Early responses of the left ventricle to pressure overload in Wistar rats.

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Short title: Early LV signaling events in pressure overload

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Abstract

The early events leading to the establishment of left ventricular hypertrophy associated to pressure overload (PO) are not well characterized. To explore these early events, aortic banding (AB) was performed in rats to induce left ventricle (LV) PO. Animals were sacrificed after 24, 48 hours or 14 days. An echocardiogram was performed before the procedure and at sacrifice. LVs were preserved for the evaluation of fibrosis, angiotensin II (AT) receptors expression and stress-related MAP kinases (Erk 1/2, JNK and p38) pathways. We observed that concentric LV hypertrophy was established after only 14 days. Collagen I and fibronectin gene expressions were decreased the first two days after AB induction whereas AT receptors mRNA levels were sharply increased. Erk 1/2 and JNK activities in LV homogenates were decreased 24 hours after AB but came back to normal after 14 days. p38 activity however was stable during the period studied. We also evaluated the presence of two phosphorylated transcription factors related to JNK signaling pathway (ATF-2 and c-Jun) in cardiomyocyte nuclei. The proportion of LV cell nuclei positive for these two activated transcription factors was significantly reduced in AB rats compared to sham. These results suggest that the early response of the LV to acute PO is to attenuate the expression of some pro-fibrotic and pro-hypertrophic signaling pathways and possibly AT signaling by decreasing Erk1/2 and JNK relative activities.

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Introduction

Cardiac hypertrophy can develop as a consequence of hypertension or some valvular heart disease. Being unable to divide, cardiac myocytes respond to parietal stress by increasing their volume thereby resulting in hypertrophy (Ruwhof and van der Laarse, 2000). Hypertrophy constitutes by itself a risk factor for the development of arrhythmias, diastolic dysfunction and progression to congestive heart failure (Hein et al., 2003;Selvetella et al., 2004). Left ventricular pressure overload resulting from hypertension or aortic valve stenosis activates a wide range of signaling pathways (Haq et al., 2001; Sadoshima et al., 1992; Sadoshima and Izumo, 1993). In addition to loading conditions, it appears that local release of neuroendocrine ligands such as angiotensin II can modulate myocyte hypertrophy and function as well as extracellular matrix (ECM) reorganization (Somsen et al., 1996). Via its various G protein-coupled receptors subtypes, angiotensin II can activate some mitogen-activated protein kinase (MAPK) signaling pathways (Baba et al., 1999; Fujii et al., 1995;Urata et al., 1989;Wolf et al., 1996). Among the members of this family, the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38-MAPK are known to be involved in this process (Booz et al., 2002;Izumi et al., 2000;Kacimi and Gerdes, 2003;Kim et al., 1999;Ruf et al., 2002). It is accepted that the ERK1/2 pathway is linked to the hypertrophic response but the respective roles of the JNK and p38 pathways are more controversial (Liang and Molkentin, 2003). Early data from studies on cultured cardiac myocytes seemed to support a coordinated action of ERK, JNK and p38 to promote hypertrophic growth (Clerk et al., 1998; Komuro et al., 1996; Zechner et al., 1997). Results from animal models (mostly genetically modified mice models) suggest that p38

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signaling does not promote the hypertrophic response and could be anti-hypertrophic in adult heart (Liao et al., 2001). Results from animal studies on JNK signaling also suggest an anti-hypertrophic role (Sadoshima et al., 2002). This difference between in vitro and in vivo studies emphasizes the complexity of the various roles of MAPK in the development of cardiac hypertrophy.

Abdominal aortic banding (AB) is a classical animal model for the study the effects of pressure overload (PO) on the left ventricle (LV) (Imamura et al., 1990;Yin et al., 1980). The early adaptations of the LV to this type of stress have received little attention.

In this study we evaluated the early effects of PO from aortic banding on the LV of Wistar rats before the compensatory remodeling is completed and fully established.

Methods

Animals: Forty male Wistar rats were purchased from Charles River (Saint-Constant QC, Canada) and randomly divided in 4 groups (n=10/gr) as follows: 1) control, 2) aortic banding (AB) 1 day, 3) AB 2 days and 4) AB 14 days. This protocol was approved by the Université Laval's animal protection committee and was consistent with the recommendations of the Canadian Council on animal care.

