

**GLUCOCORTICOID-INDUCED HYPERTENSION  
AND CARDIAC INJURY:  
EFFECTS OF MINERALOCORTICOID AND  
GLUCOCORTICOID RECEPTOR ANTAGONISM**

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ABSTRACT

Glucocorticoids are widely administered for the treatment of various disorders, although their long-term use results in adverse effects associated with glucocorticoid excess. We investigated the pathophysiological roles of glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) in the cardiac changes induced by exogenous corticosterone in rats. Corticosterone or vehicle was injected twice daily in rats from 8 to 12 weeks of age. The effects of the GR antagonist RU486, the MR antagonist spironolactone, or both agents on corticosterone action were also determined. Corticosterone induced hypertension, left ventricular (LV) fibrosis, and LV diastolic dysfunction. Neither RU486 nor spironolactone affected corticosterone-induced hypertension, whereas spironolactone, but not RU486, attenuated the effects of corticosterone on LV fibrosis and diastolic function. Corticosterone also increased cardiac oxidative stress and inflammation in a manner sensitive to spironolactone but not to RU486. The corticosterone-induced LV atrophy was not affected by either RU486 or spironolactone. Our results implicate MRs in the cardiac fibrosis and diastolic dysfunction, but not MRs or GRs in the cardiac atrophy, induced by corticosterone. Neither MRs nor GRs appear to contribute to corticosterone-induced hypertension.

Key Words: glucocorticoids, hypertension, cardiac atrophy, diastolic dysfunction

INTRODUCTION

Glucocorticoids are used widely for the treatment of patients with various conditions including

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autoimmune and allergic diseases as well as lymphoproliferative disorders.<sup>1</sup> Although exogenous glucocorticoids normally act as potent anti-inflammatory agents, their long-term administration results in several adverse effects associated with glucocorticoid excess.<sup>2</sup> Such glucocorticoid excess is manifested by a variety of symptoms and signs, including central obesity with moon face, osteoporosis, myopathy, and cardiovascular disorders such as hypertension and atherosclerosis.<sup>3</sup> Cardiovascular complications are among the more important such manifestations for predicting the morbidity and mortality of individuals with glucocorticoid excess,<sup>3</sup> with glucocorticoid treatment also having been associated with heart failure.<sup>4</sup>

Both the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) are widely expressed in the cardiovascular system, including the arterial wall and the myocardium, with glucocorticoids acting directly to maintain vascular tone and to modify vascular inflammatory, proliferative, and remodeling responses to injury.<sup>5,6</sup> Corticosterone, the endogenous glucocorticoid of rodents, manifests the same affinity for the MR as does aldosterone and is present in blood at concentrations two orders of magnitude as great as those of aldosterone.<sup>7</sup> Whereas endogenous glucocorticoids bind both the MR and the GR *in vivo*, aldosterone binds specifically to the MR.

Many pathological states characterized by muscle atrophy are associated with an increase in circulating glucocorticoid levels, suggesting that glucocorticoids might trigger such atrophy.<sup>8</sup> Indeed, administration of high doses of glucocorticoids induces muscular atrophy in humans and animals.<sup>9,10</sup> Studies have suggested that glucocorticoids inhibit protein synthesis and stimulate protein degradation in skeletal muscle.<sup>11,12</sup> An effect of glucocorticoids on the breakdown of specific skeletal muscle proteins has been described in some<sup>13</sup> but not all<sup>14</sup> studies. Administration of high doses of glucocorticoids to animals induces not only a reduction in muscle mass but also muscle dysfunction.<sup>15</sup> The molecular mechanisms by which glucocorticoid excess induces cardiovascular injury have remained unclear, however.

We have now investigated the effects of exogenous corticosterone on blood pressure as well as on cardiac remodeling and function in rats, and we have explored the pathophysiological roles of GRs and MRs in such effects.

## METHODS

### *Animals and experimental protocols*

Male inbred Sprague-Dawley (SD) rats were obtained from Japan SLC (Hamamatsu, Japan) and were handled in accordance with the guidelines of Nagoya University Graduate School of Medicine as well as with the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996). The animals were fed normal laboratory chow containing 0.36% NaCl after 6 weeks of age, and both the diet and tap water were provided *ad libitum* throughout the experimental period. At 8 weeks, the rats were randomly allocated to four groups: (1) the CTC group ( $n = 6$ ), in which they were administered corticosterone (Sigma, St. Louis, MO, USA) at a dose of 20 mg per kilogram of body weight per day; (2) the CTC+RU group ( $n = 6$ ), in which they were administered both corticosterone (20 mg/kg per day) and RU486 (Sigma) at 2 mg per day; (3) the CTC+SPL group ( $n = 6$ ), in which they were administered both corticosterone (20 mg/kg per day) and spironolactone (Sigma) at 20 mg/kg per day; and (4) the CTC+RU+SPL group ( $n = 8$ ), in which they were administered corticosterone plus RU486 plus spironolactone (at the same doses as in the other groups). Corticosterone was injected *s.c.* twice a day, RU486 was injected *s.c.* once a day, and spironolactone was administered orally via a gastric tube once a day, with all drugs being given to the animals from 8 to 12 weeks of age. The doses of corticosterone, RU486, and spironolactone were determined on the basis of the results of

