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Effects of Adiponectin on Calcium Handling Proteins in Heart Failure with Preserved Ejection Fraction

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Abstract

Background—Despite the increasing prevalence of heart failure (HF) with preserved ejection fraction (HFpEF) in humans, there remains no therapeutic options for HFpEF. Adiponectin (APN), an adipocyte-derived cytokine exerts cardioprotective actions and its deficiency is implicated in the development of hypertension and HF with reduced ejection fraction. Similarly APN deficiency in HFpEF exacerbates left ventricular hypertrophy (LVH), diastolic dysfunction and HF. However, the therapeutic effects of APN in HFpEF remain unknown. We sought to test the hypothesis that chronic APN overexpression protects against the progression of HF in a murine model of HFpEF.

Methods and Results—APN transgenic (APNTG) and wild-type (WT) mice underwent uninephrectomy, a continuous saline or *d*-aldosterone infusion and given 1.0% sodium chloride drinking water for 4-weeks. Aldosterone-infused WT mice developed HFpEF with hypertension, LVH and diastolic dysfunction. Aldosterone infusion increased myocardial oxidative stress and decreased sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) protein expression in HFpEF. Although total phospholamban (PLN) protein expression was unchanged, there was decreased expression of PKA-dependent PLN phosphorylation at Ser16 and CaMKII-dependent PLN phosphorylation at Thr17. APN overexpression in aldosterone-infused mice ameliorated LVH, diastolic dysfunction, lung congestion and myocardial oxidative stress without affecting blood pressure (BP) and LVEF. This improvement in diastolic function parameters in aldosterone-infused APNTG mice was accompanied by preserved protein expression of PKA-dependent phosphorylation of PLN at Ser16. APN replacement prevented the progression of aldosterone-induced HFpEF, independent of BP, by improving diastolic dysfunction and modulating cardiac hypertrophy.

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Disclosures
None.

Conclusions—These findings suggest that APN may have therapeutic effects in patients with HFpEF.

Keywords

adiponectin; heart failure with preserved ejection fraction; left ventricular hypertrophy; diastolic dysfunction; oxidative stress; calcium handling proteins

Heart failure with preserved ejection fraction (HFpEF), also known as “diastolic heart failure”, is a clinical syndrome characterized by signs and symptoms of heart failure (HF) with preservation of left ventricular (LV) ejection fraction (LVEF)[1]. HFpEF accounts for up to 50% of all patients presenting with HF[2], yet there remains no therapies for HFpEF. In addition to associated comorbidities, there are likely many divergent pathophysiological mechanisms similar to HF with reduced EF (HFrEF). HFpEF is highly prevalent in obese individuals[3, 4] and hypertension remains *the* major cause of HFpEF[2, 5]. Emerging evidence also indicates that factors secreted by adipocytes play a role in hypertension-related diseases[6–8].

Adiponectin (APN), an adipocyte-derived cytokine, is abundant in human plasma[9] with low APN levels seen in diseases such as hypertension, coronary artery disease, obesity and insulin resistance[10–12]. As such in experimental models, APN deficiency exacerbates the development of obesity-related hypertension[13], adverse cardiac remodeling[14, 15] in ischemia-reperfusion injury[16] and myocardial infarction[17]. Recently, we showed that lack of APN in a murine model of diastolic HF, increased the propensity to develop diastolic HF and diastolic dysfunction[18]. Although, hypoadiponectinemia in aldosterone-induced HFpEF exacerbates hypertension, LVH, diastolic dysfunction, and HF[18], the pathophysiological role and therapeutic effects of APN repletion in HFpEF are unknown. We thus sought to test the hypothesis that chronic APN overexpression protects against the progression of HFpEF and sought to investigate the proposed mechanism.

Methods

An expanded Materials and Methods section is available in the Online Data Supplement. The Institutional Animal Care and Use Committee at Boston University School of Medicine approved all study procedures related to the handling and surgery of the mice. APN transgenic (APNTG) and wild-type (WT) mice in a C57BL/6J background were generated as previously described[19].

