Time-course of decompensation after angiotensin II and high-salt diet in Balb/CJ mice suggests pulmonary hypertension-induced cardiorenal syndrome

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Abstract

Genetic background of a mouse strain determines its susceptibility to disease. C57BL/6J and Balb/CJ are two widely used inbred mouse strains that we found react dramatically differently to angiotensin II and high-salt diet (AngII+Salt). Balb/CJ show increased mortality associated with anuria and edema formation while C57BL/6J develop arterial hypertension but do not decompensate and die. Clinical symptoms of heart failure in Balb/CJ mice gave the hypothesis that AngII+Salt impairs cardiac function and induces cardiac remodelling in male Balb/CJ, but not in male C57BL/6J mice. To test this hypothesis, we measured cardiac function using echocardiography, before treatment and every day for seven days during treatment with AngII+Salt. Interestingly, pulsed wave Doppler of pulmonary artery flow indicated increased pulmonary vascular resistance and right ventricle systolic pressure in Balb/CJ mice, already 24 hours after starting AngII+Salt treatment. In addition, Balb/CJ mice showed abnormal diastolic filling indicated by reduced early and late filling and increased isovolumic relaxation time. Further, Balb/CJ exhibited lower cardiac output compared to C57BL/6J even though they retained more sodium and water, as assessed using metabolic cages. Left posterior wall thickness increased during AngII+Salt treatment but did not differ between the strains. In conclusion, AngII+Salt treatment causes early restriction of pulmonary flow, reduced left ventricular filling and cardiac output in Balb/CJ, which results in fluid retention and peripheral edema. This makes Balb/CJ a potential model to study the adaptive capacity of the heart for identifying new disease mechanisms and drug targets.

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Introduction

Heart failure is one of the leading causes of death worldwide (26) and is characterized by insufficient cardiac output, required for normal tissue function, because of impaired relaxation and thereby filling and/or contraction (11). Typical signs of heart failure are dyspnea, reduced exercise tolerance, fatigue and edema (20). C57BL/6J and Balb/CJ are two widely used inbred mouse strains that we accidently found react dramatically differently to a combination of angiotensin II and high-salt diet (AngII+Salt). Balb/CJ mice develop massive edema within 4-6 days of treatment associated with substantial mortality. C57BL/6J on the other hand develop arterial hypertension and some renal damage as previously shown, but do not decompensate or die (6). It is interesting to note that Balb/CJ has been found to be more susceptible to a number of diseases and syndromes in the literature, some have been directly tied to differences in T-cell mediated innate immunity (8, 13), others to increased oxidative stress (7). Given that the symptoms of high mortality and edema are indicative of heart failure, it is interesting to note that Balb/CJ has been found to be more sensitive to heart disease in a number of different models (12, 17, 24), but the very fast apparent decompensation after combined AngII and high dietary salt has not been reported previously and the mechanism is unknown.

Currently, there are many chronic heart failure models, but very few that show this kind of rapid deterioration (11). It is therefore of interest to study the cardiovascular response to AngII and high-salt diet in Balb/CJ mice, which could potentially work as a model of acute decompensation. Acute decompensation of heart failure refers to new or worsening signs and symptoms of heart failure, such as dyspnea and congestion, which rapidly escalates and requires urgent treatment (19). Diagnosis of acute decompensated heart failure is associated with high mortality and healthcare costs (25).

In a previous study, focusing on the renal response to AngII and high-salt diet in Balb/CJ and C57BL/6J, we found that Balb/CJ mice retain more sodium and water compared to C57BL/6J mice (14). However, we did not find any systematic effects on glomerular filtration rate, indicating that severe renal failure is probably not the cause of the high mortality. Considering the clinical symptom of heart failure in Balb/CJ mice, we hypothesized that AngII and high-salt diet impairs cardiac function and induces cardiac remodelling in Balb/CJ, but not in C57BL/6J mice. To test this hypothesis, cardiac systolic and diastolic function were measured, as well as pulmonary artery flow, blood pressure, and ventricular and atrial weight. Further, fluid balance was assessed to validate the previous findings of increased sodium and water retention in Balb/CJ mice.

Methods

Animals

Male BALB/cJBom (Balb/CJ) and C57BL/6J mice at 6 weeks-of-age were used in the experiments (Taconic, Denmark). Animals were treated in accordance with NIH guidelines for treatment of experimental animals, and the protocol was approved by committees for animal experiments at University of Bergen and Uppsala. Animals were housed at 20-25°C, 45-50% humidity, 12/12 hours light/dark cycle, with free access to food and water. They were fed standard pelleted food with 0.27-0.3% sodium, or high-salt diet with 3% or 4% sodium (Special Diets Services, Witham, UK).

Study design and treatment

Mortality frequency from the preliminary study was included in a previous publication (14), but time-to-event data was deemed important in relation to present study of the decompensation time-course. In the preliminary study sham operated animals and animals treated with a combination of 1 µg/min/kg angiotensin II (AngII) and 4% sodium diet for five weeks were used. AngII (Sigma-Aldrich) or saline was infused using subcutaneous osmotic minipumps implanted under isoflurane anaesthesia (1007D, Alzet, Cupertino, CA). Buprenorphine (0.05-0.1 mg/kg) was used for analgesia. Blood pressure was measured before treatment and five weeks after treatment or before euthanasia in edematous mice.

In the follow-up study, animals were treated with 0.5 µg/min/kg AngII and 3% sodium diet for seven days. High mortality in the preliminary study necessitated lower dose in this study to be

able to investigate the underlying pathophysiology. Before treatment, baseline sodium and fluid balance, blood pressure measurement and echocardiography were performed. Thereafter, blood pressure measurement and echocardiography were performed every day for seven days; the first measurements performed 24 hours after treatment initiation. Sodium and fluid balance were measured in treated Balb/CJ mice when >20% reduction in cardiac output was estimated with echocardiography or when symptoms of decompensation indicated the need for euthanasia. To balance the experimental groups, every time fluid and sodium balance was measured in one Balb/CJ mouse, fluid and sodium balance was measured in one randomly picked C57BL/6J mouse. For the same reason, every time a Balb/CJ mouse died or had to be euthanized, a randomly picked C57BL/6J mouse was euthanized as well.