Abdominal aortic banding: Under ketamine/xylazine anesthesia, the abdominal aorta was dissected above renal arteries. An 18-gauge needle was placed alongside the artery and both were tightly tied together. The needle was then quickly removed to create a standardized reduction of the size of the aortic lumen. At sacrifice, the whole heart was removed and each chamber were dissected and weighted separately, the LV was snap frozen in liquid nitrogen and then kept at -80°C until use. A LV mid-section was also fixed and paraffin-mounted for histological studies.

Echocardiography: A complete two-dimensional, M-mode and Doppler echocardiogram was performed under anesthesia as previously published preoperatively and just before sacrifice (Plante et al., 2003; Plante et al., 2004a; Plante et al., 2004b). Briefly, the thorax was shaved and the animal was put on a dorsal decubitus position. Imaging of the heart was done using a 12 MHz ultrasound probe and a Sonos 5500 echographer (Philips Medical Imaging, Andover, MA). Imaging depth was adjusted for each animal to obtain the largest possible 2D image in the imaging sector. Left ventricular dimensions and septal and posterior wall thicknesses were obtained in the parasternal long axis view using M-mode imaging. Relative wall

thickness (RWT) was calculated as the ratio of the sum of the wall thicknesses (septal and posterior) to the end-diastolic diameter. Filling parameters were measured by pulsed Doppler analysis of mitral E wave and A waves. (Plante et al., 2003; Plante et al., 2004a; Plante et al., 2004b). Indexation was made for both the body weight of the animal and its tibial length.

Semi-quantitative analysis of mRNA accumulation by RT-PCR: Total RNA extraction from the LV, reverse transcription and DNA amplification by PCR of collagens type I and III, fibronectin, pro-metalloprotease 2 (proMMP2) as well as the angiotensin II receptor subtypes (ATIa, ATIb and ATII) mRNAs as well as data analysis were carried out as described previously (Plante et al., 2004a; Plante et al., 2004b) using the following primer pairs: *glyceraldehyde phosphate dehydrogenase (GAPDH)*, 5'-ATCCCATCACCATCTTCCAG-3' and 5'-CCATCACGCCACAGTTTCC-3'; *collagen type 1 (Col1):* 5'-TGTTCGTGGTTCTCAGGGTAG-3' and 5'-TTGTCGTAGCAGGGTTCTTTC-3'; *Col3:* 5'-CGAGGTAACAGAGGTGAAAGA-3' and 5'-AACCCAGTATTCTCCGCTCTT-3' and *pre-Matrix metalloprotease 2 (MMP2):* 5'-CTATTCTGTCAGCACTTTGG-3' and 5'-CAGACTTTGGTTCTCCAACTT-3' ; *fibronectin (FN)* 5'-GAGAGATCTGGAGGTCAT-3' and 5'-GGGTGACACCTGAGTGAA-3' ; *AT1a receptor* 5'-GCACACTGGCAATGTAATGC-3' and 5'-GTTGAACAGAACAAGTGACC-3' ; *AT1b* 5'-GCCTGCAAGTGAAGTGATTT-3' and 5'-TTTAACAGTGGCTTTGCTCC-3' ; *AT2* 5'-GCATGAGTGTTGATAGGTACCAAT-3' and 5'-CCCATAGCTATTGGTCTTCAGCAGATG-3'. Denaturation, annealing and amplification temperatures were 94, 60 (50 for Col3, 55 for MMP2) and 68°C, respectively.

Cardiomyocyte cross-sectional area (CSA) and evaluation of LV fibrosis: Sections from paraffin-embedded mid-LV portions were stained using the Trichrome-Masson method. Three sections / slide from at least 9-10 animals per group were studied for the evaluation of cross sectional area (CSA) as previously described (Plante et al., 2004b). These sections were also used to evaluate the proportion of LV sub-endocardial (inner third) fibrosis as the blue/red ratio using an image analysis system (Image-Pro Plus, Version 4.5, Media Cybernetics, Silver Springs, MD).