previous studies.<sup>16-18)</sup> Untreated SD rats fed normal laboratory chow after 6 weeks of age remain normotensive, and such animals administered the vehicle for corticosterone served as age-matched controls (CNT group,  $n = 6$ ). At 12 weeks, all animals were anesthetized by i.p. injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) and were subjected to echocardiographic analysis. The heart was subsequently excised, and left ventricular (LV) tissue was separated for analysis.<sup>19)</sup>

#### *Corticosterone preparation*

Corticosterone was reconstituted from a vial containing 500 mg of lyophilized powder with 1 mL of ethanol followed by 24 mL of polyethylene glycol 400 (Sigma) to yield a suspension of 20 mg/mL.<sup>16)</sup>

#### *Blood pressure measurement and echocardiographic analysis*

Systolic blood pressure (SBP) and heart rate were measured weekly in conscious animals by tail-cuff plethysmography (BP-98A; Softron Co., Tokyo, Japan). At 12 weeks of age, rats were subjected to transthoracic echocardiography as described previously.<sup>20)</sup> In brief, M-mode echocardiography was performed with a 12.5-MHz transducer (Aplio SSA-700A; Toshiba Medical Systems, Tochigi, Japan). LV end-diastolic (LVDd) and end-systolic (LVDs) dimensions as well as the thickness of the interventricular septum (IVST) and LV posterior wall (LVPWT) were measured, and fractional shortening (FS) was calculated as:  $[(LVDd - LVDs)/LVDd] \times 100\%$ . For assessment of Doppler-derived indices of LV function, both LV inflow and outflow velocity patterns were simultaneously recorded by pulsed-wave Doppler echocardiography. For assessment of LV diastolic function, we calculated the peak flow velocities at the mitral level during rapid filling (E) and during atrial contraction (A), the E/A ratio, the isovolumic relaxation time (IRT), and the deceleration time (DcT). Both the isovolumic contraction time (ICT) and ejection time (ET) were also determined, and the Tei index, which reflects both LV diastolic and systolic function, was calculated as follows:  $Tei\ index = (ICT+IRT)/ET$ .<sup>18)</sup>

#### *Histology and immunohistochemistry*

LV tissue was fixed in ice-cold 4% paraformaldehyde for 48 to 72 h, embedded in paraffin, and processed for histology as described.<sup>21)</sup> To evaluate macrophage infiltration into the myocardium, we performed immunostaining for the monocyte-macrophage marker CD68 with frozen sections (thickness, 5  $\mu$ m) that had been fixed with acetone. Endogenous peroxidase activity was blocked by exposure of the sections to methanol containing 0.3% hydrogen peroxide. Sections were incubated at 4°C first overnight with mouse monoclonal antibodies to CD68 (clone ED1, diluted 1:100; Chemicon, Temecula, CA, USA) and then for 30 min with Histofine Sample Stain Rat MAX PO (Nichirei Biosciences, Tokyo, Japan). Immune complexes were visualized with diaminobenzidine and hydrogen peroxide, and the sections were counterstained with hematoxylin. Image analysis was performed with NIH Scion Image software (Scion, Frederick, MD, USA).

#### *Superoxide production*

NADPH-dependent superoxide production by homogenates prepared from freshly frozen LV tissue was measured with an assay based on lucigenin-enhanced chemiluminescence as described previously.<sup>22)</sup> The chemiluminescence signal was sampled every minute for 10 min with a microplate reader (WALLAC 1420 ARVO MX/Light; Perkin Elmer, Waltham, MA, USA), and the respective background counts were subtracted from experimental values. Lucigenin chemiluminescence was expressed as relative light units per milligram of protein. Superoxide production in tissue sections was examined with the use of dihydroethidium (Sigma) as described.<sup>23)</sup> Dihydroethidium is rapidly oxidized by superoxide to yield fluorescent ethidium, and the sections

were examined with a fluorescence microscope equipped with a 585-nm long-pass filter. As a negative control, we performed staining with dihydroethidium after incubation of sections with superoxide dismutase (300 U/mL) and confirmed that this procedure abolished the fluorescence (date not shown). The average of dihydroethidium fluorescence intensity values was calculated with the use of NIH Image software (ImageJ).<sup>24)</sup>