Tail-cuff blood pressure

Blood pressure was measured with tail-cuff after warming the animals at 32°C (CODA-6, Kent Scientific, Torrington, CT). Before the blood pressure measurements were performed, the animals were allowed to acclimatize to the tube, by keeping them in the tube covered with a dark blanket for 10-15 min. Three acclimatization cycles were followed by five measurement cycles for each animal. The five measurement cycles were averaged to obtain systolic and diastolic blood pressure, and heart rate for that animal.

Echocardiography

Echocardiography was performed under light isoflurane anaesthesia (0.9%) for maximum of 15 minutes using Vevo1100 ultrasound (Visual Sonics, Canada). Cardiac output, stroke volume,

ejection fraction and heart rate were quantified in parasternal long-axis view. Left ventricle posterior wall thickness was measured by M-mode positioned at the largest diameter in parasternal long-axis view, carefully avoiding inclusion of the papillary muscle. Mitral valve flow and pulmonary artery flow were assessed with Pulsed wave Doppler.

Fluid and sodium balance

Water and food intake, and urine excretion were measured for 24 hours using metabolic cages (MMC100, Hatteras Instruments, NC). To reduce the stress associated with metabolic caging, the upper part of the metabolic cages were covered in black plastic film. Sodium was measured in urine collected with metabolic cages, using flame photometry (Instrumentation Laboratory, Massachusetts, USA).

Lung water content and heart weight

Lung tissue was blotted dry, weighed and dried at 67°C for 16 hours. The heart was excised, left and right atria, and left and right ventricle dissected and weighed. Heart weight was corrected for tibia length.

Statistical analysis

Results are shown as mean ± standard error (SEM). Data obtained from multiple independent groups were analyzed using two-way ANOVA and Tukey's post-hoc test. Data not fulfilling the assumption of independency were analyzed with linear mixed effects model using a restricted maximum likelihood fit. Individual contrasts of least-squares means were adjusted using Tukey's

method. $p<0.05$ was accepted as significant. Kaplan-Meier survival analysis was performed using death or premature sacrifice as end-point censoring animals euthanized at experiment completion.

Results

Balb/CJ show increased mortality after treatment with AngII and high-salt diet

In the preliminary study, Balb/CJ and C57BL/6J mice were treated with a combination of 1µg/kg/min AngII and 4% sodium diet (AngII+Salt). No AngII+Salt treated Balb/CJ survived the full 37 day-experiment. They developed significant subcutaneous edema on the breast, forepaws and neck and had to be sacrificed (Figure 1A left). In addition, ascites was noted in several Balb/CJ at sacrifice, and many of the animals were anuric. In contrast, 90% of C57BL/6J mice survived without symptoms (Figure 1A right). Blood pressure did not increase in Balb/CJ mice treated with AngII+Salt, however, some mice developed hypotension (Figure 1B). C57BL/6J on the other hand developed hypertension. In summary, these early experiments showed that Balb/CJ were significantly more sensitive to AngII+Salt than C57BL/6J, and appeared to develop acute decompensation. High morbidity and fast progression in Balb/CJ necessitated less intense treatment (AngII 0.5 µg/kg/min and 3% sodium) and shorter follow-up to investigate the physiological mechanisms reproducibly. Therefore, in all following experiments the AngII dose was reduced to 0.5 µg/kg/min and sodium content in food to 3%. Mortality data presented in this paper have previously been used in a meta-analysis to estimate the overall effect of AngII+Salt on mortality in Balb/CJ and C57BL/6J (14). However, time-to-event mortality data are presented here, because of its importance in relation to the sequential cardiac function measurements.

Cardiac output decreases in Balb/CJ and increases in C57BL/6J in response to AngII+Salt treatment

To study the sequence of circulatory dysfunction Balb/CJ and C57BL/6J mice were treated with AngII+Salt for seven days. Echocardiography was performed before the treatment was started and every day during the seven day treatment period. During AngII+Salt treatment Balb/CJ mice had lower cardiac output compared to C57BL/6J mice (Figure 2A). This difference was apparent at day 3 and the mice started dying or had to be sacrificed because of edema from day 4. Reduced cardiac output was not caused by decreased systolic function since ejection fraction did not differ between the strains and was quite constant over time (Figure 2B). Balb/CJ had lower stroke volume compared to C57BL/6J (Figure 2C), without a compensatory increase in heart rate (Figure 2D).

AngII+Salt causes abnormal left ventricle filling in Balb/CJ mice

A decrease in cardiac output in Balb/CJ was a consequence of reduced filling, as can be seen as lower end-diastolic and end-systolic volume over time (Table 1). To asses filling of the heart and thereby diastolic function, mitral valve flow was measured with pulsed wave Doppler. Interestingly, passive filling (Peak E, Figure 3A, 3D) was higher in Balb/CJ than in C57BL/6J at baseline, resulting in a higher E/A ratio (Figure 3C). Both passive and active filling (Peak A, Figure 3B) of the left ventricle were reduced in Balb/CJ over time, while isovolumic relaxation time increased (Table 1), indicating abnormal diastolic function. This reduction was seen already at day 2, thus preceding reduction in cardiac output.

AngII+Salt increases pulmonary and systemic vascular resistance in Balb/CJ

Pulmonary artery flow acceleration time to ejection time ratio (AT/ET), measured by pulsed wave Doppler has been shown to estimate right ventricle pressure and thereby pulmonary pressure in a reliable and reproducible way (31). Lower AT/ET correlates with higher right ventricle and pulmonary artery pressure. Pulmonary AT/ET decreased in Balb/CJ already at day 1, thus 24 hours following treatment initiation (Figure 4A). Systolic blood pressure (Figure 5A) and mean arterial blood pressure (Figure 5C) increased at day 2 in Balb/CJ mice, but not in C57BL/6J. AngII+Salt treatment had different effect on systolic- and diastolic blood pressure, and mean arterial blood pressure over time in Balb/CJ compared to C57BL/6J (Figure 5 A-C). Interestingly, Balb/CJ had lower heart rate both at baseline and during treatment (Figure 5D). Increased pulmonary pressure as well as systemic blood pressure indicates an increase in vascular resistance in Balb/CJ during AngII+Salt treatment. Balb/CJ mice did not develop pulmonary edema (Table 2) indicating that the increase in pulmonary pressure is primary and not a result of left ventricle diastolic dysfunction.