Experimental Model

Twelve-week old APNTG mice and WT littermates were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally). They underwent uninephrectomy and intraperitoneal implantation of osmotic mini-pumps (Durect Corp., Cupertino, CA) that delivered a continuous infusion of either saline or 0.15 μg/hr *d*-aldosterone (Sigma-Aldrich Co., St. Louis, MO) for 4 weeks. See online supplement for mice groups.

Physiological Measurements

Heart rate and blood pressure (BP) were measured weekly using a noninvasive tail-cuff BP analyzer, BP-2000 Blood Pressure Analysis System (Visitech Systems, Inc., Apex, NC)[18, 20].

Echocardiography

Transthoracic echocardiography was performed at the end of 4 weeks using the Vevo 770 High-Resolution *In* Vivo Micro-Imaging System and a Real-Time Micro Visualization 707B Scanhead (VisualSonic Inc., Toronto, Ontario, Canada)[18, 20]. For details of LV structure, function and Doppler measurements see online supplement.

Mice were sacrificed 4 weeks after saline or aldosterone infusion and biomarker, organ weight, tissue analysis and cardiomyocyte sizes were determined. Myocardial oxidative stress by 3-nitrotyrosine staining and immunoblotting of calcium handling proteins and signaling pathway were also measured. qRT-PCR for atrial natriuretic peptide (ANP) mRNA expression was also determined. See online supplement for all details.

Statistical Analysis

Data are expressed as mean± standard error of the mean (SEM). For comparisons of multiple groups, Kruskal–Wallis 1-way ANOVA was performed with a post hoc Dunn test for multiple comparisons. Paired data were evaluated by Mann–Whitney test. $P<0.05$ values were considered significant. All statistical analyses were performed using Graph Pad Prism (GraphPad Software, Inc., La Jolla, CA).

Results

General characteristics

There were no deaths in the mice during the 4-week period. Characteristics of WT and APNTG mice 4-weeks after saline or aldosterone infusion are summarized in Table 1. Body weights (BW) were comparable between WT and APNTG mice regardless of saline or aldosterone infusion.

Hemodynamic parameters

Heart rates (HR) were comparable between WT and APNTG mice regardless of saline or aldosterone infusion (Table 1). Systolic BP (SBP) was measured weekly (Supplemental Figure 1) and was significantly increased by 4-weeks of aldosterone infusion in WT-aldosterone (127±3 vs. 105±2 mmHg; $P<0.01$) and APNTG-aldosterone mice (125±4 vs. 100±2 mmHg; $P<0.01$) compared with their respective saline-infused controls. There was no difference in SBP between the aldosterone-infused WT and APNTG mice.

Serum aldosterone levels

Serum aldosterone levels in WT-aldosterone (6,542.6±324.2 vs. 626.0±66.4 pg/mL; $P<0.01$) and APNTG-aldosterone mice (5,317.4±551.7 vs. 810.7±85.9 pg/mL; $P<0.01$) were significantly elevated compared with respective saline-infused mice. There was no

difference in serum aldosterone levels between WT and APNTG mice regardless of saline or aldosterone infusion (Table 1).

Serum adiponectin levels

Serum APN levels in APNTG-saline mice (27.0±1.0 μg/mL) were ~1.9 times higher than in WT-saline mice (14.5±1.3 μg/mL; P<0.01). Serum APN levels in APNTG-aldosterone mice (29.2±0.3 μg/mL) were significantly elevated vs. APNTG-saline mice (27.0±1.0μg/mL; P<0.05). There was no difference in APN levels between WT-saline and WT-aldosterone mice (Table 1).

LV structure and systolic function

Echocardiographic parameters for LV structure and systolic function are summarized in Table 2. Aldosterone infusion significantly increased total wall thickness (TWT) and LV mass in WT-aldosterone (1.04±0.02 mm and 140.4±6.5 mg) and APNTG-aldosterone mice (0.93±0.02 mm and 110.9±12.7 mg). Consistent with these findings, the heart weight (HW) to BW ratio (HW/BW) was also significantly increased in the WT-aldosterone (5.69±0.15) and APNTG-aldosterone mice (5.14±0.07; Table 1). There were no significant differences in cardiac hypertrophy between WT-saline and APNTG-saline mice (Table 1 and 2). However, cardiac hypertrophy in APNTG-aldosterone mice was significantly less vs. WT-aldosterone mice (P<0.01 for TWT; P<0.05 for LV mass and P<0.05 for HW/BW). There was no difference in LV chamber size and LVEF between the WT and APNTG mice regardless of saline or aldosterone infusion (Table 2).

