial region compared with WKY. There were no significant differences in collagen volume fraction in subendocardial and subepicardial regions between SHR, SHR-N and SHR-E. (Table 4).

DISCUSSION

Our results show that short-term (48 h) i.v. infusion of esmolol ($300 \mu\text{g kg}^{-1} \text{min}^{-1}$) in adult SHR induces early regression of LVH. This is the first study to show an association between early regression of LVH and short-term administration of a β -blocker. Although previous clinical and experimental animal studies have demonstrated that β -blockers can reverse LVH, they have all been conducted with drugs administered in the long term.^{4–8}

Effects of esmolol on SAP, HR and left ventricular hypertrophy

Clinical echocardiographic studies show different patterns in LV geometry in relation to arterial hypertension. LV geometry has been shown to affect both LV function and prognosis. In our study, increasing RWT and LVMI revealed concentric hypertrophy at 14 months of age in the SHR model. This geometric pattern, which indicates the most unfavorable prognosis for hypertensive patients with LV hypertrophy, is considered the typical response to hypertension.^{11,16}

Our results revealed that esmolol reduced SAP and HR in SHR. This decrease in SAP and HR coincided with less concentric hypertrophy, because LVMI and RWT were lower in SHR after 48 h of treatment with esmolol than SHR treated with vehicle. In the present study, LVMI in SHR-E remained elevated at the limit of

statistical significance with respect to WKY ($P = 0.05$), indicating the transition from concentric to eccentric LV hypertrophy. This transition may have favorable implications for prognosis, in addition to the recognized positive effect of reducing LVMI.¹⁷ Regression of LVM with antihypertensive therapy (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β -adrenergic blockers, calcium channel blockers and diuretics) has been reported, although only after long-term treatment.^{18,19} In the present study, together with the early reduction of LVM, early changes in CSA of left ventricular cardiomyocytes were observed. Esmolol restored LVM more completely and faster than other antihypertensive drugs (mainly β -adrenergic blockers). This effect may be attributed in part to the fact that short-term i.v. administration of esmolol exerts a greater hypotensive effect than other β -blockers (for example, i.v. metoprolol and propranolol at similar degrees of β -blockade).²⁰ This reduction of LVH was produced by a β blocker-specific effect (nitroglycerin showed no alterations in LVM, HW, cardiac metabolism or histological parameters).

Effects of esmolol on left ventricular function

At 14 months of age, SHRs present compensated hypertrophy²¹ with a profile of diastolic dysfunction and concomitant supernormal systolic function, which probably reflects pre-heart failure. Our results are consistent with Cingolani *et al.*,²² in which that they confirm the existence of differences in systolic function between adult SHR and WKY (as estimated by ejection fraction and FS). In this context, SHR show enhanced systolic function because of compensated hypertrophy in response to the increased LV wall stress caused by arterial hypertension. β -Blockade, despite its known negative inotropic effect, has been shown to improve ventricular function when administered in the long term.⁵ Our results showed that there was a trend toward a reduction of hypercontractility in SHR-E. Cardiac β_1 -adrenergic receptors are decreased in SHRs,²³ whereas β_2 adrenergic receptors are unchanged²⁴ or even increased.²⁵ This reduction in the number of β_1 adrenergic receptors might affect the response of the heart to drugs that act upon myocardial inotropism.²⁶ Therefore, the effect of

Figure 3 (a, b) PET/CT (PET/CT) images of myocardial ^{18}F -FDG uptake from a Wistar-Kyoto rat treated with vehicle (WKY), a spontaneously hypertensive rat treated with vehicle (SHR), a spontaneously hypertensive rats treated with nitroglycerin (SHR-N) and a spontaneously hypertensive rat treated with esmolol (SHR-E) (a). There were no significant differences in standardized uptake value (SUV) in either SHR-E ($n=3$) or WKY ($n=3$). Values are given as mean $\pm$ s.d. Statistically significant differences are shown: † $P<0.01$ vs. WKY; ‡ $P<0.01$ vs. SHR; †† $P<0.01$ vs. SHR-E, corrected for multiple comparisons-Bonferroni (b).