AngII+Salt induces left ventricle hypertrophy both in Balb/CJ and C57BL/6J mice

Left ventricle posterior wall thickness, measured by M-mode in parasternal long-axis view, increased in both strains during AngII+Salt treatment (Table 1), indicating left ventricle remodelling. Left ventricle and right ventricle weight after treatment did not differ between the strains (Table 2). However, there was a tendency to higher right atrium weight in Balb/CJ mice, which may indicate fluid congestion and increased right ventricular pressure as would be expected in pulmonary hypertension.

AngII+Salt treated Balb/CJ retain more sodium and water compared to C57BL/6J

Fluid and sodium balance was measured in Balb/CJ and C57BL/6J before treatment and when decompensation could be detected through symptoms or as reduced cardiac output by echocardiography. Urine and sodium excretion was lower in AngII+Salt treated Balb/CJ mice compared to AngII+Salt treated C57BL/6J mice (Figure 6). Food (Balb/CJ 2.72±1.01 g, C57BL/6J 6.78±1.64 g) and water intake (Balb/CJ 4.83±1.74 ml, C57BL/6J 10.75±6.78 ml) was slightly higher in C57BL/6J, although not significantly. Ratio of excreted urine to water intake was higher in C57BL/6J (0.65±0.08 vs 0.26±0.08). Some Balb/CJ mice did not excrete any urine at all during the 24-hour measurement period, which is indicative of acute kidney injury and potentially cardio-renal syndrome.

Discussion

The main finding of this study is that the decompensation of Balb/CJ mice treated with AngII+Salt follows initially higher systemic and increased pulmonary artery pressure, associated with cardiac hypertrophy, abnormal left ventricle filling and lower cardiac output compared to C57BL/6J mice. C57BL/6J mice on the other hand develop hypertension and cardiac hypertrophy, with low mortality and absence of edema. The results confirm our hypothesis that AngII+Salt impairs cardiac function and induces cardiac remodelling in Balb/CJ mice.

Already 24 hours following treatment initiation, a difference in pulmonary artery AT/ET was seen between the strains. Balb/CJ mice had lower pulmonary AT/ET indicating higher pulmonary pressure. Interestingly, Balb/CJ has previously been shown to be more susceptible to pulmonary vascular muscularization when exposed to cigarette smoke, which may be connected to increased susceptibility to pulmonary hypertension (22). Balb/CJ mice also had higher systemic systolic blood pressure compared to C57BL/6J mice. Increase in systemic and pulmonary blood pressure

indicates increased overall responsiveness to angiotensin II in this strain. The increase in overall responsiveness may be a result of increased expression of angiotensin II Type I Receptors (AT1R) or decreased expression of angiotensin II Type II Receptors (AT2R), the latter suggested to have vasodilatory effects (29, 32). Further, Balb/CJ may have a reduced ability of metabolizing angiotensin II, either by converting angiotensin II to Ang 1-7 or by internalizing the angiotensin II bound AT1R (3, 23). Angiotensin II has also been shown to stimulate release of endothelin-1, which plays a major role in pulmonary hypertension (4, 5). Whether any of these mechanisms is responsible for the exacerbated phenotype in Balb/CJ mice needs to be addressed in future studies.

Balb/CJ mice also show abnormal diastolic filling during AngII+Salt treatment. Similarly, left ventricular diastolic dysfunction is seen in patients with idiopathic pulmonary hypertension, characterized by reduced end-diastolic and end-systolic volume, reduced peak E and E/A ratio and increased isovolumic relaxation time (15). Patients with primary heart failure with preserved ejection fraction also show reduced peak E, E/A ratio and increased isovolumic relaxation time, although increased end-diastolic and end-systolic volume (15). It is important to note that this model was not initially designed to be similar to human disease, but is originally an interesting difference between two common mouse strains. However, we would like to stress that there are similarities to human pulmonary hypertension and acute decompensation.

Balb/CJ mice treated with AngII+Salt had lower cardiac output compared to C57BL/6J mice. Interestingly, Balb/CJ had lower cardiac output even though they had higher sodium and water reabsorption, which should increase blood volume, venous return and cardiac output (2, 27). Increased fluid reabsorption is a common finding in patients with pulmonary hypertension and

heart failure with preserved ejection fraction (16, 28). However, the increased sodium and water reabsorption in this model may be aggravated by an enhanced action of angiotensin II on the kidneys to stimulate sodium retention. Angiotensin II is also known to stimulate release of aldosterone, which further increases sodium retention (21). Whether angiotensin II stimulated aldosterone release differs between the strains needs further exploration in future studies.

Sodium and urine excretion were measured as 24-hour collections using metabolic cages. The method has some limitations since mice can be sloppy drinkers and some of the urine will evaporate during the collection period. However, high salt diet increases urine excretion, thereby reducing the role of urine evaporation in the treated groups, and 24-hour urinary biochemistry assessed using metabolic cages is similar in Balb/CJ and C57BL/6J (21). Metabolic caging is a stressful situation for the animals, evident by the decreased weight in both strains by day 1, which in turn may affect sodium and urine excretion. Even though the metabolic cages were covered with black plastic film to reduce the stress, the stress associated with metabolic caging is the major limitation of sodium and fluid balance measurements. In addition, the surgery to implant the osmotic minipump is an additional stressor and probably contributes to the initial weight decrease. Interestingly, stress seems to be part of the model as we find slightly improved survival in animals that do not undergo as many investigations, for example the animals used for earlier microarray experiments (14).

Angiotensin II is known to induce cardiac hypertrophy irrespective of its effect on systemic blood pressure (1, 18). Interestingly, AngII+Salt induces left ventricle remodelling in both strains as indicated by increased left ventricle posterior wall thickness, but only one of them decompensates. Cardiac hypertrophy is a compensatory mechanism for increased work load, but

the molecular pathway for induction of hypertrophy, cell death and fibrosis are similar. As a consequence, small changes in the molecular pathway may result in heart failure instead of physiological cardiac remodelling (10). One such pathway is the TGFβ signalling pathway which may stimulate hypertrophic growth via TGFβ activated kinase 1 (TAK1) or induce apoptosis and heart failure via small mothers against decapentaplegic (SMADs) signalling (9, 10). Thus, angiotensin II may be stimulating slightly different signalling pathways in Balb/CJ and C57BL/6J, which results in decompensation in Balb/CJ and compensation in C57BL/6J.

