The role of angiotensin receptor-1 blockade on electromechanical changes induced by left ventricular hypertrophy and its regression

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Abstract

Objective: The aims of this study were to: investigate the role of angiotensin in mediating changes to myocardial electromechanical properties during the development and regression of left ventricular hypertrophy (LVH) generated by constriction of the thoracic aorta; and identify any role of angiotensin-1 receptor blockade on ameliorating changes to these electromechanical properties.

Methods: LVH was induced in guinea-pigs by constricting the ascending aorta (AC groups). After 42±3 days, the constriction was either removed or left in place. Following the second operation animals were fed losartan (10 $mg \cdot kg^{-1} \cdot day^{-1}$) or saline for 42±3 days. Sham-operated animals served as controls. In other groups, LVH was generated by subcutaneous angiotensin II (200 $ng \cdot kg^{-1} \cdot min^{-1}$) infusion for 42±3 days with or without losartan administration (AT groups), and compared to animals undergoing aortic constriction for a similar period. Electromechanical changes were recorded in isolated left ventricular myocardial preparations.

Results: Wet and dry heart-to-body weight ratios (HBR) increased significantly in the AC and AT models, compared to control. Losartan prevented the increase of HBR in the AT group. Removal of the constriction allowed LVH to regress to control. The force-frequency relationship was reduced in both models and recovered fully on regression. However, the two models generated different electrophysiological changes: in the AC group longitudinal conduction velocity was reduced and transverse conduction increased, with a consequent reduction of the anisotropic conduction ratio: on regression recovery was only partial; action potential duration was prolonged and did not recover. In the AT group electrophysiological changes were limited, only an increase of transverse conduction, and a reduction of the anisotropic conduction ratio were observed. Losartan had no effect on HBR or electromechanical variables in the aortic constricted animals, nor did it affect the extent of recovery in animals with regression of LVH.

Conclusions: The electromechanical changes to hypertrophied myocardium are different in these two models of LVH. Moreover, losartan was ineffective in modulating the consequences of hypertrophy induced by constriction of the thoracic aorta.

Introduction

Left ventricular hypertrophy (LVH) is a significant predictor of cardiovascular mortality and morbidity arising from both ventricular arrhythmias and myocardial failure [1,2]. Electrophysiological changes occur during hypertrophy, in particular to action potential (AP) duration, and to conduction due to alterations of the active and passive electrical properties of the myocardium [3-5], and these are likely to contribute to the risk of re-entrant arrhythmias and sudden death.

Regression of LVH by pharmacological and surgical procedures has been shown in animal models of hypertrophy [6,7] and in humans with aortic stenosis and hypertension [8,9]. However, this is not always accompanied by normalisation of the associated pathophysiology, in particular electrophysiological abnormalities [6], and indeed the prognosis associated with previous LVH remains poor [10]. Furthermore, it is difficult to separate out the effects of drug-induced regression of LVH from the action of the drugs themselves or the reduction of blood pressure.

The renin-angiotensin system has been implicated in the development of some models of hypertrophy [11], but its involvement in the changes to the physiological properties of myocardium that occur during hypertrophy has not been investigated. Although systemic increases of angiotensin (AT) have not been observed in hypertrophy induced by constriction of the thoracic aorta [12] it remains possible that AT may mediate some of the functional changes. The purpose of this study was twofold: to investigate the potential role of AT in this context, by comparing the electromechanical changes induced in hypertrophied myocardium by aortic constriction or infusion of AT; and to determine the role that blockade of angiotensin-1 receptors may play in ameliorating the consequences of hypertrophy and reversal of changes on regression of hypertrophy.

Methods

Induction of left ventricular hypertrophy (LVH) and regression from LVH. Ten groups of adult male Dunkin-Hartley guinea-pigs (600-800 g) were used to study the pathophysiology of LVH, seven by constriction of the ascending aorta (including sham-operated controls), and three by angiotensin infusion: the protocols are shown in figure 1. The basic treatment block was 42 or 84 days, as in this model these periods generate LVH without development of heart failure [3,6]. In groups 2, 3, 4, 5 and 7 LVH was induced by placing a high-density plastic clip (internal diameter 2.0 mm) around the ascending aorta. Animals were anaesthetised with Na pentobarbitone (0.12 mg Sagatal, Rhone Merieux Ltd, Harlow) and 0.1 mg diazepam for injection (Phoenix Pharma Ltd, Gloucester), followed by inhalation of a 49%/49%/2%, N_2O/O_2/halothane mixture. Animals were intubated and ventilated at 100 cycles per minute, with an O_2 flow of 0.4 $l \cdot min^{-1}$ and the aorta exposed via a left thoracotomy, as previously described [13]. Groups 1 and 6 were sham-operated controls that underwent the same procedures, without clip placement. After 42±3 days, animals in groups 6 and 7 were killed, and the hearts removed rapidly for *in vitro* experiments. Animals in groups 1-5 at this time underwent a right thoracotomy and the plastic clip was either left in place (age-matched LVH, groups 2 & 3), removed (de-banded groups 4 & 5), or had a second sham-operation (group 1). After this second procedure, animals in groups 2 - 5 were daily given an oral gavage containing losartan in 1 ml isotonic saline (10 $mg \cdot kg^{-1}$, Merck, Sharp & Dohme [14], groups 3 & 5), or saline alone (groups 2 & 4).

With group 9 guinea-pigs, LVH was induced by infusion of angiotensin II for 42±3 days (200 $ng \cdot kg^{-1} \cdot min^{-1}$, Sigma; [15]), by subcutaneous osmotic mini-pumps inserted under anaesthesia: group 8 animals received saline infusion. Finally, group 10 animals also received 42 days of angiotensin infusion, with losartan also administered as described above.