MAP Kinase Assay: Modifications of previously published methods were used here (Braz et al., 2003; Takeishi et al., 2001). Pieces of LV tissue were homogenized using a glass potter in 10 volumes of ice-cold buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1mM EDTA, 1mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1mM β-glycerophosphate, 1mM sodium orthovanadate, 1% protease inhibitor cocktail (Sigma, Markham ON, Canada)) and then incubated for 20 min. Protein concentration of the supernatant from a 30-min centrifugation in a tabletop centrifuge of the homogenate was determined by the Lowry method. This supernatant (2 mg of protein) was then pre-cleared using10 μL of Protein A/G Plus agarose (Santa Cruz Biotechnology, Santa Cruz, CA) at 4C for 90 min. Samples were then centrifuged at 1000 x g for 5 minutes and 500 μg of protein (Erk 1/2 and JNK) or 250 μg (p38) were incubated with 2 μg of respective antibody overnight at 4C (anti-ERK1/2 rabbit polyclonal antibody, Upstate, Charlottesville, VA , anti-JNK 2 mouse monoclonal antibody and anti-p38 mouse monoclonal antibody, Santa Cruz Biotechnology). Immnunoprecipitation was completed the next day by incubating the samples with 10 μL of protein A/G Plus agarose for 1h at

4C. Beads were then collected after slow-speed centrifugation, washed once with homogenisation buffer, 2 times with LiCl buffer (500 mM LiCl, 100 mM Tris-HCl pH 7.6, 01% Triton X-100, 1 mM DTT) and once with the Kinase buffer (25 mM Hepes pH 7.4, 25 mM $MgCl_2$, 25 mM β-glycerophosphate, 1mM EGTA, 1 mM DTT, 1 mM sodium orthovanadate). The kinase assay was performed in a total volume of 30 μL of Kinase buffer containing 100 μM ATP and 2 μg of respective GST-fusion protein substrate (JNK: c-jun, Erk 1/2: Elk-1 and p38: ATF-2 purchased from Cell Signaling Biotechnology, Beverly, MA) for 30 minutes at 30°C. The reaction was stopped by adding 10 μL of Laemmli buffer 4X. Samples were separated by SDS-PAGE. Proteins were transferred on a nitrocellulose membrane, blocked in Tris buffered saline-Tween 0.001% containing 3% milk (p38 and ERK 1/2) or 3% bovine serum albumin (JNK) for 1 hour at room temperature. Membranes were then incubated for 2 hours at room temperature with the respective primary antibody (anti-phospho-c-jun rabbit polyclonal antibody,1:1000, anti-phospho-Elk-1 mouse monoclonal antibody, 1:250 or anti-phospho-ATF-2 rabbit polyclonal antibody, 1:500) (Santa Cruz Biotechnology).

MAP kinase protein content: Twenty-five μg of cytosolic LV extract were separated by SDS-PAGE. Membranes were immunolabeled with either an anti-JNK 2 mouse monoclonal antibody (1:500), anti-Erk1/2 rabbit polyclonal antibody (1:1000) or an anti-p38 mouse monoclonal antibody (1:500).

Nuclear expression of phospho-c-Jun and phospho-ATF2 in LV sections:
Consecutive paraffin-mounted mid-ventricular sections of the left ventricle (5/group)

were labeled with either an anti-phospho-c-jun rabbit polyclonal antibody, (1:1000) or an anti-phospho-ATF-2 (1:500) rabbit polyclonal antibody. Views (3/sections) from the inner half (endocardial) and the outer half (epicardial) of the LV wall were digitalized and assigned a random number. Analysis of the labeled sections was then done by two blinded observers using image analysis software (SigmaScan Pro, Version 4, Saugus, MA). Briefly, the number of positively-stained nucleus positive over the total number of nuclei was evaluated for each view and is expressed as a percentage.

Statistical analysis: Results are presented as mean ± SEM unless specified otherwise. One-way analysis of variance was performed to compare serial data. Statistical significance was set at a $p<0.05$ using post-hoc Tukey's test. Data and statistical analyses were performed using GraphPad Prism version 4.02 for Windows, GraphPad Software, (San Diego CA).

Results

Survival rate: All animals in the sham group and in AB groups survived until the end of the protocol. None of the animals required medical treatment for acute heart failure.

Development of LV and RV hypertrophy (Table 1)

The evolution of LV and right ventricle (RV) masses are summarized in Table 1. Indexation for both the LV and the RV mass was made using tibial length to account for animal growth (body weight not used for indexation due to acute weight loss in animals at days 1 and 2 post AB). At 14 days, LV hypertrophy is clearly established as illustrated by the significant increase in indexed LV weight compared to sham rats. The same was true for the RV after 14 days but to a lesser extent.