Diastolic function

Echocardiographic parameters (mitral and tissue Doppler) for LV diastolic function in the mice are summarized in Table 2; Figure 1A–B. *Mitral Doppler:* Aldosterone infusion significantly increased peak E velocity in the WT-aldosterone vs. WT-saline mice and in APNTG-aldosterone vs. APNTG-saline mice (Table 2; Figure 1B). The peak E velocity in APNTG-aldosterone mice was however, significantly lower than in WT-aldosterone mice (P<0.01). Aldosterone infusion significantly increased peak A velocity in APNTGaldosterone vs. APNTG-saline mice, but not in WT-aldosterone vs. WT-saline mice (P=NS). The resultant ratio of peak E velocity to peak A velocity (E/A) was significantly higher in WT-aldosterone (2.17±0.17) vs. WT-saline mice (1.45±0.04; P<0.01). This increase in E/A ratio in WT-aldosterone mice, indicating impaired LV compliance, was attenuated in APNTG-aldosterone mice (1.50±0.09; P<0.01). Deceleration time (DT) was significantly shortened in WT-aldosterone vs. WT-saline mice (P<0.01). The shortened of DT in WT-aldosterone mice, indicates abnormal LV relaxation and was attenuated in APNTG-aldosterone mice (P<0.01). There was no difference in isovolumic relaxation time (IVRT) between WT and APNTG mice regardless of saline or aldosterone infusion. *Tissue Doppler:* (Table 2) Aldosterone infusion significantly decreased peak e' velocity in WT-aldosterone vs. WT-saline mice (P<0.01) and APNTG-aldosterone mice vs. APNTG-saline mice (P<0.01). However, the peak e' velocity in APNTG-aldosterone mice was significantly higher than in WT-aldosterone mice (P<0.01). The resultant ratio of peak E velocity to peak e' velocity (E/e') was significantly higher in WT-aldosterone mice compared with WT-

saline mice (P<0.01). The increase in E/e' in WT-aldosterone mice, indicates elevated diastolic filling pressure and was significantly attenuated in APNTG-aldosterone mice (P<0.01).

APNTG-aldosterone mice showed a reduction in the ratio of peak E velocity to peak A velocity compared with WT-aldosterone mice. However relative to the saline-infused mice this ratio of peak E velocity to peak A velocity was elevated. The reduction in E/A ratio in APNTG-aldosterone mice is due to a decrease in peak E velocity demonstrating an improvement in early transmitral flow (E wave) in the restrictive filling pattern. Despite a reduction in E/A ratio in APNTG mice the E/e' remained elevated suggesting elevated filling pressures or restrictive filling.

Lung congestion

Aldosterone infusion significantly increased wet/dry lung weight, an indicator of pulmonary congestion, in WT-aldosterone vs. WT-saline mice (P<0.01) and APNTG-aldosterone vs. APNTG-saline mice (P<0.05; Table 1). There was no difference in wet/dry lung weight, between saline-infused WT and APNTG mice. However, the wet/dry lung weight in APNTG-aldosterone mice was significantly lower compared with WT-aldosterone mice indicating less pulmonary congestion (P < 0.05).

LV cardiomyocyte hypertrophy

Aldosterone infusion significantly increased LV cardiomyocyte C/S area in the WT-aldosterone and APNTG-aldosterone mice compared with respective saline-infused mice (P<0.01 for both; Figure 2A–B). Consistent with these findings, ANP mRNA expression, a molecular marker of cardiomyocyte hypertrophy, was increased in the LV of WT-aldosterone vs. WT-saline mice (P<0.01) and APNTG-aldosterone vs. APNTG-saline mice (P<0.05; Figure 2C). There was no difference in the LV cardiomyocyte C/S area and ANP mRNA expression in saline-infused WT and APNTG mice. However, both LV cardiomyocyte C/S area and ANP mRNA expression in APNTG-aldosterone mice were significantly decreased vs. WT-aldosterone mice (P<0.01 and P<0.05, respectively; Figure 2).