All procedures conformed to The Guide for the Care and Use of Laboratory Animals (National Institute of Health (NIH) Publication No. 85-23, revised 1996) and UK Guidelines in The Operation of Animals (Scientific Procedures) Act, 1986**.**

FIGURE 1 NEAR HERE

Isolated preparations. Animals were weighed, sacrificed by cervical dislocation and the hearts rapidly removed and weighed. Heart-to-body weight ratio (HBR) was used to assess the extent of hypertrophy and regression. Lung-to-body weight ratio was used to assess a lack of heart failure if the value was similar in experimental and sham-operated groups. Parallel cohorts of animals for groups 1, 2, 4, 6, 7, 8 and 9 were prepared to measure the dry weight of the left ventricle to test the hypothesis that and increase of HBR was due to a greater tissue mass rather than accumulation of oedema. The left and right ventricles were separately weighed, placed in an oven at 80°C and dried for 48 hours.

Left ventricular papillary muscles were isolated from experimental hearts, mounted in a superfusion bath, secured at one end to a fixed hook and at the other end to an isometric tension transducer, and superfused with Tyrodes's solution at 4 $ml \cdot min^{-1}$, at 37°C. Tyrode's contained (mM): NaCl 118, KCl 4.0, $NaHCO_3$ 24, $MgCl_2$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 0.4, glucose 6.1, Na pyruvate 5.0, pH 7.40±0.2, gassed with 95% O_2 and 5% CO_2. Muscles were up to 6mm long, <2mm wide, and <1mm thick to minimise hypoxic damage to the tissue.

The force-frequency relationship was quantified as the ratio of peak isometric tensions generated at 1.6 and 0.8 Hz stimulation frequencies ($T_{1.6/0.8}$). Field-stimulated action potentials were recorded with 10 MΩ, 3M KCl-filled microelectrodes at 1 Hz. AP duration was the time from the upstroke to 95% repolarisation (APD_{95}). Conduction velocity along the longitudinal axis (θ_L) was measured by stimulating the muscle at one end via insulated Ag-AgCl electrodes, with pulses at 1.5x

threshold to minimize extracellular current spread, and recording APs at distances, *d*, more than 1 mm from the stimulation site. The distance *d* was measured with a Vernier scale on the microelectrode micromanipuator, and checked with an eyepiece graticule under x40 magnification, both methods were accurate to 100 µm. θ_L was calculated as the distance:delay ratio over several values of *d*. Transverse conduction velocity, θ_T, was measured using large extracellular stimulating electrodes parallel to the muscle axis, and restricting the extracellular volume, under these conditions AP conduction is transverse to the axis [16]. We have shown previously [4,5] that these stimulating conditions provide one-dimensional, planar conduction in the axis of interest, with an error of < 5% in other dimensions. The time constant of the action potential base or foot, τ_{ap}, was calculated from a semilogarithmic plot of the initial 10-12 mV depolarisation of the conducted AP.

Statistical analyses. Results are expressed as mean ± SD, or when several separate measurements were made from the same preparation as SE. Comparison between groups was assessed using ANOVA with Bonferroni *post hoc* tests. The null hypothesis was rejected when $p<0.05$.

Results

Development of cardiac hypertrophy and regression from hypertrophy. The magnitude of cardiac hypertrophy was assessed in two ways: the wet heart-to-body weight ratio (HBR); and the dried left ventricular-to-body weight ratio (DLVBR). Table 1 shows that both variables increased after constriction for 42 days (group 7) and 84 days (group 2), with respect to their respective age-matched controls (groups 6 and 1, respectively). Furthermore, in the group that was de-banded after 42 days constriction (group 4) HBR and DLVBR decreased significantly to values not different from the age-matched control. HBR and DLVBR after 42 and 84 days constriction were not significantly different. Thus, the effect of the debanding procedure after 42 days could be measured after ventricular hypertrophy had developed to a steady level. Table 1 also shows that angiotensin, compared to saline, infusion for 42 days (groups 8 & 9) also increased HBR and DLVBR, to values similar to the time-matched aortic constriction groups. Thus, with respect to generation of hypertrophy the methods of thoracic aortic constriction and angiotensin infusion produced comparable results.

The lung-to-body weight ratio in the combined control groups (sham constriction and saline infusion, groups 1, 6 & 8) was 4.44±0.65 $g \cdot kg^{-1}$; values in all remaining groups were not significantly different from this value.

TABLE 1 NEAR HERE

Electromechanical changes during hypertrophy and its regression. Table 2 summarises the electromechanical properties of myocardium after 84 days aortic constriction, and also after 42 days of constriction and 42 days of de-banding, compared to 84-day sham-operated controls. Some data is in confirmation of previous findings [4-6]. It should be noted that the changes to the 84-day constricted group were quantitatively similar to changes after 42 days constriction (for the latter see 'AC' data in figure 2B). Preparations from control animals showed a positive staircase, as

evidenced by values for $T_{1.6/0.8}$ greater than 1.0. This variable was significantly reduced in the hypertrophied hearts but recovered completely in the regressed hearts. Absolute twitch strength at 1Hz, time-to-peak tension and twitch duration were similar in all experimental groups (sham-operated values were respectively: 2.15±1.40 $mN \cdot mm^{-2}$; 132±3.7 ms; 289±8.1 ms).

TABLE 2 NEAR HERE

However, electrophysiological changes associated with LVH showed less complete reversibility. Table 2 shows that APD_{95} was prolonged in hypertrophy and remained so after LVH regression. Action potential conduction velocity in the longitudinal axis of the preparation, θ_L, was reduced in LVH, but was increased in the transverse axis, θ_T, resulting in a reduction of the anisotropic conduction ratio, θ_L/θ_T. Regression of hypertrophy only partially ameliorated these conduction changes; thus θ_L only partially recovered to an intermediate value, although transverse conduction, θ_T, returned to control. The anisotropy ratio also returned to a value not significantly different from the control value. By contrast, the resting membrane potential, E_m, was unaffected by LVH and its regression. The maximum rate of action potential (AP) depolarisation (dV/dt_{max}), and the time constant of the action potential foot, τ_{ap} - factors importantly determined by the magnitude of the Na^+ current - were also unaltered in LVH and its regression. dV/dt_{max} and τ_{ap} were measured from action potentials conducting in the longitudinal (L) or transverse (T) axes. In groups shown in table 2, and in all other experimental groups, these values were greater in the transverse compared to the longitudinal axis; this significance will be considered in the Discussion. Figure 2A illustrates the rising phases of longitudinally and transversely conducting action potentials. The thicker lines (arrowed) drawn over the subthreshold regions of the rising phase indicate the regions used to calculate τ_{ap}. The delay, *d*, between the start of the stimulus artefact and the peak of differential response (lower traces) was used to calculate conduction velocity – see also figure legend). All

further interventions had no effect on the values of membrane potential, dV/dt_{max} or τ_{ap} so their values are not further reported.