Reduced cardiac function in Balb/CJ is corroborated by the clinical finding of edema. However, the reduction of cardiac output estimated in our data does not appear severe enough to be uniformly lethal. This may indicate additional contributing mechanisms, but may equally well be hidden because of limitations inherent to the model and design. Anesthesia is necessary for the accurate measurement of mouse cardiac function with ultrasound, but anesthesia also reduces sympathetic drive and may thus mask some of the differences between the strains. Further, as the mice start to decompensate they no longer tolerate anesthesia, much like in human pulmonary hypertension (30), so there is a limit to how ill the mice can be before the number of animals lost to investigation becomes unacceptable for ethical reasons. Finally, the time resolution of our measurements, at once per day, may be too low to detect the final progression of decompensation and a continuous telemetric approach may be warranted in future studies.

In conclusion, AngII+Salt treatment causes early restriction of pulmonary flow, reduced left ventricular filling and cardiac output in male Balb/CJ, which results in fluid retention and peripheral edema. The pattern of peripheral edema, unchanged lung fluid, and diastolic left

ventricular failure is most indicative of right ventricular failure, which is consistent with pulmonary hypertension and fluid overload as the precipitating mechanisms. C57BL/6J on the other hand do not show this aggravated phenotype, indicating that there is a genetic difference between these strains, which makes Balb/CJ more sensitive to treatment with AngII+Salt. In future studies, male Balb/CJ may be used as a model to study the adaptive capacity of the heart, which may help us identify new disease mechanisms and drug targets.

Perspective and significance

Some patients are more prone to circulatory decompensation with the same injury or pathology. However, the reasons for this are not entirely clear but may include genetic predisposition, and unappreciated differences in the underlying pathophysiology. Decompensation is a complex process where cardiac overload and fluid congestion leads to progressive heart failure. The hormone angiotensin II (AngII) is known to play a role in vascular contractility, salt and fluid retention as well as cardiac contractility. This paper describes a new model of critical circulatory decompensation in Balb/CJ mice treated with a combination of AngII and high-salt diet with signs of early pulmonary flow restriction as in vasoreactive pulmonary hypertension. On the other hand, the mouse strain C57BL/6J is resistant to this treatment. As a new animal model this provides a new setting in which to study factors that predispose to pulmonary vasoconstriction, fluid retention and cardiac decompensation. In addition, the naturally occurring genetic variation between Balb/CJ and C57BL/6J may be used to identify novel genetic determinants for these phenotypes. Further, pulmonary hypertension after AngII suggests that clinical use of AngII as a vasopressor may warrant a higher level of monitoring, e.g. Swan-Ganz catheterization.

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Table legends

Table 1. Echocardiographic measurements in Balb/CJ and C57BL/6J

Echocardiographic measurements were obtained during control period (Day 0) and every day during treatment with angiotensin II and 3% sodium diet (day 1-7). AET = Left ventricle ejection time. IVCT = Left ventricle isovolumic contraction time. IVRT = Left ventricle isovolumic relaxation time. cIVRT = corrected isovolumic relaxation time. IVRT was corrected for differences in heart rate by using formula IVRT/square root of RR interval. MPI = myocardial performance index calculated from (IVRT+IVCT)/AET. MV decal T = Mitral valve deceleration time. PA Peak Vel = Pulmonary artery peak velocity. ESV = End-systolic volume. EDV = End-diastolic volume. LVPW = Left ventricle posterior wall thickness. * indicates $p < 0.05$ treatment vs. control within strain. # indicates $p < 0.05$ between strains with same treatment.

Table 2. Heart weight and lung water content after treatment with angiotensin II and 3% sodium diet

Heart weight corrected for tibia length. RV = Right ventricle. LV = Left ventricle. S = Septum. RA = Right atrium.

Figure legends

Figure 1.

Pilot study results from Balb/CJ and C57BL/6J mice treated with 1 μg/min/kg AngII and 4% sodium showing survival (A) and blood pressure measured before treatment and five weeks after treatment or before sacrifice in edematous mice (B). 10 animals were used in each group. * indicates $p < 0.05$ treatment vs. control within strains. # indicates $p < 0.05$ between strains with same treatment by two-way ANOVA.

Figure 2.

Left ventricle systolic function in Balb/CJ and C57BL/6J mice during control (day 0) and treatment with 0.5 μg/min/kg AngII and 3% sodium (AngII+Salt, day 1-7). AngII+Salt treatment reduces cardiac output (A) and stroke volume (B) in Balb/CJ over time without effecting ejection fraction (B) or heart rate (D). 8 Balb/CJ and 8 C57BL/6J mice were used in the experiment, however, the number of animals decreased after day 4 because of death or euthanasia (see Table 1). Because of low power, animals after day 5 were not included in the statistical analysis. The data were analyzed with linear mixed effects model and individual contrasts of least-squares means were adjusted using Tukey's method. # indicates $p < 0.05$ between strains with same treatment. † indicates significant strain difference and ‡ indicates significant interaction of time and strain.

Figure 3.

Left ventricle diastolic function in Balb/CJ and C57BL/6J mice during control (day 0) and treatment with 0.5 μg/min/kg AngII and 3% sodium (AngII+Salt, day 1-7). Peak E velocity (A), Peak A velocity (B) and E/A ratio (C) are decreased in treated Blab/CJ mice indicating abnormal left ventricle filling. (D) shows representative mitral valve flow velocity curve in Balb/CJ and C57BL/6J before treatment and after treatment. 8 Balb/CJ and 8 C57BL/6J mice were used in the experiment, however, the number of animals decreased after day 4 because of death or euthanasia (see Table 1). Because of low power, animals after day 5 were not included in the statistical analysis. The data were analyzed with linear mixed effects model and individual contrasts of least-squares means were adjusted using Tukey's method. * indicates $p < 0.05$ treatment vs. control within strains. # indicates $p < 0.05$ between strains with same treatment. † indicates significant strain difference and ‡ indicates significant interaction of time and strain.

Figure 4.