Myocardial fibrosis

Aldosterone infusion significantly increased the area of myocardial fibrosis in WT-aldosterone vs. WT-saline mice (P<0.01) and APNTG-aldosterone vs. APNTG-saline mice (P<0.05; Figure 3). Myocardial fibrosis in APNTG-aldosterone mice was significantly less than the WT-aldosterone mice (P<0.05).

Myocardial oxidative stress

Myocardial oxidative stress, assessed by 3-nitrotyrosine staining, was markedly increased in WT-aldosterone mice vs. WT-saline mice. The increase in nitrotyrosine staining in WT-aldosterone mice was attenuated in APNTG-aldosterone mice (Figure 4A–B). There was a 54% reduction in nitrotyrosine staining in APNTG-aldosterone mice (P<0.05 vs. WT-aldosterone mice).

Calcium handling proteins: SERCA2a and PLN

Four weeks of aldosterone infusion significantly decreased SERCA2a protein expression in WT-aldosterone vs. WT-saline mice (0.71-fold; P<0.01) and APNTG-aldosterone vs. APNTG-saline mice (0.66-fold; P<0.05). The decrease in SERCA2a protein expression was comparable between WT-aldosterone and APNTG-aldosterone mice (Figure 5A). There was no difference in PLN protein expression between WT and APNTG mice regardless of saline or aldosterone infusion (Figure 5B). Four weeks of aldosterone infusion significantly decreased phosphorylation of PLN at Ser16 in WT-aldosterone vs. WT-saline mice (0.55-fold; P<0.01; Figure 5C). However, this decrease in PLN phosphorylation at Ser16 in WT-aldosterone mice was significantly attenuated in APNTG-aldosterone mice (P<0.05; Figure 5C). Four weeks of aldosterone infusion significantly decreased phosphorylation of PLN at Thr17 in WT-aldosterone vs. WT-saline mice (0.73-fold; P<0.01) and APNTG-aldosterone vs. APNTG-saline mice (0.68-fold; P<0.01; Figure 5D). PLN phosphorylation of at Thr17 was similar between WT-aldosterone and APNTG-aldosterone mice (Figure 5D).

PKA expression

Four weeks of aldosterone infusion significantly decreased PKA C-α protein expression in WT-aldosterone vs. WT-saline mice (0.78-fold; P<0.01). This decrease of PKA C-α protein expression in WT-aldosterone mice was however significantly attenuated in APNTG-aldosterone mice (P<0.05; Figure 6).

CaMKII expression

There was no difference in CaMKII protein expression between WT and APNTG mice regardless of saline or aldosterone infusion (Figure 7A). Four weeks of aldosterone infusion significantly decreased phosphorylation of CaMKII in WT-aldosterone vs. WT-saline mice (0.65-fold; P<0.01) and APNTG-aldosterone vs. APNTG-saline mice (0.66-fold; P<0.05; Figure 7B). There was no difference in phosphorylation of CaMKII between WT-aldosterone and APNTG-aldosterone mice.

APN supplementation (Figure 8A–B)

To examine whether Ad-APN supplementation ameliorated aldosterone-induced diastolic dysfunction *in vivo*, both WT-saline and WT-aldosterone mice were treated with either adenovirus-APN (Ad-APN) or adenovirus-β-galactosidase (Ad-βgal). Ad-APN or Ad-βgal were injected into the jugular vein of mice 14 days after surgery. This dose of Ad-APN raises APN levels in the physiological range and contains the 3 isoforms[14, 21] that are present in mice with the hexamer form being dominant. Neither Ad-APN nor Ad-βgal had an effect on SBP in WT-saline and WT-aldosterone mice (data not shown).