Effects of angiotensin II infusion. Table 1 shows that angiotensin II infusion (n=7) also increased significantly HBR and DLVBR. Figure 2B shows the electromechanical properties with angiotensin infusion and compares these with aortic constriction for 42 days. It shows that the changes accompanying myocardial hypertrophy were different in the two models. The $T_{1.6/0.8}$ ratio was reduced from 1.55±0.02 to 1.24±0.02, similar to that in the aortic constriction group. However, the prolongation of the action potential, and reduction of longitudinal conduction velocity were absent in the angiotensin group, although there was a similar increase of transverse conduction velocity and a consequent reduction, albeit smaller, of the anisotropic conduction ratio. Control data from sham-operated (group 6) and saline-infused (group 8) animals were identical; for clarity only the latter control data are shown in figure 2. Figure 2C shows sample action potentials from the control, aortic-constricted and angiotensin-infused groups.

FIGURE 2 NEAR HERE

Effects of the angiotensin II type-1 receptor (AT_1) antagonist, losartan, on electromechanical changes during hypertrophy. Forty-two days after initiating aortic constriction, two groups of animals were fed losartan: during the continued presence of the constriction (group 3); after removal of the constriction (group 5). The objective was to determine if AT1-receptor antagonism influenced changes to myocardial electromechanical properties during these periods. To determine if the losartan dose, and route of administration, were adequate, a control series of experiments was carried out whereby losartan was administered during angiotensin infusion via osmotic mini-pumps. In three separate experiments losartan administration alone had no effect on HBR (2.66±0.24 *vs* 2.44±0.18 $g \cdot kg^{-1}$, saline *vs* losartan) or any electromechanical variable (data not shown).

Figure 3A shows that losartan prevented the increase of HBR induced by angiotensin infusion (group 10 *vs* group 9 animals); the value of 2.93±0.41 $g \cdot kg^{-1}$ was significantly less than the angiotensin group, and not different from the saline-infusion group. Furthermore, losartan prevented the angiotensin-mediated changes to electromechanical properties reported above, i.e. the $T_{1.6/0.8}$ ratio (1.59±0.08 *vs* 1.24±0.04, $p<0.05$, with and without losartan respectively). It may be concluded that the administration of losartan effectively antagonised the effect of infused angiotensin on cardiac growth and functional myocardial properties.

FIGURE 3 NEAR HERE

The possible involvement of angiotensin, through a losartan-sensitive mechanism, in altering electromechanical function during LVH maintained by aortic constriction was therefore examined. Figure 3B shows that 42-days administration of losartan did not reduce HBR. Furthermore, figure 4 shows that treatment with losartan had no effect on the variables altered during aortic constriction. Thus, the $T_{1.6/0.8}$ ratio was attenuated, APD_{95} was prolonged, longitudinal conduction velocity was reduced, transverse conduction increased, and the anisotropic conduction ratio decreased: all to similar extents to those without losartan.

FIGURE 4 NEAR HERE

Effects of losartan on electromechanical changes during regression

Figure 3C shows that 42-days oral administration of losartan after removal of the aortic constriction had no influence on the return of HBR to the control value. Figure 5 also shows that losartan also had no effect on the complete recovery of $T_{1.6/0.8}$ after regression of LVH, and that the action potential also remained prolonged. For reference, the data for the maintained-LVH group are also shown. Equally, longitudinal conduction velocity, θ_L, only partially recovered in the deconstricted group, with or without losartan; the values in both groups were significantly different from both the sham-operated and the maintained-LVH groups. The transverse conduction, and hence the

computed conduction anisotropy data were not completely comparable between the de-banded groups with and without losartan. With losartan the mean value of transverse conduction velocity did reduce after de-banding, but the recovery was statistically incomplete.

It may be concluded that losartan was able to prevent the generation of cardiac hypertrophy induced by angiotensin infusion, along with the limited number of electromechanical changes observed in this model of cardiac hypertrophy. However, losartan had no effect on ameliorating the magnitude of hypertrophy, and the associated electromechanical changes, after aortic constriction. Furthermore, losartan, at least over 42-days administration, had no effect on the regression process, in particular the electrophysiological changes that persisted after cardiac size had normalised were unaffected by treatment with losartan.

Discussion

Alterations to electromechanical function in LVH and its regression. In confirmation of previous work [4,6], constriction of the guinea-pig ascending aorta for about 42 days generated cardiac growth as evidenced by a larger heart-to-body weight ratio (HBR) and an increase of dry heart weight. This was completely reversible on removal of the constriction, after a period comparable to the constriction phase. Morphological regression was accompanied by recovery of contractile function, as evidenced by the staircase response; however, several electrophysiological changes either remained (APD) or recovered only partially (conduction velocity). Whether these variables would eventually normalise after a longer period of de-banding is unclear and requires further study. The risk of arrhythmias in humans is not always reduced with regression of LVH [17], which may in part be due to incomplete recovery of electrophysiological function. Our study concords with a canine model of volume-overload hypertrophy, which showed that despite regression of hypertrophy QT interval remained prolonged [18]. In contrast, a feline model of pressure-overload hypertrophy showed that with regression of wall thickness, ventricular fibrillation thresholds and the ease of induction of ventricular arrhythmias reverted to control levels [19].

A rise of the intracellular $[Na^+]$ is an early characteristic of LVH and we have postulated that this is partly responsible for electromechanical changes by indirectly affecting gap junction conductance [3,20]. However, this cannot be the sole explanation, because of the heterogenous changes to conduction velocity. Our data are consistent with a change also to gap junction redistribution around the myocyte, as observed in other models of LVH [21]. However, we do not know the rate and extent of recovery of these cellular and morphological changes during regression of LVH.