LV echocardiographic data (Table 2)

Table 2 depicts the evolution of LV dimensions, absolute and relative wall thickness (index of concentric LV remodeling) and ejection fraction in rats with aortic banding. During the course of the protocol, the end-diastolic diameter (EDD) as well as the end-systolic diameter (ESD) did not differ significantly from sham animals. On the other hand, wall thicknesses (septal (SW) and posterior (PW) walls) were significantly increased as soon as 48 hours post-AB. As a consequence, an increase in relative wall thickness (concentric remodeling) was present at day 2 post-AB induction. Ejection fraction remained stable among all groups. Indexation of echocardiographic values to tibial length had no impact on the results or on statistical significance (not shown).

Left atria, lung weight and diastolic function

Table 3 depicts the evolution of left atrial (LA) size and left ventricular diastolic filling pattern evaluated by Doppler in rats with AB. As soon as 2 days after AB induction, we observed an enlargement of the LA diameter as assessed by echocardiography. At 14 days, lung weight was significantly increased. The mean diastolic E/A ratio remained normal for all the groups. However, if we consider individually the animals having an E/A ratio outside the 1.4 and 2.7 E/A ratio normal range (Plante et al., 2003), several rats at day 2 and 14 days had out of range E/A ratio whereas in sham control rats and at day 1 groups, all had a normal E/A ratio.   Mitral E wave downslope tended to increase in all AB groups but this trend did not reach statistical significance.

LV fibrosis and myocyte hypertrophy

We did not observe any increase in sub-endocardial fibrosis in our AB animals (results not shown). Cross-sectional area (CSA) of cardiomyocytes was also evaluated in LV sections from all animals and we observed 14 days after AB a 12% increase (p<0.05) for this parameter (results not shown).

Extracellular matrix components mRNA expression in AB rats. (Figure 1)

Collagen I and fibronectin mRNA levels were decreased in the first two days following AB induction. A similar trend was observed for collagen III mRNA levels. After two weeks of pressure overload, these levels were similar to controls. We observed a similar pattern of regulation for proMMP2 mRNA levels in these samples.

Angiotensin II receptors gene expression in the LV of AB rats (Figure 2)

We evaluated the LV expression of the genes encoding for the AT1a, AT1b and AT2 receptors in our AB rats. We observed a significant increase in AT1a mRNA levels 48 hours post-AB induction. AT1b, and AT2 mRNA levels were both more strongly increased in all AB rats compared to controls as early as 1 day post AB.

MAP kinases regulation in pressure overload (Figures 3 and 4)

We evaluated the protein content and activity of MAP kinases Erk1/2, JNK and p38. As illustrated in Figure 3, during the acute phase of pressure overload, the protein content as evaluated by immunoblotting of Erk1/2 and JNK in the LV was increased 24 hours after AB and was back to control levels after 14 days. Levels of p38 remained stable during the same period. We then assayed the activity of these kinases in these LV samples. The level of formation of phospho-erk-1 (Erk1/2) and phospho-ATF2 (p38) were similar for all groups. However, phosphorylation of c-jun by JNK in the LV of AB rats was severely decreased after 24 hours. After correction for the protein content present in the assay for each kinase, we observed that the specific activities of Erk1/2 and JNK were decreased 24 hours after AB induction but returned to normal levels after 14 days. In order to evaluate the current levels of activation of two transcription factors related to the JNK and p38 signaling pathways in the LV, we measured the presence of the phosphorylated forms of c-Jun and ATF-2 in the nucleus of the LV myocardial cells. The proportion of cell nuclei positive for either c-Jun or ATF-2 phosphorylated forms was significantly decreased in total LV cells (myocytes and fibroblasts) as soon as 24 hours post-AB. This effect was seen in sub-endocardial as well as mid-wall sections of VG and was still present 14 days after AB (Fig. 4).

Discussion

Left ventricle pressure overload results in the development of concentric hypertrophy. Molecular events leading to cardiac hypertrophy have been studied extensively in the past and a dichotomy has been reported between observations made *in vitro* (cardiomyocyte culture) and *in vivo* (animal models) relatively to the respective roles of the JNK and p38 signaling pathways (Liang and Molkentin, 2003). Although the activation of these pathways seems to lead to a hypertrophic response in cultured cardiac myocytes, a protective (anti-hypertrophic) role has been suggested for these signaling pathways in the intact adult heart although they may also promote the transition from a compensated state towards dilated cardiomyopathy (Liang et al., 2003; Liang and Molkentin, 2003; Nishida et al., 2004; Sadoshima et al., 2002). In the current study, we have concentrated our attention towards the early events following the induction of LV pressure overload in adult rats by aortic banding.