Pulmonary artery flow in Balb/CJ and C57BL/6J mice during control (day 0) and treatment with 0.5 μg/min/kg AngII and 3% sodium (AngII+Salt, day 1-7). Pulmonary artery acceleration time to ejection time ratio (A) is reduced in Balb/CJ at day 1, indicating increased pulmonary vascular resistance. (B) shows representative pulmonary artery flow curve in Balb/CJ and C57BL/6J before and after treatment. 8 Balb/CJ and 8 C57BL/6J mice were used in the experiment, however, the number of animals decreased after day 4 because of death or euthanasia (see Table 1). Because of low power, animals after day 5 were not included in the statistical analysis. The data were analyzed with linear mixed effects model and individual contrasts of least-squares means were adjusted using Tukey's method. * indicates $p < 0.05$ treatment vs. control within

strains. # indicates $p < 0.05$ between strains with same treatment. † indicates significant strain difference and ‡ indicates significant interaction of time and strain.

Figure 5.

Tail-cuff blood pressure in Balb/CJ and C57BL/6J mice during control (day 0) and treatment with 0.5 µg/min/kg AngII and 3% sodium (AngII+Salt, day 1-5). AngII+Salt treatment increases systolic blood pressure (A), diastolic blood pressure (B) and mean arterial blood pressure (C) in Balb/CJ over time. Heart rate is lower in Balb/CJ compared to C57BL/6J both at baseline and during treatment. 8 Balb/CJ and 8 C57BL/6J mice were used in the experiment, however, the number of animals decreased after day 4 because of death or euthanasia (see Table 1). Day 6 and 7 are not shown because of missing data in Balb/CJ during those days. The data were analyzed with linear mixed effects model and individual contrasts of least-squares means were adjusted using Tukey's method. * indicates $p < 0.05$ compared to control. # indicates $p < 0.05$ between strains with same treatment. † indicates significant strain difference and ‡ indicates significant interaction of time and strain.

Figure 6.

Fluid and sodium balance in Balb/CJ and C57BL/6J mice treated with 0.5 µg/min/kg AngII and 3% sodium (AngII+Salt). Urine (A) and sodium excretion (B) is lower in AngII+Salt treated Balb/CJ mice compared to C57BL/6J mice. Number of animals is 4-6 in each group. The data were analyzed with linear mixed effects model and individual contrasts of least-squares means

were adjusted using Tukey's method. * indicates $p < 0.05$ compared to control. # indicates $p < 0.05$ compared to C57BL/6J with same treatment.