Of note was a lack of change to the AP upstroke (dV/dt_{max}), or the time constant of the AP foot (τ_{ap}) associated with any of the interventions. However, both were hastened in transverse conduction

pathways, indicative of discontinuous conduction in the myocardial syncitium [22]. Its significance is that reduction of longitudinal conduction velocity in LVH, and the failure to normalise completely on regression of LVH, would emphasise the low safety factor in this axis (i.e. increase the likelihood of conduction failure), and increase the possibility of re-entrant arrhythmias [23]. Both dV/dt_{max} and τ_{ap} are importantly determined by the magnitude and/or kinetics of the Na^+ inward current, and implies these did not alter significantly during any intervention. We propose that the persistent changes to conduction in LVH result mainly from alterations to unit gap junction conductance and their redistribution around the myocyte. Moreover it is important to identify conditions that may ameliorate the effect of LVH on altered electrophysiological variables, and if recovery during regression could be hastened. For this reason we investigated the role of angiotensin and AT1 receptor blockade by losartan.

The pathology associated with LVH and the role of angiotensin. Changes to myocardial function in humans are also associated with an increase of afterload, e.g. with aortic stenosis. In the intact heart this can be manifest as increased QT dispersion [24], and in isolated tissues as prolongation of the action potential and slowed conduction [25]. Furthermore, regression of hypertrophy reduced the electrophysiological changes, but incompletely [24,26], and thus mirror results with our guinea-pig model. Many animal models of hypertrophy are associated with upregulation of the renin-angiotensin system, with an increase of systemic angiotensin-II (AII) and aldosterone levels [27]. The evidence from this study is that angiotensin does not play a prominent role in the changes to electromechanical function associated with thoracic aortic constriction (AC). Firstly, the changes occurring with angiotensin-induced hypertrophy were quite different from those evoked by AC. Secondly losartan had no effect, at the dose used (10 $mg \cdot kg^{-1} \cdot day^{-1}$) on changes evoked by AC, although it prevented completely the changes induced by angiotensin infusion.

Most studies using AT_1 receptor antagonists and ACE inhibitors have focused on regression of hypertrophy itself, and fewer on their ability to reverse associated electromechanical changes. In keeping with our conclusions, other observations have shown that losartan did not regress LVH associated with thoracic AC, nor improve contractile function [28,29]. Concerning other models of hypertrophy AT_1 receptor blockade did not reverse electrophysiological changes, nor prevent the development of arrhythmias [30] or improve survival [31].

Whether ACE inhibitors effect regression of LVH in this model requires further study. In rats the ACE inhibitor perindropril prevented the age-related increase in HBR independent of its antihypertensive effects, but did not prevent APD prolongation [32]. However, chronic treatment of SHR rats with capropril restored APD with regression of hypertrophy [33]. Studies in other models of cardiac hypertrophy and failure have shown differential effects of ACE inhibition and AT_1 receptor antagonism [34,35]. One possibility is that ACE inhibitors, but not AT_1 receptor antagonists, inhibit degradation of bradykinin into non-active metabolites, as bradykinin itself can inhibit progression of hypertrophy [36].

However, in hypertrophy generated by renovascular hypertension both AT_1 receptor antagonists and ACE inhibitors normalize ventricular electrophysiology assessed by ventricular fibrillation thresholds, action potential duration and effective refractory periods [37-40]. This model is more similar to our angiotensin-infusion model, but it should be stressed that the electromechanical properties that we described above were not identical to those in this pressure-overload hypertrophy

In conclusion, induction of LVH by different methods generates variable electromechanical changes to myocardium. Regression of cardiac growth is not accompanied by complete recovery of function, and in particular electrophysiological changes that could predispose the heart to arrhythmias remain altered. Losartan, at a dose sufficient to prevent angiotensin-induced LVH, had

no effect on regressing hypertrophy induced by thoracic aortic constriction, nor did it facilitate recovery of function during natural regression of cardiac growth. Further studies may reveal if higher doses, or longer treatment periods with AT_1 receptor blockers, ACE inhibitors or direct aldosterone blockers, affects electromechanical changes with aortic constriction.

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Figure Legends

Figure 1. The experimental groups used in the study. Groups 1-5 were animals undergoing two procedures at 0 and 42 days. Groups 2-5 had as a first procedure aortic constriction; at day 42 the constriction was either left in place with a sham procedure (groups 2, 3), or removed (groups 4, 5). After the second procedure animals were gavaged with either saline (groups 2, 4) or losartan (groups 3, 5). Group 1 animals had two sham-procedures and were age-matched controls. Experiments were carried out at day 84. Groups 6-10 were animals undergoing one procedure at day 0. Group 7 animals had aortic constriction, and were compared to sham-operated group 6 animals. Group 8-10 animals had osmotic mini-pump insertion, filled with saline (group 8) or angiotensin (groups 9, 10). During this period animals were gavaged with saline (group 9) or losartan (group 10).

Figure 2. Electromechanical responses to angiotensin-II (AT) infusion and thoracic aortic constriction (AC). A: The depolarising phases (upper traces) of action potentials propagating in the longitudinal (L) or transverse (T) axis of the preparation. The thicker lines (arrowed) over the experimental traces were analysed to calculate the time constant of the foot of the action potential, τ_{ap}. Lower traces are the derivatives of the upper traces. The conduction velocity was calculated as the ratio of distance between stimulating and recording electrodes and the delay *d*. In the examples, from an 84-day aortic constricted (group 2) animal, the velocities were 59.6 $cm \cdot s^{-1}$ (L) and 22.4 $cm \cdot s^{-1}$ (T). B: Values or the $T_{1.6/0.8}$ twitch tension ratio; action potential duration (APD), longitudinal (θ_L) and transverse (θ_T) conduction velocities and the anisotropic conduction ratio, θ_L/θ_T in saline (Sal) or angiotensin-II (AT) infused animals, or aortic constricted animals (AC), groups 7-9, * $p<0.05$ with respect to saline, control values. Mean data ± SD, except APD_{95} values, mean ± SE. C: Examples of action potentials recorded from a saline-infused animal (control), an angiotensin-II infused animal and a 42-day aortic-constricted animal.