Ad-APN attenuated the aldosterone-induced changes in diastolic dysfunction: E/A ratio WT-aldosterone treated with Ad-APN (2.4) vs. WT-aldosterone mice treated with Ad-βgal (1.75; P<0.01). Similarly, E/e' decreased 34% vs. WT-aldosterone mice treated with Ad-βgal (P<0.01).

Discussion

In this study, aldosterone-infused WT mice developed hypertension, LVH, diastolic dysfunction, and increased lung congestion while maintaining a preserved LVEF, thus resulting in HFpEF. Aldosterone infusion also increased myocardial oxidative stress, decreased SERCA2a protein expression, decreased PKA-dependent PLN phosphorylation at Ser16, and decreased CaMKII-dependent phosphorylation of PLN at Thr17. Chronic hyperadiponectinemia ameliorated LVH, diastolic dysfunction, and lung congestion without effects on BP or LVEF in HFpEF mice. Chronic APN overexpression also decreased myocardial nitrotyrosine staining, a measure of oxidative stress. The improvement of diastolic dysfunction parameters was associated with preserved PKA-dependent PLN phosphorylation at Ser16. In addition, APN supplementation with Ad-APN improved measures of diastolic dysfunction in HFpEF mice infused with aldosterone.

We previously showed that APN deficiency in aldosterone-induced HFpEF mice exacerbated hypertension and LVH[18]. Although it has been reported that hypoadiponectinemia is a risk factor for hypertension[11], the therapeutic effect of APN on hypertension is largely unknown. Ohashi, et al. reported that adenovirus-mediated overexpression of APN ameliorated obesity-related hypertension in mice[13]. However, in our study, transgenic mice with chronic APN overexpression and Ad-APN supplementation of WT mice did not affect BP. This difference may be due to pathophysiological differences of targeted experimental models and less likely due to the difference of APN levels in each experimental model. APN levels in our APNTG mice were about 2-fold higher than those in WT mice[19], and although we did not measure APN levels in the APN supplementation experiments, it was likely similar to adenovirus-mediated APN levels in Ohashi's study where APN levels were about 6-fold higher than those at baseline. Further studies are needed to determine the therapeutic effect of APN in hypertension, but the importance of our study is that chronic APN overexpression ameliorates the progression of LVH, independent of alterations in BP. Aldosterone-induced LVH, which is composed of LV cardiomyocyte hypertrophy and myocardial fibrosis[20, 22] is accompanied by oxidative stress via mineralocorticoid receptor activation[23–25]. Thus, suppression of oxidative stress might be an important therapeutic target in HFpEF[26, 27]. Several studies have recently shown that APN exerts its cardioprotective effect by inhibiting oxidative stress[28–30]. Consistent with these findings, we showed that APN overexpression diminished aldosterone-induced myocardial 3-nitrotyrosine production, a marker of oxidative stress. APN likely mitigates aldosterone-induced adverse cardiac remodeling partly by suppressing oxidative stress[29, 30]. In a high fat experimental model, APN prevented platelet aggregation by attenuating oxidative and nitrosative stress[31] and modulated ROS metabolite levels and increased antioxidant levels in an ischemia/reperfusion porcine model[32]. Mitochondrial targeted antioxidant peptide, SS-31 may prove to be a therapeutic option in HFpEF patients as it targets mitochondrial ROS, and modulates LVH, fibrosis, and LV diastolic dysfunction in an experimental model[33].

Both cardiomyocyte hypertrophy and myocardial fibrosis contribute to impaired active relaxation and increased passive stiffness of the LV, and subsequently leads to diastolic dysfunction and clinical HF[34, 35]. In our study, hyperadiponectinemia decreased cardiac

hypertrophy and improved some measures of diastolic dysfunction, independent of BP. Thus, chronic hyperadiponectinemia may improve diastolic dysfunction and HF by ameliorating LVH[18]. In human studies, it has been reported that low plasma APN is associated with diastolic dysfunction[36].