#### *Quantitative RT-PCR analysis*

Total RNA was extracted from LV tissue and treated with DNase with the use of a spin-vacuum isolation kit (Promega, Madison, WI, USA). Complementary DNA was synthesized from 2 µg of total RNA by reverse transcription (RT) with random primers (Invitrogen, Carlsbad, CA, USA) and MuLV Reverse Transcriptase (Applied Biosystems, Foster City, CA, USA). Real-time polymerase chain reaction (PCR) analysis was performed as previously described<sup>25)</sup> with a Prism 7700 Sequence Detector (Perkin Elmer) and with primers and TaqMan probes specific for cDNAs encoding insulin-like growth factor-1 (IGF-1: 5'-GACCAAGGGGCTTTACTTCAAC-3', 5'-CCGGAAGCAACTCATCCAC-3', and 5'-CCGTCTGTGGTGCCCTCGAATGC-3' as the forward primer, reverse primer, and TaqMan probe, respectively; GenBank accession no. NM\_001082477), myostatin (5'-GACAGTGAGAGAGAGGGCAATG-3', 5'-TGGCTTCTATTCTGGAGTACCTTG-3', and 5'-TCTCCACGCACACGCATTACACAGCC-3', respectively; NM\_019151), FOXO1 (5'-CATACCAAGGCCATCGAGAG-3', 5'-CACTCTTCACCATCCACTCGTAG-3', and 5'-CGGAGAAGAGGGCTCACCTGTGCGCA-3', respectively; XM\_342244), FOXO3 (5'-AGACCAGCCACCTTTTCTTCC-3', 5'-CTGAGCGAGTCCGAAGTGAG-3', and 5'-TCGCACTACGGCAACCAGACTCCA-3', respectively; NM\_001106395), atrogen-1 (5'-CCGGCCTTCAAAGGTCTCAC-3', 5'-CGCTCAGCCTCTGCATGATG-3', and 5'-ACCGACCTGCCTGTGTGCTTACAAGT-3', respectively; NM\_133521), muscle ring finger-1 (MuRF-1: 5'-GCCATCCTGGACGAGAAGAAG-3', 5'-GATCAGGGCCTCGATGAAGTC-3', and 5'-CTCCTCCTGCTCCTGAGTGATCCGCTG-3', respectively; NM\_080903), transforming growth factor-β1 (TGF-β1),<sup>20)</sup> connective tissue growth factor (CTGF),<sup>22)</sup> collagen type I,<sup>26)</sup> and monocyte chemoattractant protein-1 (MCP-1).<sup>22)</sup> Reagents for the detection of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (Applied Biosystems) were used to quantify rat GAPDH mRNA as an internal standard.

#### *Statistical analysis*

Data are presented as means ± SEM. Differences among groups of rats at 12 weeks of age were assessed by one-way factorial analysis of variance (ANOVA); if a significant difference was detected, intergroup comparisons were performed with the Fisher multiple-comparison test. The time course of SBP was compared among groups by two-way repeated-measures ANOVA. A *P* value of <0.05 was considered statistically significant.

## RESULTS

#### *Physiological analysis*

Body weight of rats was significantly smaller in the CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups than in the CNT group at 12 weeks of age (Table 1). Tibial length was shorter in CTC rats than in CNT rats, and this effect of corticosterone was prevented by coadministration of RU486 either alone or together with spironolactone, suggesting that inhibition of bone growth by corticosterone was mediated via GRs. Whereas CNT rats maintained a normal SBP throughout the experimental period, CTC rats showed a substantial increase in SBP at 9

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weeks of age and thereafter. This increase in SBP apparent in CTC rats was not affected by treatment with RU486, spironolactone, or both agents (Fig. 1, Table 1). Heart rate was similar among the five experimental groups (Table 1). LV weight and the ratio of LV weight to tibial length were significantly decreased in CTC rats compared with CNT rats, and these differences were not affected by treatment with RU486 or spironolactone alone or together (Table 1). The ratio of LV weight to right ventricular weight was similar among the five experimental groups.

#### Cardiac remodeling, function, and gene expression

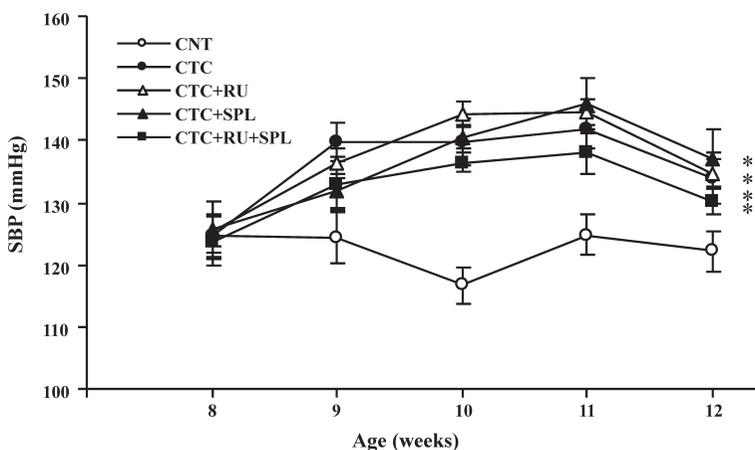
IVST, LVDD, LVDs, and LVPWT were significantly smaller and FS was significantly larger in CTC rats than in CNT rats, and these effects of corticosterone were not modified by treatment with RU486, spironolactone, or both drugs (Table 2). E/A was decreased and IRT and DcT were increased in CTC rats compared with CNT rats (Table 2). These changes of indices of LV relaxation were significantly altered in CTC+SPL and CTC+RU+SPL.