Figure 3. Heart-to-body weight ratios (HBR) of the experimental groups. A: the effect of angiotensin-II infusion in the absence (AT) or presence of losartan (groups 8-10, figure 1). B: The effect of feeding animals with saline or losartan during the period of 42-84 days aortic constriction (AC) – groups 1-3. The effect or feeding losartan following de-banding (groups 1, 4, 5). * $p<0.05$ with respect to sham-operated control values, § $p<0.05$ with respect to AC values. Mean data ± SD

Figure 4. Electromechanical variables recorded in aortic-constricted (AC) animals fed with saline or losartan (groups 1-3). Values of the $T_{1.6/0.8}$ ratio; action potential duration (APD), longitudinal (θ_L) and transverse (θ_T) conduction velocities and the anisotropic conduction ratio, θ_L/θ_T are shown. * $p<0.05$ with respect to sham-operated control values. Mean data ± SD, except APD_{95} values, mean ± SE.

Figure 5. Electromechanical variables recorded in de-banded animals fed with saline or losartan (groups 1, 4 and 5). Values of the $T_{1.6/0.8}$ ratio; action potential duration (APD), longitudinal (θ_L) and transverse (θ_T) conduction velocities and the anisotropic conduction ratio, θ_L/θ_T are shown. For comparison the values recorded in age-matched aortic-constricted (AC) animals (group 2) are shown. * $p<0.05$ with respect to sham-operated control values, § $p<0.05$ with respect to AC values. Mean data ± SD, except APD_{95} values, mean ± SE.

Figure 1

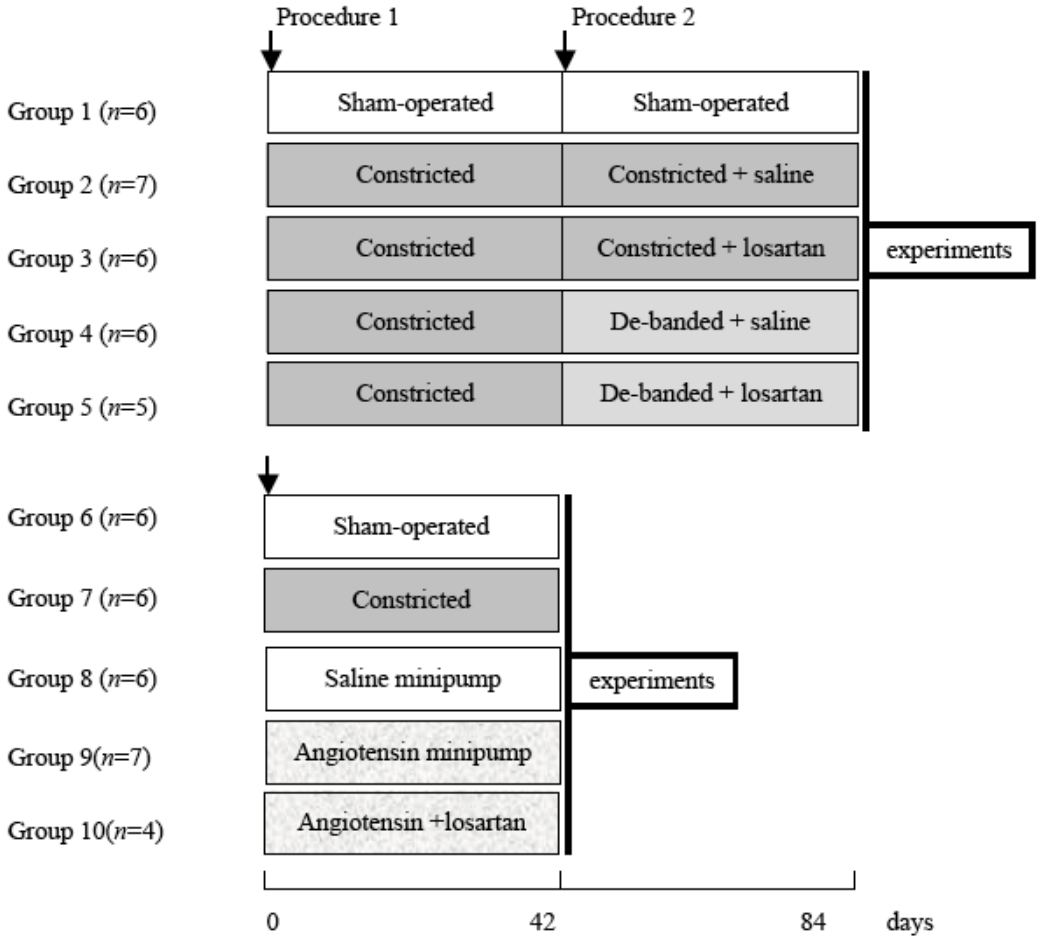
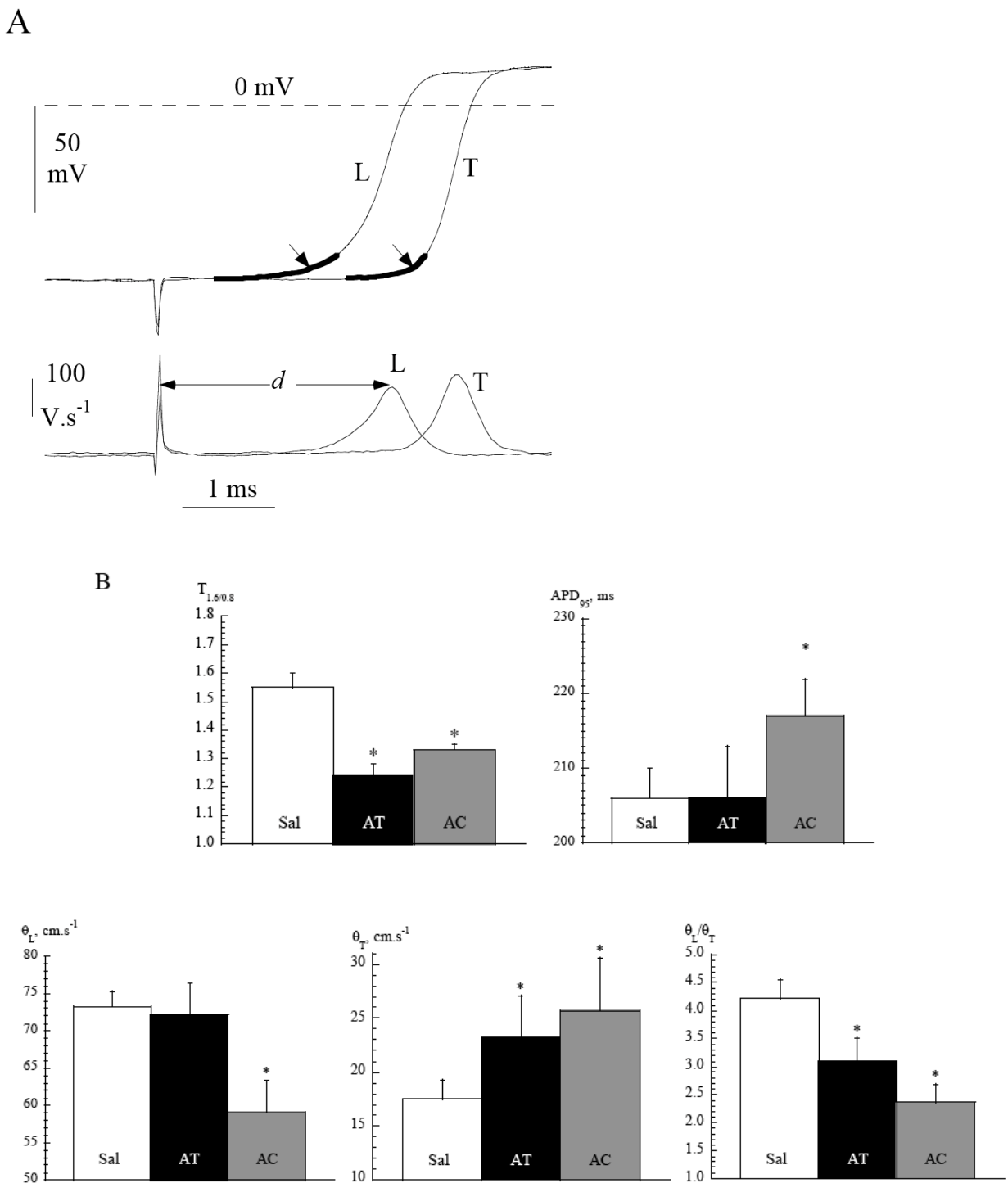