Diastolic intracellular calcium handling is a major determinant of LV relaxation[35, 37]. Dephosphorylated PLN is an inhibitor of SERCA2a, but PKA-catalyzed (or CaMKII), phosphorylation of PLN results in the dissociation of PLN from SERCA2a, thus activating this Ca^{2+} pump and augmenting SERCA2a activity. In our study, chronic aldosterone infusion decreased SERCA2a protein expression and PLN phosphorylation at both Ser16 and Thr17, but did not alter PLN protein expression. In addition, PKA C-α, an active catalytic subunit of PKA, and protein expression and phosphorylation of CaMKII, an indicator of CaMKII activity, were both decreased by chronic aldosterone infusion. Accumulating evidence indicates that β-adrenergic receptor stimulation regulates PKA and CaMKII activity[38–40]. Additionally, it has also been reported that Ser16 phosphorylation is mainly affected by PKA activity whereas Thr17 phosphorylation is affected by CaMKII activity[38, 39, 41]. Although β-adrenergic signaling affected by chronic aldosterone infusion remains to be clarified in this study, decreased PKA and CaMKII activity suggest that β-adrenergic signaling is down-regulated by chronic aldosterone infusion. Collectively, our data indicates that chronic aldosterone infusion decreased PKA-dependent phosphorylation of PLN at Ser16 and CaMKII-dependent phosphorylation of PLN at Thr17, presumably followed by β-adrenergic signaling downregulation, and subsequently suppressed SERCA2a protein expression. We did not measure intracellular Ca^{2+} concentrations. However, evidence indicates that diastolic intracellular calcium handling is mainly regulated by SERCA2a and its modulator PLN[35, 37, 41]. Thus, changes in SERCA2a protein expression and/or phosphorylation of PLN in our study, may be associated with abnormal intracellular diastolic calcium handling and subsequent diastolic dysfunction, as demonstrated by impaired LV compliance (increased E/A), abnormal LV relaxation (shortened DT), and elevated diastolic filling pressure (increased E/e'). Nonetheless, in our study hyperadiponectinemia and chronic APN overexpression ameliorated these measures of diastolic dysfunction in HFpEF indicating that the improvement in these measures might be due to alterations in calcium handling protein signaling.

At cellular and molecular levels, APN induces Ca^{2+} influx via AdipoR1 and subsequently activates CaMKK, AMPK and SIRT1 in skeletal muscle[42]. However in the myocardium, SERCA2a protein expression in chronic aldosterone infusion was not affected by chronic APN overexpression. Likewise, CaMKII activity and CaMKII-dependent phosphorylation of PLN at Thr17 were both decreased and to comparable levels. Yet chronic APN overexpression ameliorated the decrease of PLN phosphorylation at Ser16, followed by preserved PKA activity. SERCA2a function is determined not only by SERCA2a protein expression[43], but also by the phosphorylation status of PLN[44]. In our study, chronic APN overexpression did not affect the SERCA2a protein expression, but improved diastolic dysfunction. These findings may be partly explained by improvement of SERCA2a function through phosphorylation of PLN. In addition, several studies have shown that APN is associated with cyclic adenosine monophosphate (cAMP)-dependent PKA signaling[45, 46].

Thus, our finding suggests that chronic APN overexpression preserves phosphorylation of PLN at Ser16 through PKA activation, and improves SERCA2a dysfunction.

Finally, the potential beneficial effects of APN may extend to the downregulation of inflammatory cytokines or the upregulation of anti-inflammatory cytokines which may impact cardiac hypertrophy, the extracellular matrix, diastolic dysfunction and HFpEF. Pro-inflammatory cytokines, such as TNF-α which is pro-hypertrophic[47], are increased in diastolic dysfunction and HFpEF[18] (Supplemental Figure 2). We recently showed that IFN-γ, a pro-inflammatory cytokine attenuated cardiac hypertrophy and is a regulator of cardiac hypertrophy in HFpEF thus disputing the notion that inflammatory cytokines mediate only adverse effects[48].