We observed after 14 days of pressure overload significant and typical macroscopic changes in the LV (increased LV mass and thicker LV walls) as well as direct and indirect evidences of diastolic dysfunction and elevated left filling pressures (increased LA diameter, lungs weight and abnormal filling parameters in some animals). These changes were typical of concentric left ventricular hypertrophy associated with pressure overload and occurred very early after only 14 days of pressure overload. Interestingly, despite the fact that the expression of all angiotensin II receptors (mostly AT1b and AT2) subtypes was clearly and quickly up-regulated suggesting an activation of the pro-hypertrophic and pro-fibrotic tissue renin-angiotensin system, mRNA levels of several ECM-related proteins were all down-regulated 24 and 48 hours after banding. The role

of the AT1b and AT2 receptors in the myocardium is still the object of debate.

Interestingly, the AT2 receptor has been shown to be involved in the LV hypertrophy of

AB rats in presence of AT1 receptor blockade suggesting a role for this receptor in the

hypertrophic response to pressure overload (Mukawa et al., 2003). We did observe two

weeks after AB a partial return to normal mRNA levels for all the ECM components we

have studied but expression levels all tended to be below normal.

Obviously, there is a discrepancy between the presence of a pro-hypertrophic and pro-

fibrotic LV stress and the levels of expression of these ECM proteins involved in

remodeling. In order to better understand this discrepancy between obvious signs of

activation of the angiotensin II-related signaling pathways at the receptor levels and a

negative response at the level of mRNA expression of several members of the ECM, we

looked at the MAP kinase signaling in the LV of our animals. Twenty-four hours after the

induction of LV pressure overload, levels of Erk 1/2 and JNK were significantly increased

while their kinase activity was equal (Erk 1/2) or less (JNK). Afterwards, after 2 and 14

days, these levels were back to normal. No changes were observed for p38 levels. It is

not clear why p38 activity failed to follow both Erk1/2 and JNK transient decreases in

relative activity. In fact, it could be argued that only the JNK pathway was down-

regulated over the period we studied our rats. It has been shown that lack of JNK1 in

mice with thoracic aortic banding was associated with faster progression of systolic

dysfunction in earlier stages (first seven days) compared to wild-type animals but

cardiac parameters of these JNK1-/- mice were indistinguishable from controls at later

times (3 months) (Tachibana et al. 2006). In our model, it is possible that the presumed

protective role of the JNK pathway was also loss in the first 24 hours leading to the

hypertrophic response. Moreover, two phosphorylated forms of transcription factors in

the nuclei of cardiomyocytes and SAPK/JNK-associated factors were also clearly reduced. Despite the inactivation of this stress-related signaling pathway and no clear activation of the pro-hypertrophic ERK 1/2 pathway, some level of remodeling was present two weeks after banding suggesting the implication of other signaling pathways either pro-hypertrophic or the inhibition of protective ones. It is possible that an imbalance between the state of activation of these pathways or a transient activation in more acute states (minutes or hours) may be sufficient to induce the hypertrophic response. MAP kinase pathways activation has been shown in the past in acute aortic banding (Fischer et al., 2001;Nadruz, Jr. et al., 2003;Nadruz, Jr. et al., 2004) either in hyper-acute settings (less than two hours of AB) or only at later times (>14 days) (Miyamoto et al., 2004). Increases in the activation of other pathways have been reported namely those related to NF-κB (Li et al., 2004), Akt, Stat3 and others (Miyamoto et al., 2004). More studies are needed to better understand this phenomenon.

In conclusion, we demonstrate the early activation of components of the renin-angiotensin system accompanied by a rapid decrease in the activity of two MAP kinase pathways (JNK and p38). In addition, we observed a transient decrease in the mRNA expression of various components of the extracellular matrix. The presence of established LV hypertrophy after 14 days suggests that these early adaptations fail to protect the LV when the pressure overload is sustained and becomes chronic.

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Figure legends:

Figure 1: Semi-quantitative evaluation of Collagen type I (ColI), Collagen Type III (Col III), Fibronectin and proMMP2 mRNA expression by RT-PCR. Results are expressed mean ± SEM as arbitrary units. (n=10/group). Day 0 or sham values were fixed at 1. *: P<0.05 and **: P<0.01 vs. day 0 (sham).

Figure 2: Semi-quantitative evaluation of AT1a, AT1b, AT2 receptors mRNA expression by RT-PCR. Results are expressed mean ± SEM (n=10/group) as arbitrary units. Day 0 or sham values were fixed at 1. *: P<0.05 and **: P<0.01 vs. day 0 (sham).