Microscopic analysis revealed that the cross-sectional area of cardiac myocytes was smaller in CTC rats than in CNT rats and that this effect of corticosterone was not influenced by

**Table 1** Physiological parameters for SD rats in the five experimental groups at 12 weeks of age.

Parameter	CNT	CTC	CTC+RU	CTC+SPL	CTC+RU+SPL
BW (g)	381.3 ± 7.2	303.8 ± 4.7*	322.7 ± 8.4*	301.3 ± 9.0*	320.0 ± 3.7*
TL (mm)	37.1 ± 0.4	35.3 ± 0.3*	36.6 ± 0.3†	36.0 ± 0.3*	36.4 ± 0.2†
SBP (mmHg)	122 ± 3.2	134.0 ± 4.2*	134.8 ± 2.3*	137.2 ± 4.6*	130.2 ± 2.0*
HR (bpm)	333.9 ± 11.6	355.4 ± 16.3	339.0 ± 11.5	348.2 ± 15.5	351.0 ± 8.4
LVW (mg)	754.5 ± 21.9	624.5 ± 11.7*	652.7 ± 18.0*	625.5 ± 21.5*	651.5 ± 15.6*
LVW/TL (mg/mm)	20.3 ± 0.5	17.5 ± 0.4*	17.8 ± 0.4*	17.4 ± 0.5*	18.0 ± 0.4*
LVW/RVW	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.03	4.1 ± 0.1	4.0 ± 0.05

Abbreviations not defined in text: BW, body weight; TL, tibial length; HR, heart rate; LVW, left ventricular weight; RVW, right ventricular weight. Data are means ± SEM ( $n = 6, 6, 6, 6,$  and  $8$  for CNT, CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups, respectively). \* $P < 0.05$  versus CNT group; † $P < 0.05$  versus CTC group.



**Fig. 1** Time course of SBP in SD rats of the five experimental groups. Data are means ± SEM ( $n = 6, 6, 6,$  and  $8$  for CNT, CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups, respectively). \* $P < 0.05$  versus CNT group.

**Table 2** Cardiac morphological and functional parameters for SD rats in the five experimental groups at 12 weeks of age.

Parameter	CNT	CTC	CTC+RU	CTC+SPL	CTC+RU+SPL
IVST (mm)	1.66 ± 0.07	1.42 ± 0.04*	1.37 ± 0.05*	1.38 ± 0.04*	1.39 ± 0.03*
LVDd (mm)	8.44 ± 0.32	7.29 ± 0.21*	7.55 ± 0.24*	7.43 ± 0.27*	7.18 ± 0.18*
LVDs (mm)	5.22 ± 0.41	3.64 ± 0.19*	4.11 ± 0.30*	3.78 ± 0.30*	3.81 ± 0.18*
LVPWT (mm)	1.64 ± 0.06	1.38 ± 0.04*	1.34 ± 0.04*	1.37 ± 0.04*	1.40 ± 0.03*
FS (%)	38.56 ± 2.89	50.23 ± 1.42*	45.99 ± 2.44*	49.38 ± 2.63*	47.14 ± 1.42*
E/A	1.90 ± 0.07	1.59 ± 0.06*	1.60 ± 0.07*	1.88 ± 0.05†	1.90 ± 0.06†‡
IRT (ms)	24.5 ± 1.3	35.2 ± 3.1*	33.7 ± 2.0*	27.8 ± 2.5†	26.7 ± 1.5†‡
DcT (ms)	32.4 ± 1.8	40.6 ± 1.2*	39.1 ± 1.4*	33.8 ± 1.3†	34.1 ± 0.6†‡
Tei index	0.47 ± 0.02	0.72 ± 0.06*	0.63 ± 0.04*	0.52 ± 0.06†	0.48 ± 0.03†‡

Data are means ± SEM ( $n = 6, 6, 6,$  and  $8$  for CNT, CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups, respectively). \* $P < 0.05$  versus CNT group; † $P < 0.05$  versus CTC group; ‡ $P < 0.05$  versus CTC+RU group.

treatment with RU486 and/or spironolactone (Fig. 2A, B). The abundance of IGF-1 mRNA in LV tissue, which correlates with myocardial growth, was reduced by corticosterone in a manner sensitive to RU486 but not to spironolactone (Fig. 2C). The amounts of mRNAs for myostatin, FOXO1, FOXO3, atrogin-1, and MuRF-1, all of which negatively regulate myocardial growth, were increased in CTC rats compared with CNT rats, and these effects of corticosterone were inhibited in both CTC+RU and CTC+RU+SPL rats but not in CTC+SPL rats (Fig. 2D–H).