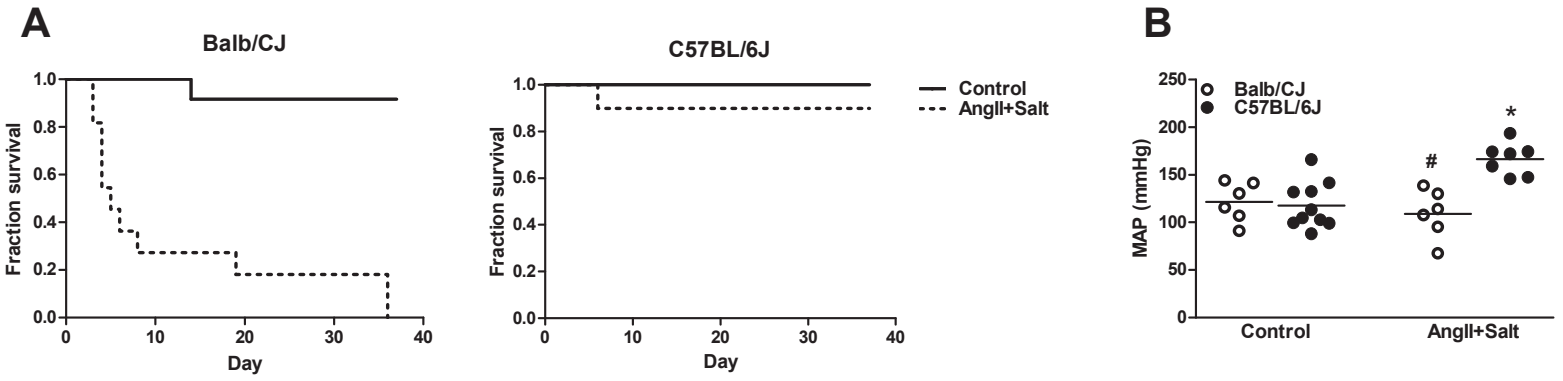
Tables

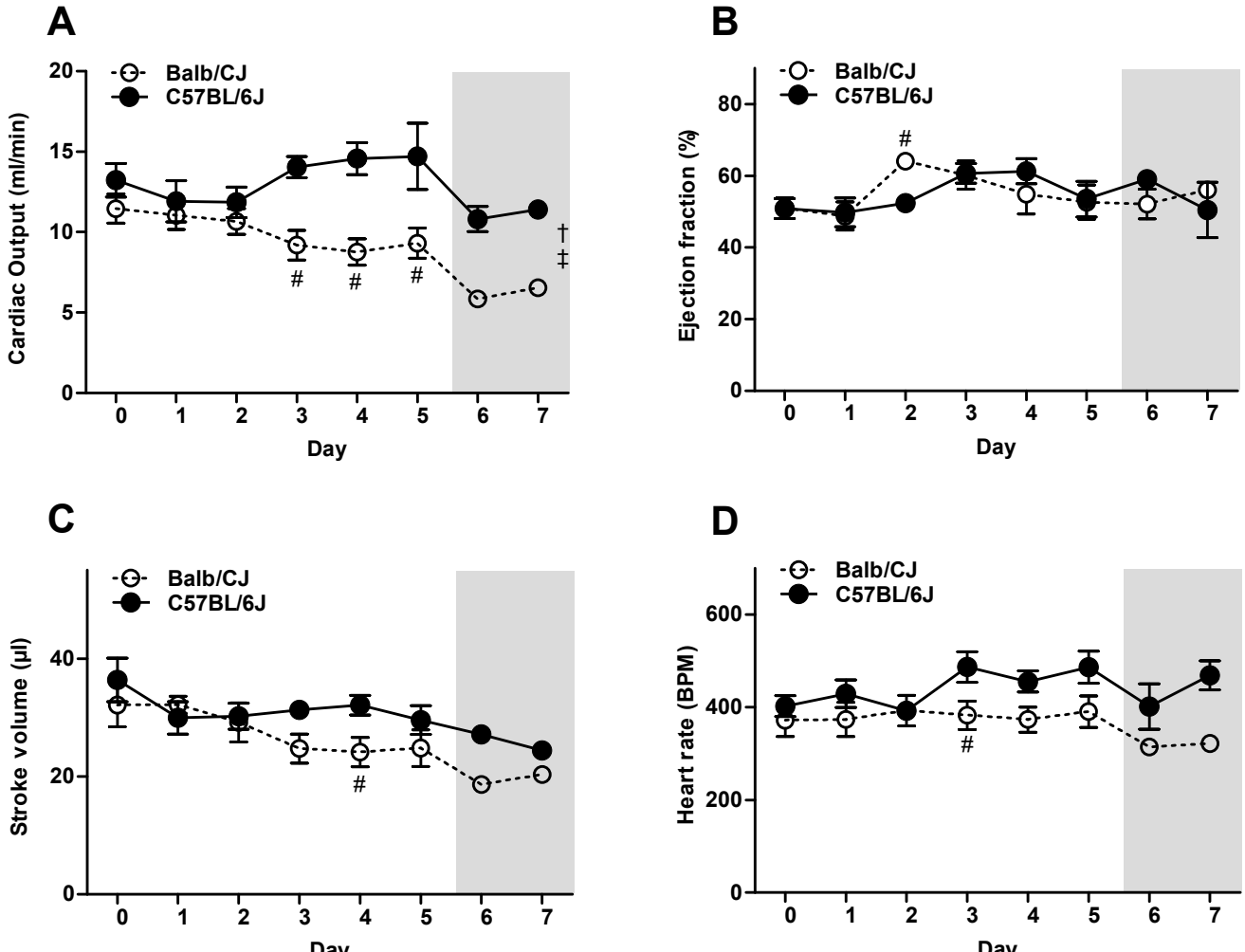
Table 1

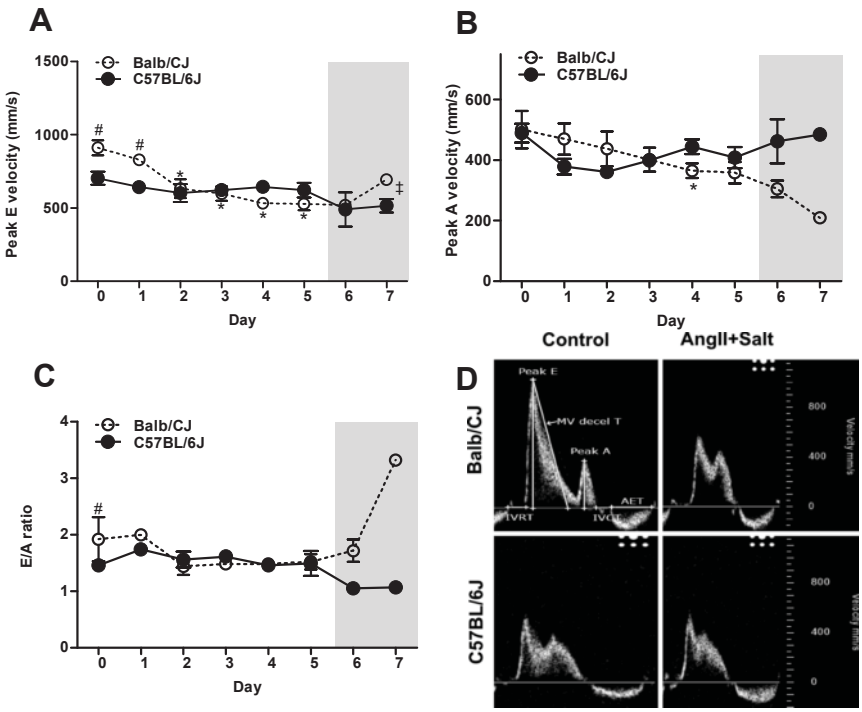
Balb/CJ	**Control**	**AngII+Salt**						
Day	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Number alive	8	8	8	8	8	6	2	1
Weight (g)	25.71±0.26	20.38±1.04*	22.55±1.21*	23.27±0.95*	23.89±1.49	23.95±1.71	20.55±0.75	17.60
Echocardiography								
AET (ms)	57.85±1.31	57.16±1.30	55.52±1.55	55.14±1.91	53.19±3.29	59.99±4.78	62.28±1.61	64.58
IVCT (ms)	21.4±1.94#	16.62±1.35#	16.31±2.19	13.77±0.98*	15.31±1.31*	17.23±0.46	17.06±2.39	23.33
IVRT (ms)	23.49±1.42	27.09±1.51	28.578±1.86	29.50±3.36	32.36±2.36#	40.06±5.67*#	40.39±0.94	39.17
cIVRT	1.75±0.09	2.07±0.10	2.06±0.10	2.09±0.11	2.38±0.17	2.85±0.44*#	2.59±0.01	2.78
MPI	0.77±0.05	0.76±0.03#	0.84±0.05	0.80±0.05	0.91±0.08#	0.95±0.03	0.92±0.00	0.97
MV decel T (ms)	36.20±2.97	42.96±4.75#	43.76±2.72	39.57±2.08#	40.46±3.52#	37.42±2.72	36.33±3.00	27.08
PA Peak Vel	645.9±44.31	684.54±25.25#	627.25±45.52	658.92±41.47	674.82±40.80	518.31±72.22#	609.23±120.77	466.98
ESV (µl)	36.61±3.39	35.67±6.09	22.38±4.02*	17.78±3.85*	22.86±5.45*	21.98±2.64	17.42±3.33	16.00
EDV (µl)	66.21±3.44	66.58±5.70	50.26±3.93*	43.75±5.52*	46.98±5.53*	46.78±3.44*	36.05±3.80	36.34
LVPW (mm)	0.66±0.03	0.60±0.05#	0.78±0.04	0.92±0.13*#	0.78±0.03	0.83±0.08	0.66±0.13	NA
C57BL/6J	**Control**	**AngII+Salt**						
Day	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Number alive	8	8	8	8	8	8	3	2
Weight (g)	28.70±1.80	22.43±0.96*	26.35±1.69*	26.92±1.40	26.38±1.08*	26.41±1.07*	24.00±0.95	22.80±1.40
Echocardiography								
AET (ms)	51.94±2.46	54.75±1.35	53.97±1.26	52.20±2.50	53.19±2.85	53.15±1.57	54.40±2.26	45.88±4.49
IVCT (ms)	16.29±2.20	21.35±1.86	18.28±2.41	15.16±1.25	13.28±0.96	17.54±1.62	18.97±1.59	16.39±0.28
IVRT (ms)	23.48±2.20	29.40±3.60	28.14±2.03	22.86±0.94	22.71±0.94	26.46±2.45	29.05±4.29	27.64±1.25
cIVRT	1.92±0.12	2.10±0.20	2.10±0.10	1.83±0.08	1.83±0.06	2.13±0.21	2.19±0.14	2.24±0.04
MPI	0.80±0.07	0.96±0.10	0.96±0.06	0.78±0.05	0.69±0.04	0.84±0.08	0.88±0.07	0.97±0.06
MV decel T (ms)	28.52±1.74	34.13±2.11	34.38±1.38	29.47±1.80	29.83±2.44	31.31±2.12	38.33	32.22
PA Peak Vel	669.92±53.50	485.37±71.97	631.94±39.31	674.71±22.41	787.21±45.56	727.57±73.37	571.65	578.78±80.93
ESV (µl)	33.39±3.23	40.65±4.47	31.23±2.76	21.06±2.30	21.23±2.95	26.50±3.62	18.77±0.51	24.87±6.71
EDV (µl)	67.15±3.84	66.71±3.87	61.60±3.82	54.85±2.00	53.33±3.39*	56.05±3.38	45.87±0.88	49.28±5.80
LVPW (mm)	0.49±0.05	0.57±0.03	0.63±0.01	0.68±0.05	0.77±0.07	0.86±0.03*	0.54	NA