Figure 3: MAP kinases content and activity in the LV of AB rats. Erk1/2, JNK and p38 protein content (upper panels) and activity (middle panels) were evaluated as described in the Material and Methods section. Bottom panels represent the activity corrected for the protein content in the assay for each kinase. Results are expressed as mean ± SEM (n=5/group). Day 0 (sham) values were arbitrarily fixed at 100. *: P<0.05 and **: P<0.01 vs. day 0 (sham).

Figure 4: Decrease of ATF-2 and c-Jun phosphorylation levels in the LV of AB rats. Numbers of LV cell nuclei positive for either p-c-Jun or p-ATF2 were evaluated in the sub-endocardial (inner third) and the mid-wall (central third) sections as described in the Material and Methods section. Typical examples of positive cell nuclei immunolabelling (arrows) for p-c-Jun or p-ATF2 are shown in the bottom section. Results are expressed in % of positive nucleus as mean ± SEM (n=5/group). *: P<0.05 vs. day 0 (sham).

Table 1: Evolution of left and right ventricular weights during the protocol

Days post-AB	0	1	2	14
	(sham controls)			
Tibial length, mm	52 ± 0.2	53 ± 0.4	53 ± 0.8	54 ± 0.2**
LV weight, mg	605 ± 15.6	640 ± 21.4	651 ± 25.6	802 ± 22.5**
iLV weight, mg/mm	11.5 ± 0.27	12.0 ± 0.35	12.1 ± 0.38	14.7 ± 0.41**
RV weight, mg	164 ± 5.5	172 ± 8.3	176 ± 9.4	190 ± 5.5**
iRV weight, mg/mm	3.1 ± 0.10	3.2 ± 0.14	3.3 ± 0.14	3.5 ± 0.10*

Values are means ± SEM (n=9-10). *: $p<0.05$ and **: $p<0.01$ vs. 0 (sham control animals). AB: aortic banding; LV: left ventricle; RV: right ventricle; i: measurement indexed to animal's tibial length.

Table 2: LV echocardiographic data

Days post-AB	0	1	2	14
	(sham controls)			
EDD, mm	7.8 ± 0.09	7.9 ± 0.12	7.9 ± 0.12	8.2 ± 0.20
ESD, mm	4.4 ± 0.11	4.2 ± 0.20	4.3± 0.18	4.5 ± 0.24
SW, mm	1.6 ± 0.03	1.7 ± 0.07	1.8 ± 0.05*	2.0 ± 0.06**
PW, mm	1.7 ± 0.02	1.7 ± 0.04	1.9 ± 0.06**	2.0 ± 0.03**
RWT	0.42 ± 0.010	0.43 ± 0.013	0.46 ± 0.009**	0.49 ± 0.017**
EF, %	69 ± 2.0	72 ± 2.3	72 ± 2.2	70 ± 2.1

Values are means ± SEM (n=9-10). *: $p<0.05$ and **: $p<0.01$ vs. day 0 (sham). AB: aortic banding; EDD: end-diastolic diameter; ESD: end-systolic diameter; SW: septal wall thickness; PW: posterior wall thickness; RWT: relative wall thickness ((SW + PW)/EDD); EF: ejection fraction.

Table 3. Left atrial size and diastolic filling parameters

Days post-AB	0	1	2	14
	(sham controls)			
LA weight, mg	23 ± 2.3	22 ± 1.2	25 ± 1.9	28 ± 2.9
iLA weight, mg/mm	0.47 ± 0.054	0.44 ± 0.024	0.41 ± 0.020	0.52 ± 0.054
LA diameter, mm	3.8 ± 0.13	4.4 ± 0.32	4.7 ± 0.21*	4.9 ± 0.22*
Lungs weight, g	1.4 ± 0.05	1.6 ± 0.07	1.9 ± 0.13	2.2 ± 0.22**
iLungs weight, mg/mm	25 ± 0.9	30 ± 1.4*	35± 2.3**	40 ± 4.2**
Mitral E/A wave ratio	2.0 ± 0.13	1.9 ± 0.13	1.9 ± 0.16	1.8 ± 0.16
E/A<1.4 or E/A>2.7	0/10	0/9	3/9	3/9
Mitral E wave downslope	1473 ± 78	1668 ± 123	1595 ± 123	1666 ± 134

Values are means ± SEM (n=9-10). *: $p<0.05$ or **: $p<0.01$ vs. 0 (sham controls).