In conclusion, chronic APN overexpression and supplementation prevented the progression of aldosterone-induced HFpEF, independent of blood pressure. The beneficial effect of APN was associated with reduced myocardial oxidative stress and modulation of intracellular calcium handling regulatory proteins. Our findings indicate that APN and its signaling pathway may be a therapeutic target for patients with HFpEF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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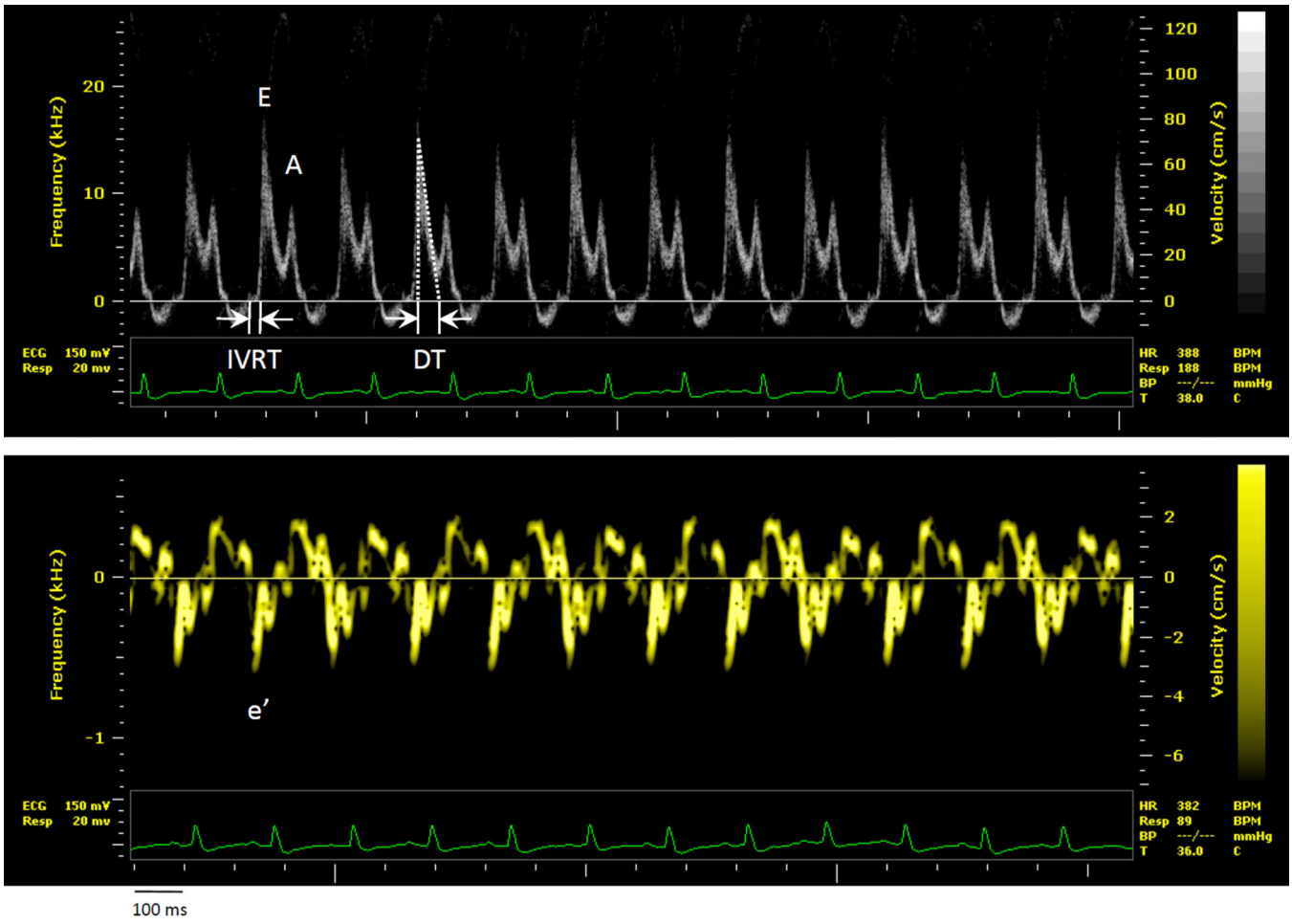