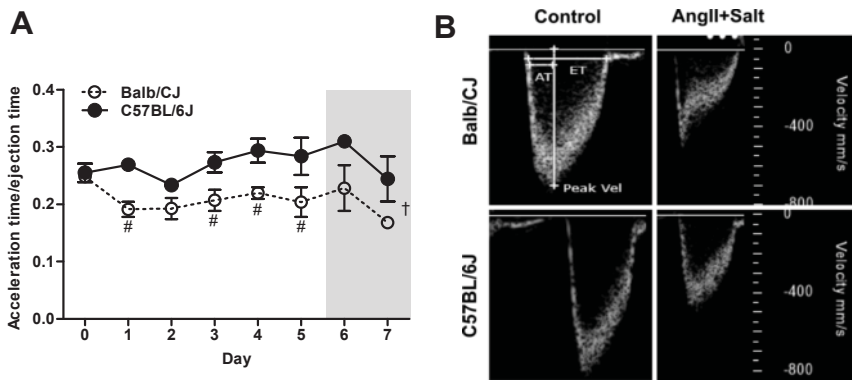
Table 2

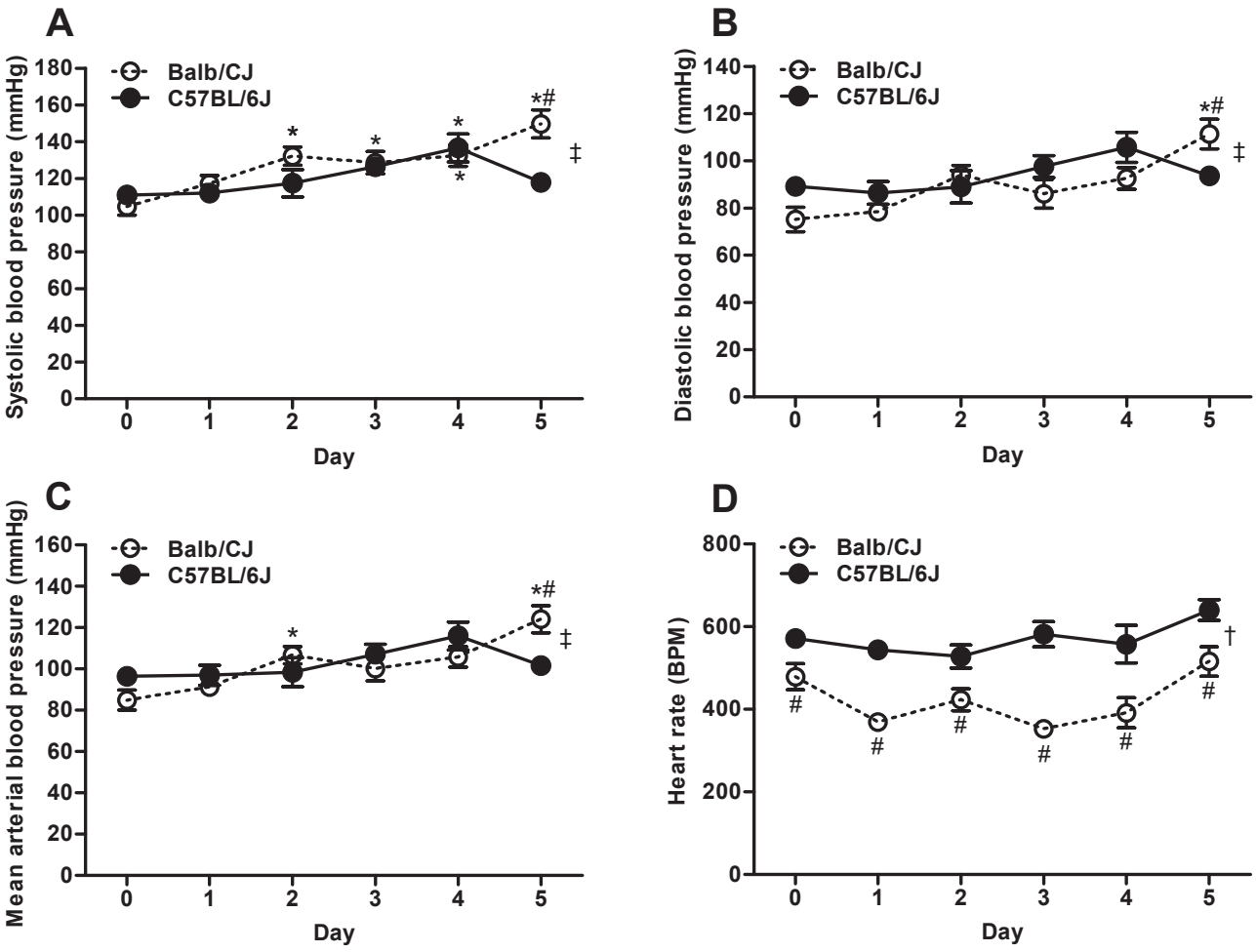
	Balb/CJ	C67BL/6J
Heart weight (mg)	80.90±4.75	72.42±2.84
Left atria weight (mg)	5.97±1.80	2.86±0.24
Right atria weight (mg)	10.52±3.49	3.34±0.61
Left ventricle weight (mg)	53.03±2.38	56.23±2.99
Right ventricle weight (mg)	12.18±0.70	12.14±1.10
RV/(LV+S)	0.23±0.02	0.21±0.02
RA/RV	0.86±0.33	0.29±0.06
Lung water content (%)	78.46±1.62	76.55±0.46