AB: aortic banding; LA: left atrium; E wave: Doppler maximal early diastolic filling wave

velocity; A wave: Doppler maximal late diastolic filling wave velocity; i: values indexed

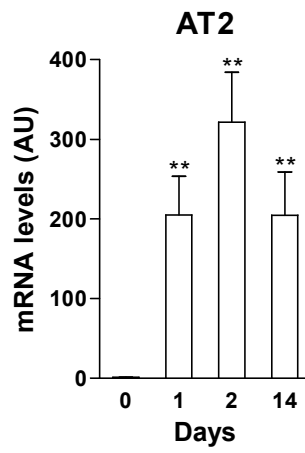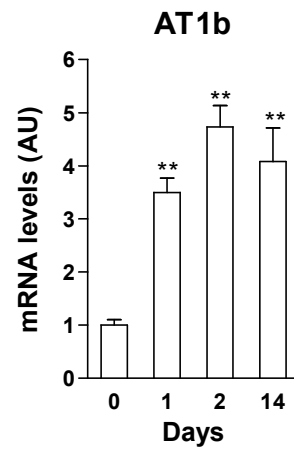for animal's tibial length.
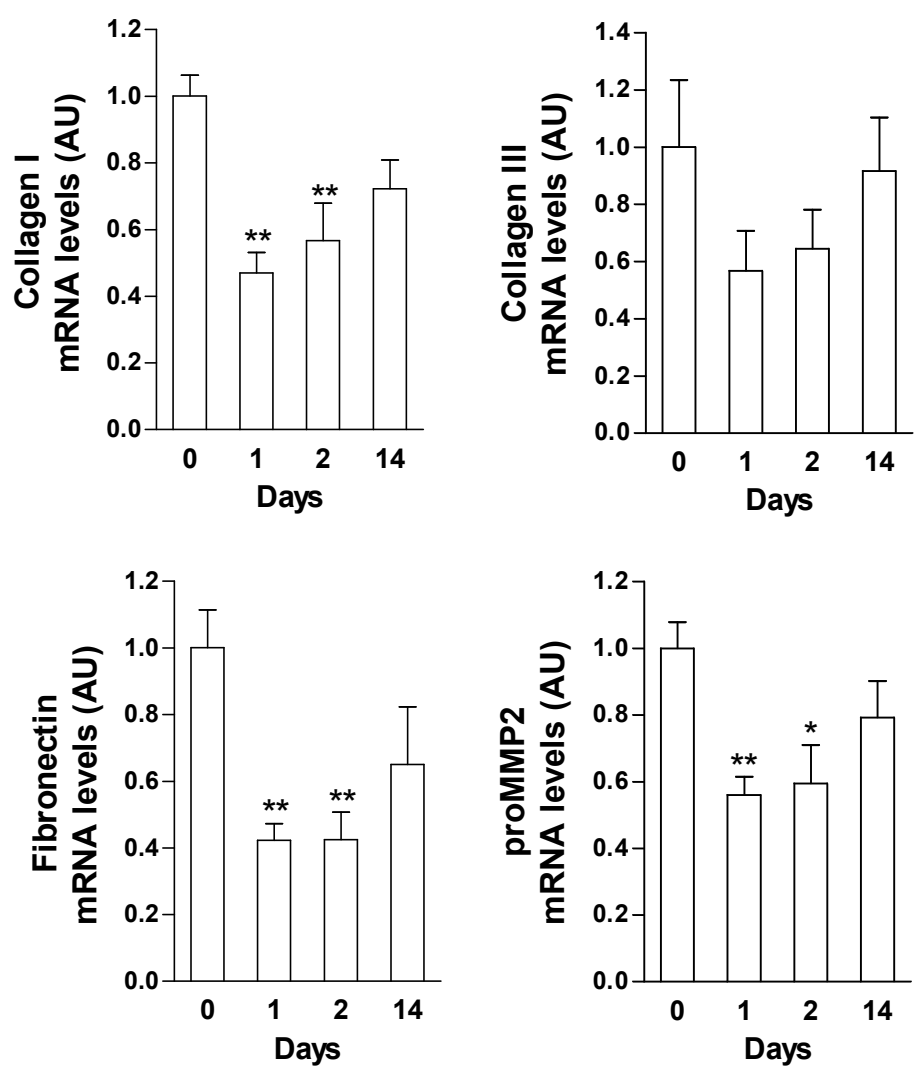