Azan-Mallory staining revealed that fibrosis in perivascular and interstitial regions of the LV myocardium was increased in CTC rats compared with CNT rats, and that this effect of corticosterone was abolished in both CTC+SPL and CTC+RU+SPL rats but not in CTC+RU rats (Fig. 3A–C). The amounts of mRNAs for collagen type I, TGF- $\beta$ 1, and CTGF in LV tissue were also increased by corticosterone, and these effects were attenuated by spironolactone but not by RU486 (Fig. 3D–F).

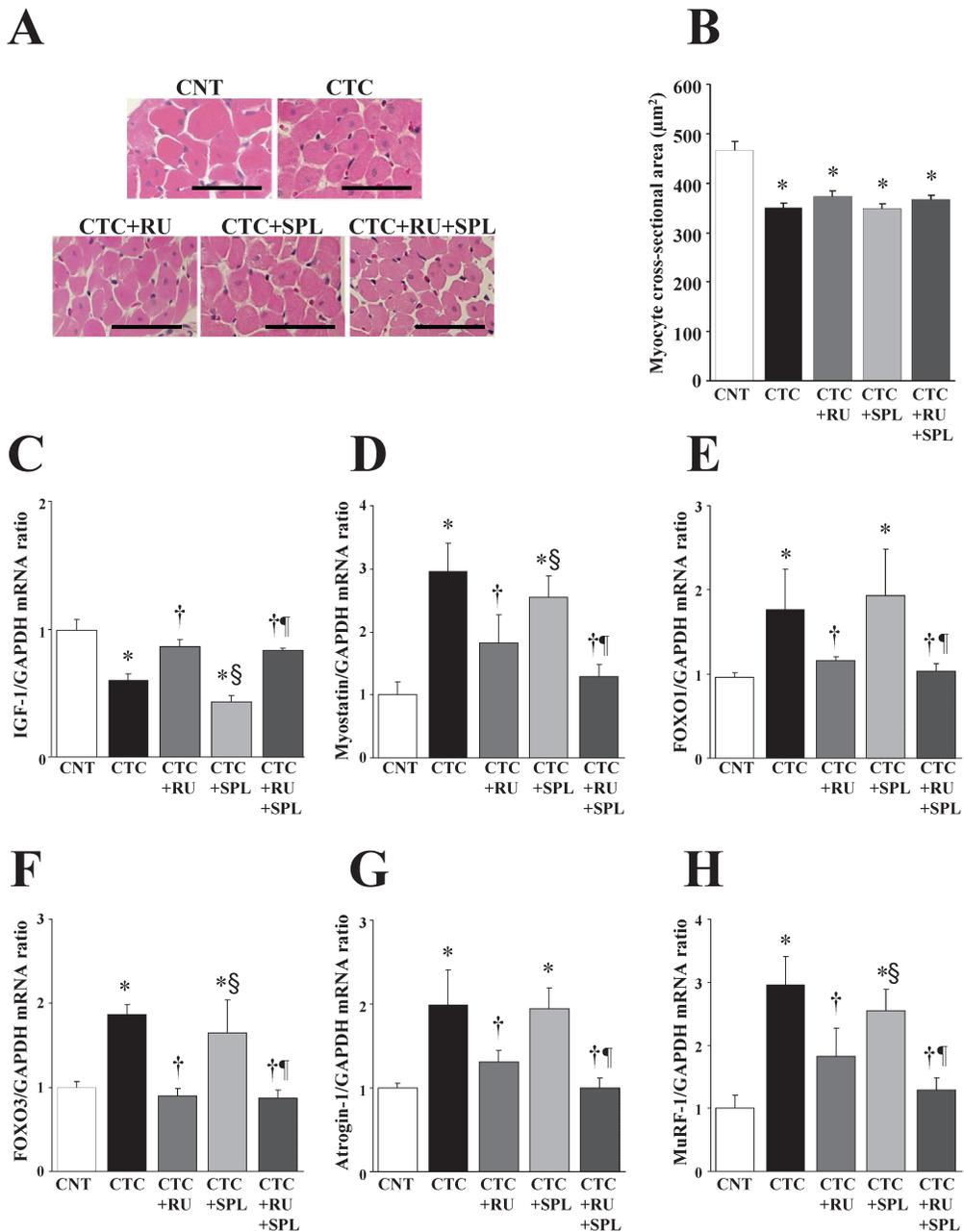
#### *Cardiac oxidative stress and inflammation*

Superoxide production in LV tissue sections, as revealed by staining with dihydroethidium, as well as NADPH oxidase activity in LV homogenates were increased for CTC rats compared with CNT rats, and these effects of corticosterone were abolished in both CTC+SPL and CTC+RU+SPL rats but not in CTC+RU rats (Fig. 4).