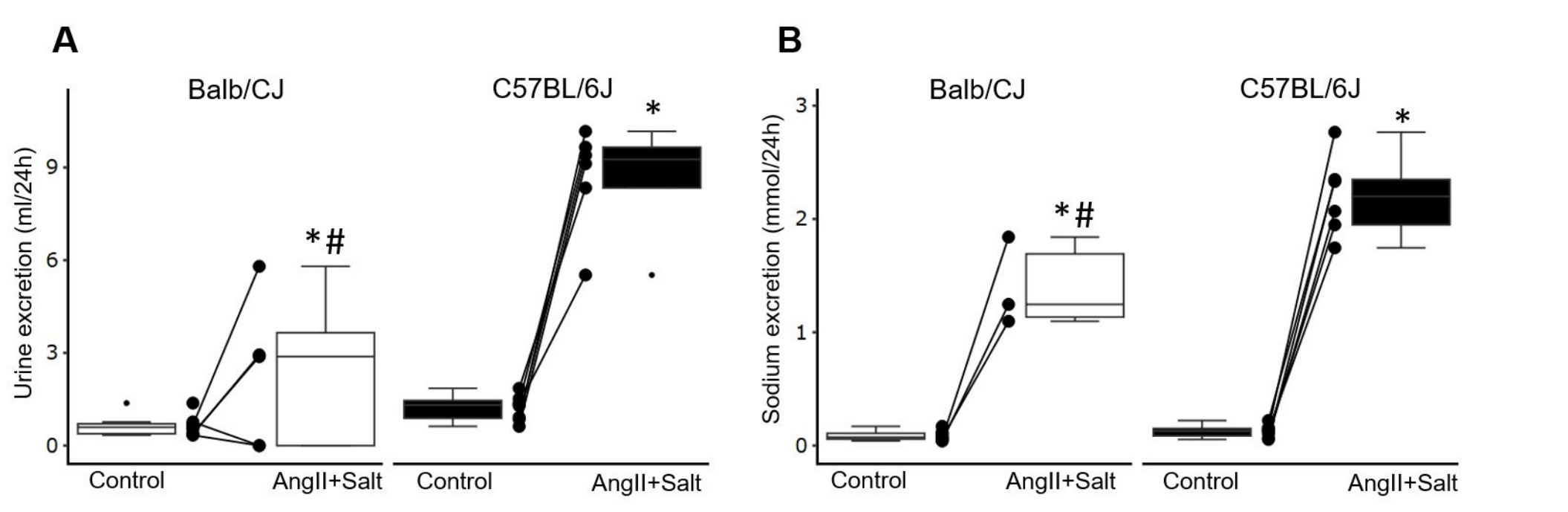

A
Balb/CJ
C57BL/6J
Fraction survival
Day
Control
AngII+Salt
B
Balb/CJ
C57BL/6J
MAP (mmHg)
Control
AngII+Salt
#
*


A
Balb/CJ
C57BL/6J
Cardiac Output (ml/min)
Day
B
Balb/CJ
C57BL/6J
Ejection fraction (%)
Day
C
Balb/CJ
C57BL/6J
Stroke volume (µl)
Day
D
Balb/CJ
C57BL/6J
Heart rate (BPM)
Day


A
Balb/CJ
C57BL/6J
Peak E velocity (mm/s)
1500
1000
500
0
0 1 2 3 4 5 6 7
Day
B
Balb/CJ
C57BL/6J
Peak A velocity (mm/s)
600
400
200
0
0 1 2 3 4 5 6 7
Day
C
Balb/CJ
C57BL/6J
E/A ratio
4
3
2
1
0
0 1 2 3 4 5 6 7
Day
Control
AngII+Salt
D
Balb/CJ
C57BL/6J
Peak E
MV decel T
Peak A
AET
IVRT
IVCT
800
400
0
Velocity mm/s


A
Balb/CJ
C57BL/6J
Acceleration time/ejection time
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
Day
#
#
#
#
†
B
Control
AngII+Salt
Balb/CJ
C57BL/6J
AT
ET
Peak Vel
0
-400
-800
Velocity mm/s


A
Balb/CJ
C57BL/6J
Systolic blood pressure (mmHg)
180
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5
Day
*
*
*
*#
‡
B
Balb/CJ
C57BL/6J
Diastolic blood pressure (mmHg)
140
120
100
80
60
40
20
0
0 1 2 3 4 5
Day
*#
‡
C
Balb/CJ
C57BL/6J
Mean arterial blood pressure (mmHg)
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5
Day
*
*#
‡
D
Balb/CJ
C57BL/6J
Heart rate (BPM)
800
600
400
200
0
0 1 2 3 4 5
Day
#
#
#
#
#
#
†


A
Balb/CJ
C57BL/6J
Urine excretion (ml/24h)
0
3
6
9
*#
*
Control
AngII+Salt
Control
AngII+Salt
B
Balb/CJ
C57BL/6J
Sodium excretion (mmol/24h)
0
1
2
3
*#
*
Control
AngII+Salt
Control
AngII+Salt