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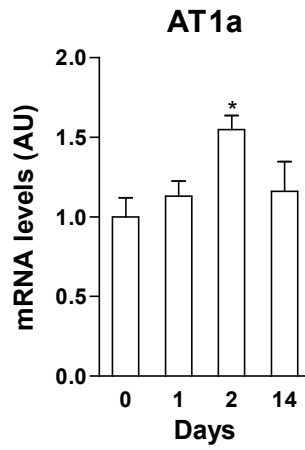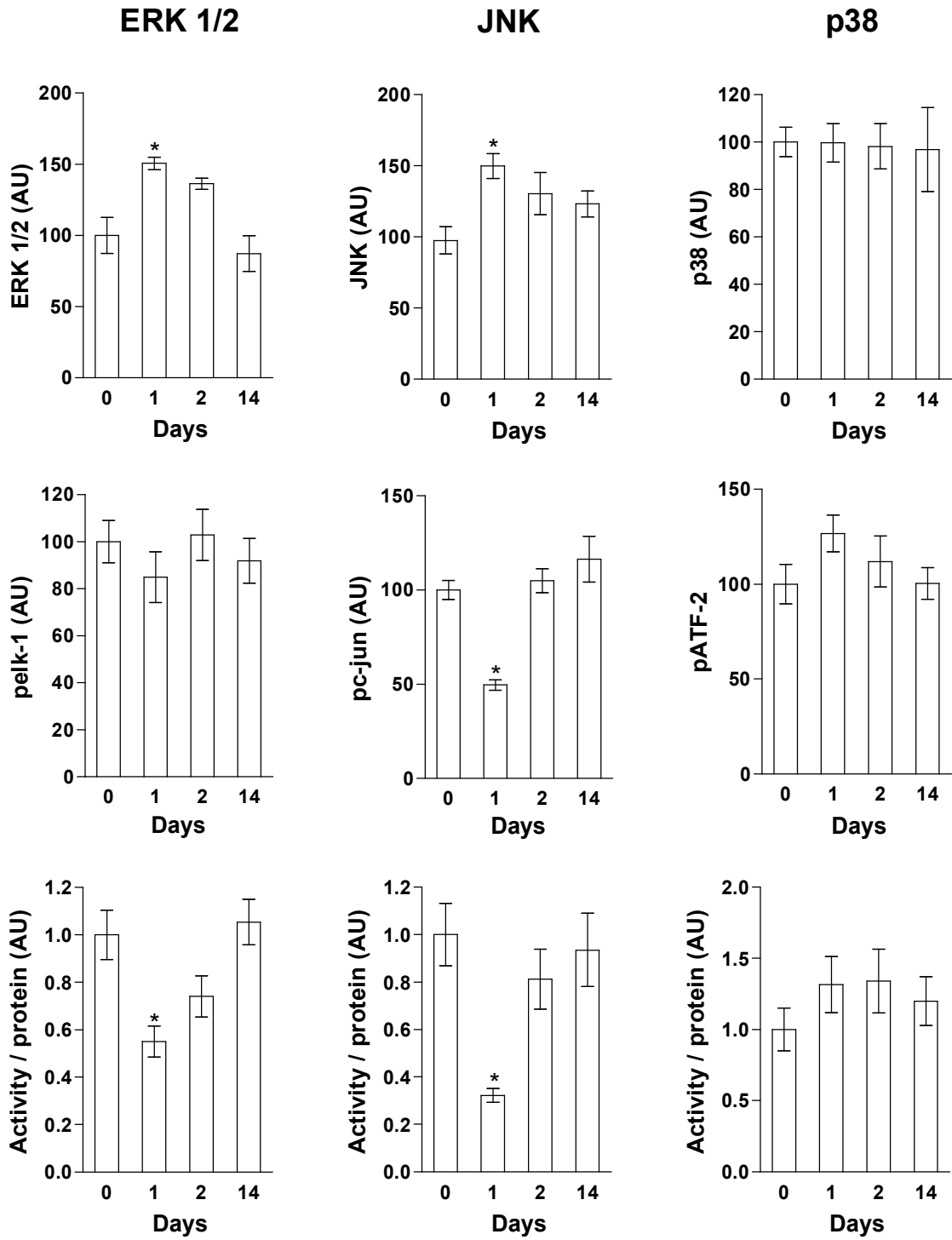
Figure 1
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1

AT1a

AT1b

AT2

Figure 2
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Figure 3
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Figure 4
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