Table 4 Histological variables in two subregions of the LV of WKY and SHR groups

	WKY (n = 9)	SHR (n = 8)	SHR-E (n = 8)	SHR-N (n = 8)	P-value ANOVA
CSA (μm^2) subepicardium	140 $\pm$ 22	321 $\pm$ 55 [‡]	165 $\pm$ 23 ^{*,#}	315 $\pm$ 12 ^{†,‡‡}	<0.001
CSA (μm^2) subendocardium	139 $\pm$ 21	283 $\pm$ 78 [‡]	142 $\pm$ 11 [#]	276 $\pm$ 21 ^{†,‡‡}	<0.001
CVF (%) subepicardium	6.04 $\pm$ 2.69	10.01 $\pm$ 2.68 [*]	11.26 $\pm$ 3.08 [†]	9.03 $\pm$ 2.54 [*]	0.020
CVF (%) subendocardium	11.80 $\pm$ 6.69	24.92 $\pm$ 4.68 [†]	24.99 $\pm$ 6.37 [†]	22.80 $\pm$ 3.96 [†]	0.009

Abbreviations: ANOVA, analysis of variance; CSA, cross-sectional area of cardiomyocytes; CVF, collagen volume fraction; SHR, spontaneously hypertensive rats treated with vehicle; SHR-E, spontaneously hypertensive rats treated with esmolol; SHR-N, spontaneously hypertensive rats treated with nitroglycerin; WKY, Wistar-Kyoto rats treated with vehicle. Statistically significant differences between WKY, SHR, SHR-N and SHR-E are shown (* $P<0.05$ vs. WKY; † $P<0.01$ vs. WKY; ‡ $P<0.001$ vs. WKY; # $P<0.001$ vs. SHR; †† $P<0.001$ vs. SHR-E). Values are given as mean $\pm$ s.d. ANOVA, CSA, CVF.

Figure 4 Examples histological sections of subepicardial region of the LV from a Wistar-Kyoto rat treated with vehicle (WKY), a spontaneously hypertensive rat treated with vehicle (SHR), a spontaneously hypertensive rats treated with nitroglycerin (SHR-N) and a spontaneously hypertensive rat treated with esmolol (SHR-E). Note that SHR-E showed lower cross-sectional area of cardiomyocytes than SHR. H&E 200x.

esmolol (β_1 -blocker) on systolic function could be altered by a decrease in the number of myocardial β_1 receptors.

Our results are consistent with Cingolani *et al.*,²² in which they confirm the presence of diastolic dysfunction in adult SHR and WKY. Although some β -blockers are associated with an improvement in diastolic function,⁴ we found that the administration of esmolol in SHR showed no significant differences in diastolic function with respect to SHR controls. Only studies on human have shown esmolol to lower HR to levels (57–68 beats per min) associated with the

recovery of diastolic function and we used an experimental animal with an initial HR of about 300 beats per min.²⁷

Effects of esmolol on myocardial glucose metabolism

Energy is generated in the heart from glucose, fatty acids and acetate, although oxidation of fatty acids is the primary source.²⁸ Handa *et al.*²⁹ showed a higher rate of glucose uptake in animal models of cardiac hypertrophy. In our study, myocardial glucose metabolism in the SHR model was more marked than in the WKY. These results are

consistent with those of other authors in this model of LVH, although the animals used were of a different age.²⁸ By reducing HR and inotropy, β -blockers should reduce myocardial energy demands and oxygen consumption,³⁰ and the pharmacological effect of β -blockade in reducing energy demands may thus facilitate biological recovery of myocytes.³¹ This has only been observed after long-term treatment with β -blockers. Our study showed that short-term treatment with esmolol (48 h) produced a marked decrease in the glucose metabolism of the hypertrophied ventricle.

In conclusion, our results show that esmolol leads to the regression of cardiac hypertrophy in experimental hypertension, because the esmolol-treated SHR showed lower LVM, lower CSA of cardiomyocytes and lower myocardial glucose metabolism than the control SHR. This is the first study to associate a regression of LVH in a markedly short period of treatment with a β -blocker. If these results were confirmed in humans, esmolol could be taken into consideration for the treatment of LVH in critical care units.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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