Procedure 1
Procedure 2
Group 1 (n=6)
Sham-operated
Sham-operated
Group 2 (n=7)
Constricted
Constricted + saline
Group 3 (n=6)
Constricted
Constricted + losartan
experiments
Group 4 (n=6)
Constricted
De-banded + saline
Group 5 (n=5)
Constricted
De-banded + losartan
Group 6 (n=6)
Sham-operated
Group 7 (n=6)
Constricted
Group 8 (n=6)
Saline minipump
experiments
Group 9(n=7)
Angiotensin minipump
Group 10(n=4)
Angiotensin +losartan
0
42
84
days

FIGURE 2


A
0 mV
50
mV
L
T
100
V.s-1
d
L
T
1 ms
B
T1.6/0.8
APD95, ms
Sal
AT
AC
θL, cm.s-1
θT, cm.s-1
θL/θT

C

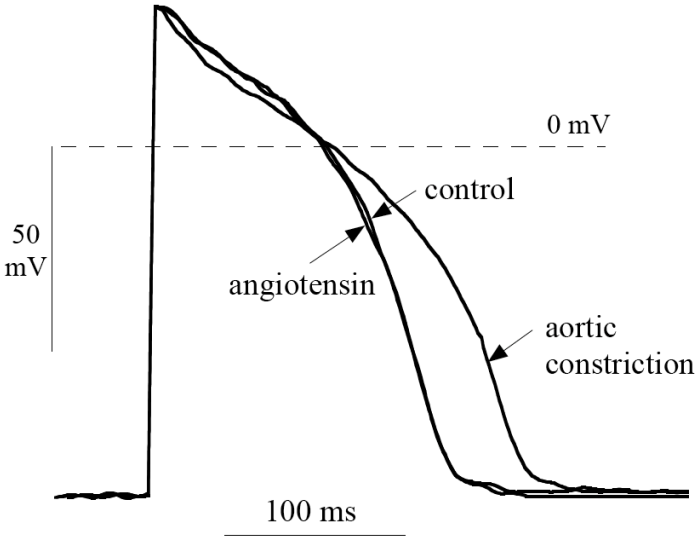


Figure 3