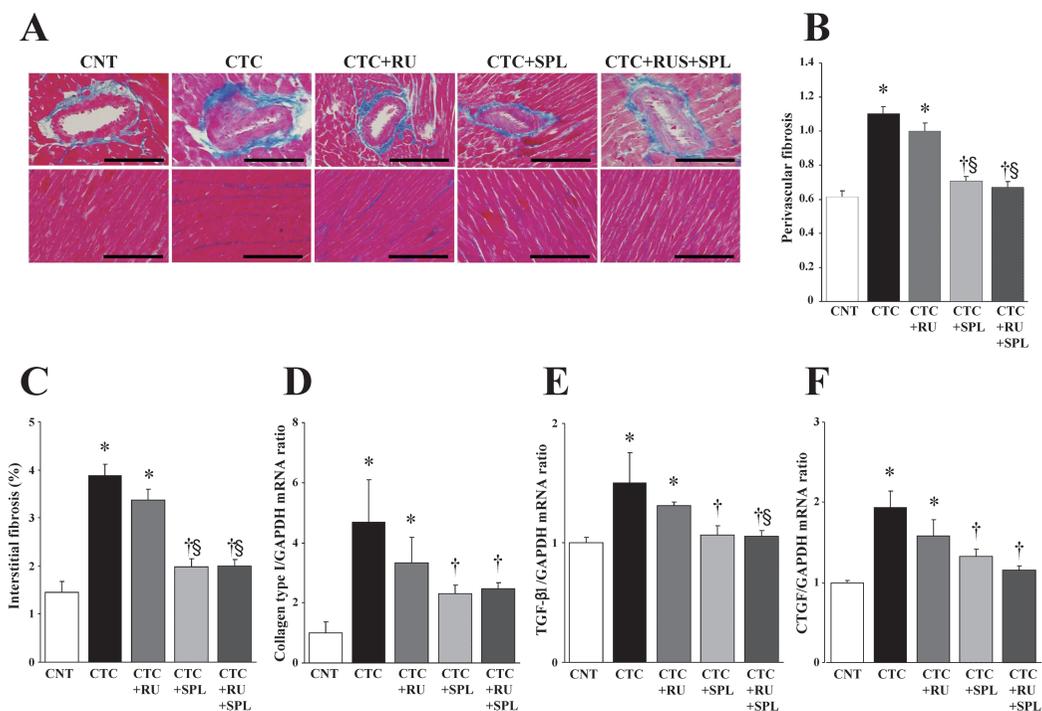
Immunostaining of the LV myocardium for the monocyte-macrophage marker CD68 revealed that corticosterone increased the number of CD68-positive cells in a manner sensitive to coadministration of spironolactone but not to that of RU486 (Fig. 5A, B). In addition, expression of the MCP-1 gene in the left ventricle was increased in CTC rats compared with CNT rats, and this effect of corticosterone was inhibited in both CTC+SPL and CTC+RU+SPL rats but not in CTC+RU rats (Fig. 5C).

## DISCUSSION

We have shown that exogenous corticosterone induced hypertension and LV diastolic dysfunction as well as LV atrophy and fibrosis in SD rats. Spironolactone attenuated the effects of corticosterone on LV fibrosis and diastolic dysfunction, as well as blocked increases in the levels of cardiac oxidative stress and inflammation induced by the glucocorticoid, without lowering blood



**Fig. 2** Cardiomyocyte size and expression of fetal-type cardiac genes in the left ventricle of SD rats in the five experimental groups at 12 weeks of age. (A) Hematoxylin-eosin staining of transverse sections of the LV myocardium. Scale bars, 50  $\mu$ m. (B) Cross-sectional area of cardiac myocytes determined from sections similar to those in (A). (C–H) Quantitative RT-PCR analysis of IGF-1, myostatin, FOXO1, FOXO3, atrogin-1, and MuRF-1 mRNAs, respectively, in LV tissue. The amount of each mRNA was normalized by that of GAPDH mRNA and then expressed relative to the mean value for the CNT group. Data in (B) through (H) are means  $\pm$  SEM ( $n = 6, 6, 6, 6,$  and  $8$  for CNT, CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups, respectively). \* $P < 0.05$  versus CNT group; † $P < 0.05$  versus CTC group; ‡ $P < 0.05$  versus CTC+RU group; § $P < 0.05$  versus CTC+SPL group.