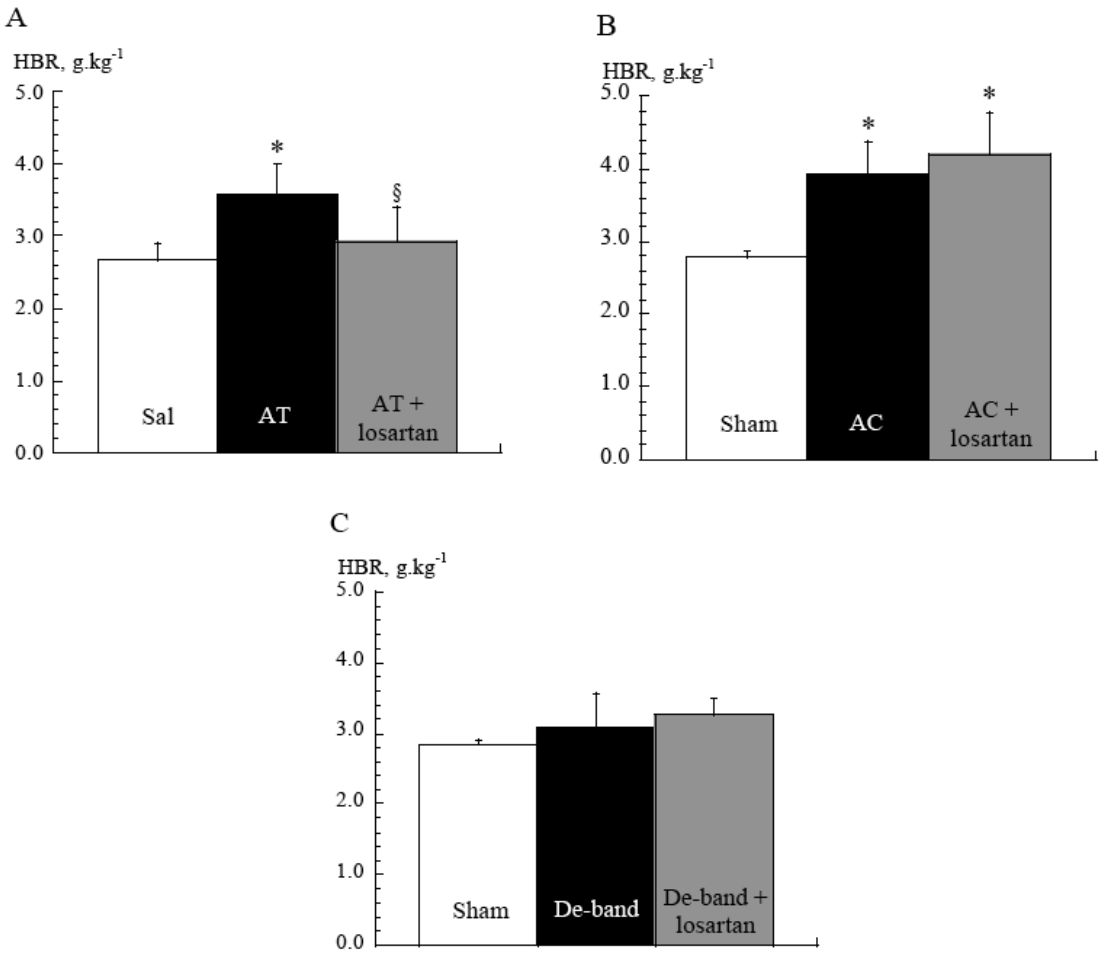

A
HBR, g.kg-1
5.0
4.0
3.0
2.0
1.0
0.0
*
§
Sal
AT
AT + losartan
B
HBR, g.kg-1
5.0
4.0
3.0
2.0
1.0
0.0
*
*
Sham
AC
AC + losartan
C
HBR, g.kg-1
5.0
4.0
3.0
2.0
1.0
0.0
Sham
De-band
De-band + losartan

Figure 4

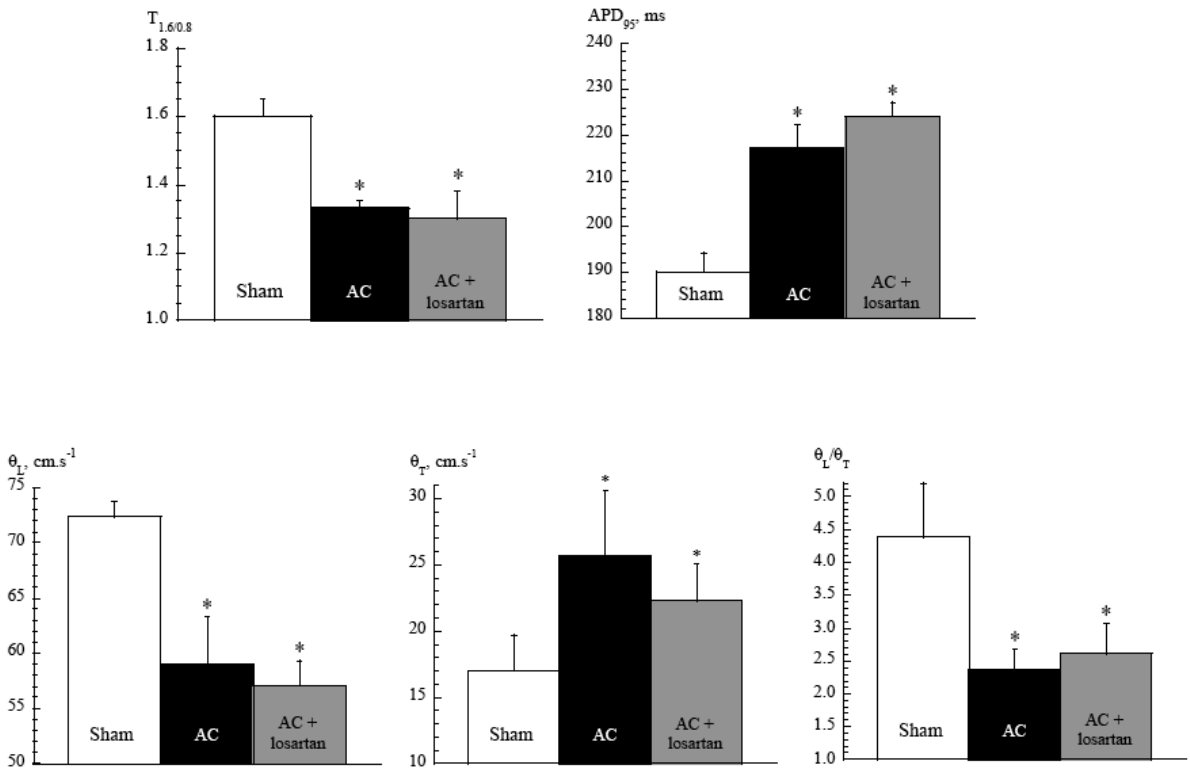

$T_{1.6/0.8}$
1.8
1.6
1.4
1.2
1.0
Sham
AC
AC + losartan
APD_{95}, ms
240
230
220
210
200
190
180
Sham
AC
AC + losartan
θ_L, cm.s^{-1}
75
70
65
60
55
50
Sham
AC
AC + losartan
θ_T, cm.s^{-1}
30
25
20
15
10
Sham
AC
AC + losartan
θ_L/θ_T
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Sham
AC
AC + losartan