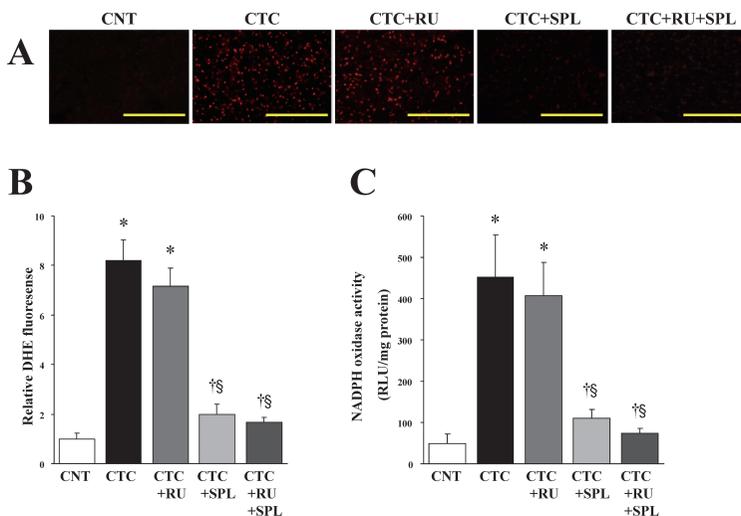


**Fig. 3** Cardiac fibrosis and related gene expression in the left ventricle of SD rats in the five experimental groups at 12 weeks of age. (A) Collagen deposition as revealed by Azan-Mallory staining in perivascular (upper panels) or interstitial (lower panels) regions of the LV myocardium. Scale bars, 200  $\mu$ m. (B, C) Relative extents of perivascular and interstitial fibrosis, respectively, in the LV myocardium as determined from sections similar to those in (A). (D–F) Quantitative RT-PCR analysis of collagen type I, TGF- $\beta$ 1, and CTGF mRNAs, respectively, in LV tissue. The amount of each mRNA was normalized by that of GAPDH mRNA and then expressed relative to the mean value for the CNT group. Data in (B) through (F) are means  $\pm$  SEM ( $n = 6, 6, 6, 6,$  and  $8$  for CNT, CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups, respectively). \* $P < 0.05$  versus CNT group; † $P < 0.05$  versus CTC group; ‡ $P < 0.05$  versus CTC+RU group.

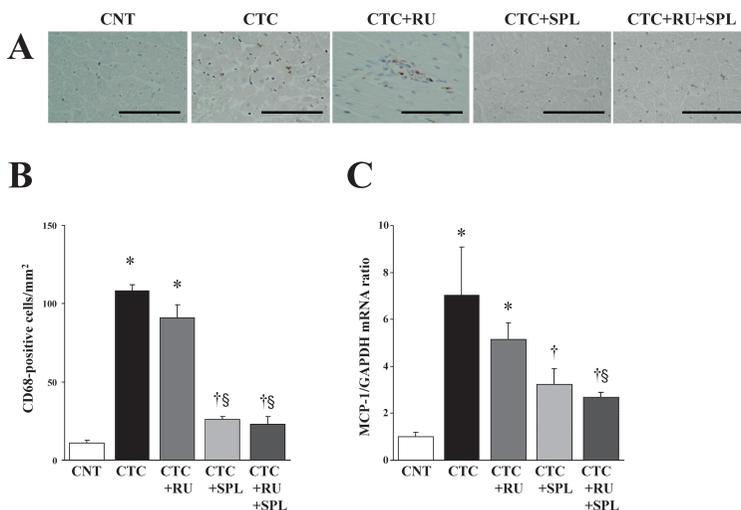
pressure. These data suggest that exogenous glucocorticoids activate cardiac MRs and thereby promote cardiac fibrosis and diastolic dysfunction in this setting. In contrast, LV atrophy was not affected by RU486 or spironolactone alone or together, although the effects of corticosterone on the expression of IGF-1 and atrophy-related genes in the heart were attenuated by RU486 alone and together. These results suggest that cardiac GRs are implicated in the expression of such genes, but that GR signaling pathways may not be important in the development of glucocorticoid-induced cardiac atrophy.

Although cortisol, the major glucocorticoid in humans, has a plethora of effects on the brain, heart, blood vessels, kidney, and body fluid compartments, the precise mechanism by which it increases blood pressure is unclear.<sup>27)</sup> Treatment with corticosterone markedly increased SBP in SD rats in the present study, and this effect was not attenuated by coadministration of RU486 and/or spironolactone. These data suggest that MRs and GRs do not mediate corticosterone-induced hypertension in SD rats. They are also consistent with previous results showing that spironolactone or RU486 did not modify cortisol- or adrenocorticotropic hormone-induced hypertension despite demonstrable anti-mineralocorticoid or anti-glucocorticoid actions in humans<sup>28)</sup> or rats.<sup>29)</sup>

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**Fig. 4** Superoxide production and NADPH oxidase activity in the left ventricle of SD rats in the five experimental groups at 12 weeks of age. **(A)** Superoxide production in interstitial regions of the LV myocardium as revealed by dihydroethidium staining. Scale bars, 200  $\mu$ m. **(B)** Dihydroethidium (DHE) fluorescence intensity determined from sections similar to those in **(A)**. Data are expressed relative to the value for the CNT group. **(C)** NADPH-dependent superoxide production in LV homogenates expressed as relative light units (RLU) per milligram of protein. Data in **(B)** and **(C)** are means  $\pm$  SEM ( $n = 6, 6, 6, 6,$  and  $8$  for CNT, CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups, respectively). \* $P < 0.05$  versus CNT group; † $P < 0.05$  versus CTC group; ‡ $P < 0.05$  versus CTC+RU group.