Figure 5

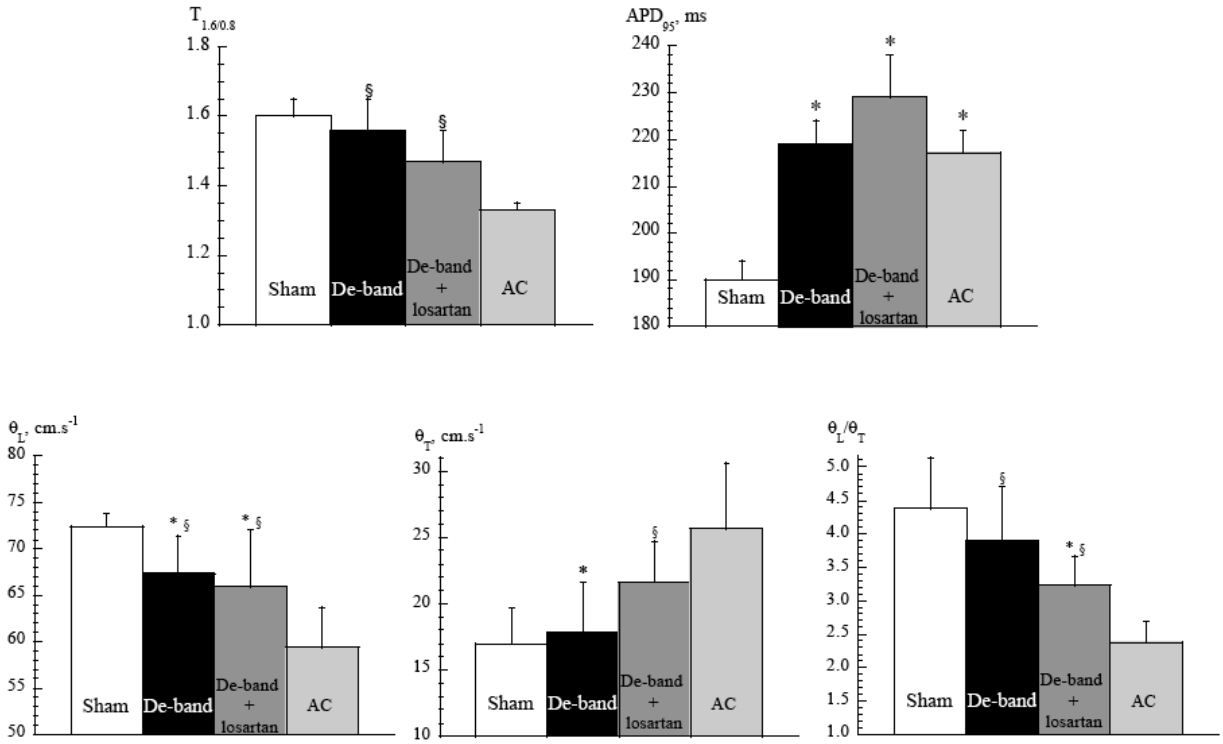

T1.6/0.8
1.8
1.6
1.4
1.2
1.0
Sham
De-band
De-band + losartan
AC
APD95, ms
240
230
220
210
200
190
180
θL, cm.s-1
80
75
70
65
60
55
50
θT, cm.s-1
30
25
20
15
10
θL/θT
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

Table 1: Measurements of heart weights during thoracic aorta constriction, de-banding of the aorta, and by angiotensin infusion. Values are mean ± S.D. * denotes $p<0.05$ with respect to sham-operation. § denotes $p<0.05$ de-banded with respect to aortic constriction.

	Heart-to-body weight ratio, $g \cdot kg^{-1}$	Dried LV-to-body weight ratio, $g \cdot kg^{-1}$
Sham-operation, 42 days, group 6	**2.84±0.07**	**0.32±0.084**
Aortic constriction, 42 days, group 7	**3.66±0.45 ***	**0.45±0.041 ***
Sham-operation, 84 days, group 1	**2.79±0.09**	**0.33±0.028**
Aortic constriction, 84 days, group 2	**3.92±0.45 ***	**0.51±0.044 ***
Aortic constriction, 42 days/de-banded, 42 days group 4	**3.08±0.20 §**	**0.37±0.041 §**
Saline infusion, 42 days, group 8	**2.66±0.24**	**0.32±0.080**
Angiotensin, 42 days, group 9	**3.57±0.44 ***	**0.46±0.042 ***

Table 2: Electromechanical properties of isolated myocardial preparations from control, aortic constricted and de-banded groups. $T_{(1.6/0.8)}$, ratio of peak tension generated at 0.8 and 1.6 Hz; APD_{95}, AP duration at 95% repolarisation. θ, conduction velocity; E_m, resting membrane potential; dV/dt_{max}, maximum AP upstroke velocity; τ_{ap}, time constant of the foot of the AP. Subscripts L and T refer to variables from action potentials conducting in the longitudinal or transverse axis of the preparation. Values are mean ± S.E.M., except θ_L, θ_T and the derived anisotropy ratio, θ_L/θ_T, which are mean ± S.D. * $p<0.05$ aortic constriction and de-banded with respect to sham-operation. § $p<0.05$ de-banded with respect to aortic constriction. #, $p<0.05$ transverse (T) *vs* longitudinal (L) for dV/dt_{max} and τ_{ap}.

	Sham-operation Group 1	**Aortic constriction Group 2**	**De-banded Group 4**
$T_{(1.6/0.8)}$	**1.60±0.05**	**1.34±0.02 ***	**1.56±0.02 §**
APD_{95}, ms	**190±4**	**217±5 ***	**219±5 ***
θ_L, $cm \cdot s^{-1}$	**72.4±1.4**	**59.6±4.3 ***	**67.3±4.1 *§**
θ_T, $cm \cdot s^{-1}$	**17.0±2.7**	**25.7±4.9 ***	**17.8±3.7 §**
θ_L/θ_T	**4.38±0.76**	**2.37±0.32***	**3.88±0.83 §**
E_m, mV	**84.5±0.6**	**85.4±1.0**	**84.7±0.4**
dV/dt_{max} (L), $V \cdot s^{-1}$	**224±16**	**214±12**	**204±13**
dV/dt_{max} (T), $V \cdot s^{-1}$	**263±25 #**	**243±17 #**	**234±12 #**
τ_{ap} (L), ms	**0.31±0.026**	**0.30±0.016**	**0.33±0.014**
τ_{ap} (T), ms	**0.22±0.015 #**	**0.24±0.011 #**	**0.25±0.010 #**