**Fig. 5** Macrophage infiltration and MCP-1 gene expression in the left ventricle of SD rats in the five experimental groups at 12 weeks of age. **(A)** Immunohistochemical analysis of LV tissue sections with antibodies to the monocyte-macrophage marker CD68. Scale bars, 200  $\mu$ m. **(B)** Density of CD68-positive cells determined from sections similar to those in **(A)**. **(C)** Quantitative RT-PCR analysis of MCP-1 mRNA in LV tissue. Results were normalized by the amount of GAPDH mRNA and then expressed relative to the mean value for the CNT group. Data in **(B)** and **(C)** are means  $\pm$  SEM ( $n = 6, 6, 6, 6,$  and  $8$  for CNT, CTC, CTC+RU, CTC+SPL, and CTC+RU+SPL groups, respectively). \* $P < 0.05$  versus CNT group; † $P < 0.05$  versus CTC group; ‡ $P < 0.05$  versus CTC+RU group.

Oxidative stress and deficiency of nitric oxide have recently been implicated in the pathogenesis of glucocorticoid-induced hypertension.<sup>30)</sup> Indeed, glucocorticoids have various effects on the nitric oxide synthase (NOS) pathway, including effects on L-arginine availability, down-regulation of inducible NOS and endothelial NOS gene expression, reduction of cofactor availability, and NOS uncoupling.<sup>31,32)</sup> Vascular inflammation and oxidative stress, induced independently of MRs and GRs, may therefore have contributed to the glucocorticoid-induced hypertension observed in the present study.

Glucocorticoids have divergent effects and the mechanisms of glucocorticoid-induced muscle atrophy still remain unclear. A new scenario emerges that considers the size of the myofiber and muscle performance to be a result not of a single pathway but of a network of signaling.<sup>33)</sup> In the present study, corticosterone-treated rats developed cardiac (and cardiomyocyte) atrophy despite the presence of sustained LV pressure overload, and this effect was not inhibited by treatment with RU486 and/or spironolactone. These data suggest that GRs and MRs might not contribute to the corticosterone-induced inhibition of LV myocardial (and cardiomyocyte) growth. Glucocorticoids inhibit the production by muscle of IGF-1,<sup>34)</sup> a growth factor that stimulates the development of muscle mass, as well as stimulate the production by muscle of myostatin,<sup>35)</sup> a growth factor that impedes the development of muscle mass.<sup>36,37)</sup> Myostatin increases the abundance of the active form of the transcription factor FOXO1, allowing for increased expression of atrophy-related genes such as those for atrogen-1 and MuRF-1.<sup>38)</sup> However, none of the atrophy-related genes have been found to be directly regulated by glucocorticoids.<sup>33)</sup> The present findings suggest that GRs are implicated in the expression of some growth- and atrophy-related genes in the heart, but that GR signaling pathways may not play a major role in this model of cardiac atrophy. While many studies including ours use 8-week-old rats to define “adulthood” in rats, rats continue to grow significantly beyond this age. For instance, a previous study using older rats (6–8 months) at the time of treatment has reported an increase in myocardial protein synthesis with corticosteroid (dexamethasone) treatment.<sup>39)</sup> From this perspective, it is possible that CTC has induced myocardial “growth retardation” rather than true atrophy.

Both GRs and MRs are expressed in the cardiovascular system, where glucocorticoids influence vascular inflammatory, proliferative, and remodeling responses to injury.<sup>5,6)</sup> Activation of MRs in the myocardium and coronary vasculature has thus been shown to induce cardiac remodeling and fibrosis, myocardial oxidative stress, and coronary vascular inflammation.<sup>40,41)</sup> Furthermore, MR activation by reactive oxygen species (ROS) increases NADPH oxidase activity.<sup>22)</sup> Corticosterone-induced hypertension may have been a stimulus for ROS production in the heart of rats in the present study. Glucocorticoid-MR complexes are activated as a result of the generation of ROS.<sup>41)</sup> Our results showing that spironolactone attenuated cardiac oxidative stress and inflammation induced by corticosterone are consistent with the previous observations that MR inhibition attenuated cardiac oxidative stress and inflammation in rodent models of chronic pressure overload.<sup>22)</sup> Spironolactone may thus have protected the heart of rats in the present study from corticosterone-induced fibrosis and diastolic dysfunction through inhibition of MRs.

In conclusion, exogenous corticosterone induced hypertension and LV diastolic dysfunction as well as LV atrophy and fibrosis in SD rats. Our results suggest that MRs mediate these effects of corticosterone on LV fibrosis and diastolic function, and that GRs are implicated in the expression of some growth- and atrophy-related genes but not in cardiac atrophy per se. In contrast, the induction of hypertension by corticosterone appears to be independent of MRs and GRs. Our data thus provide important insight into the mechanisms underlying corticosterone-induced cardiac injury. Given that the actions of glucocorticoids are complex, however, further studies are required to identify the precise mechanisms of glucocorticoid-induced hypertension and cardiac injury.

## DISCLOSURES

None.

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