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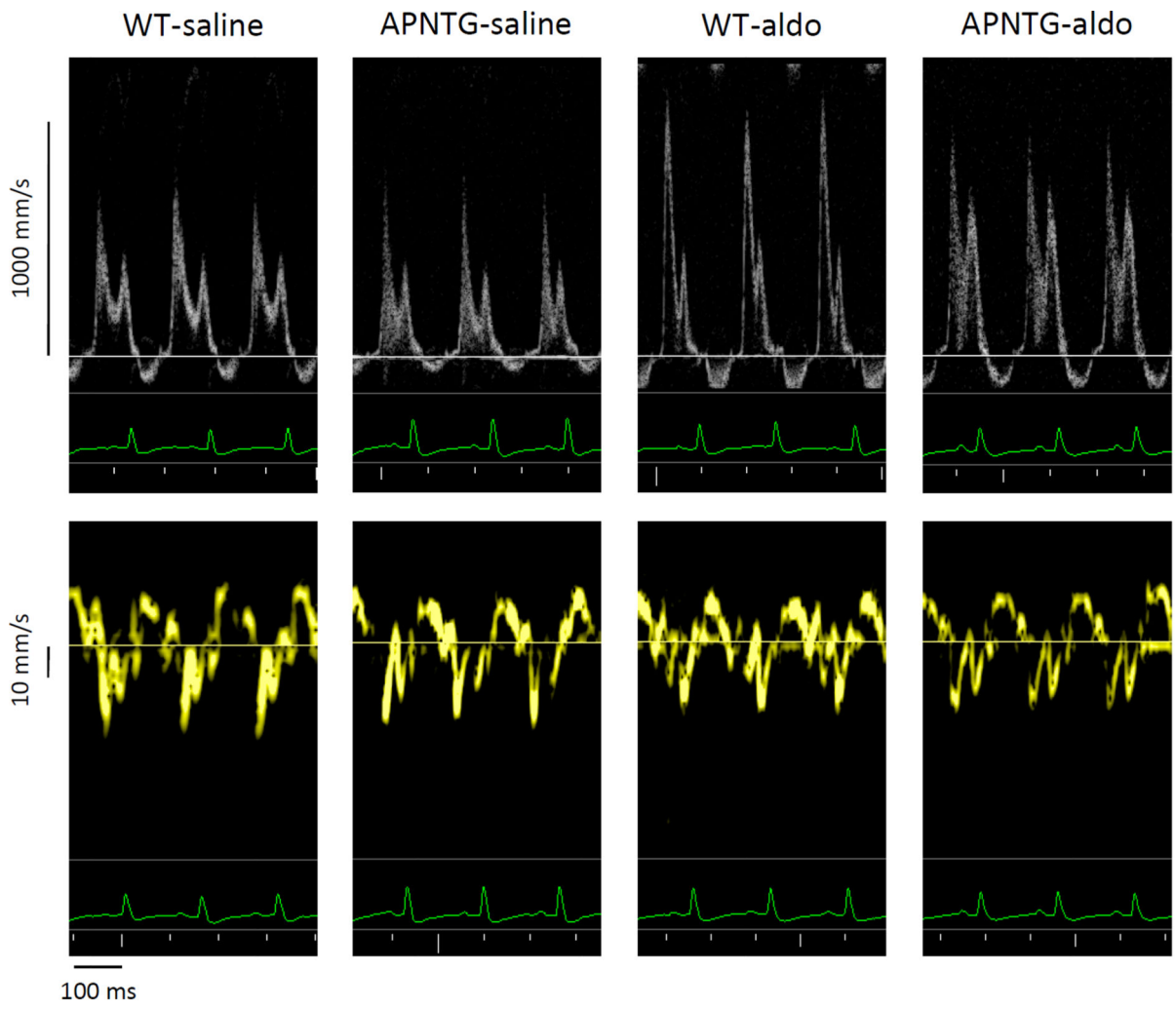


Figure 1.

Representative pulse wave and tissue Doppler images in WT and APNTG mice 4 weeks after saline or aldosterone infusion. A) WT-saline; B) WT-saline, APNTG-saline, WT-aldosterone, and APNTG-aldosterone. IVRT indicates isovolumetric relaxation time, E; peak early transmitral flow velocity, A; peak late transmitral flow velocity, DT; early filling deceleration time, e'; peak early diastolic myocardial velocity.

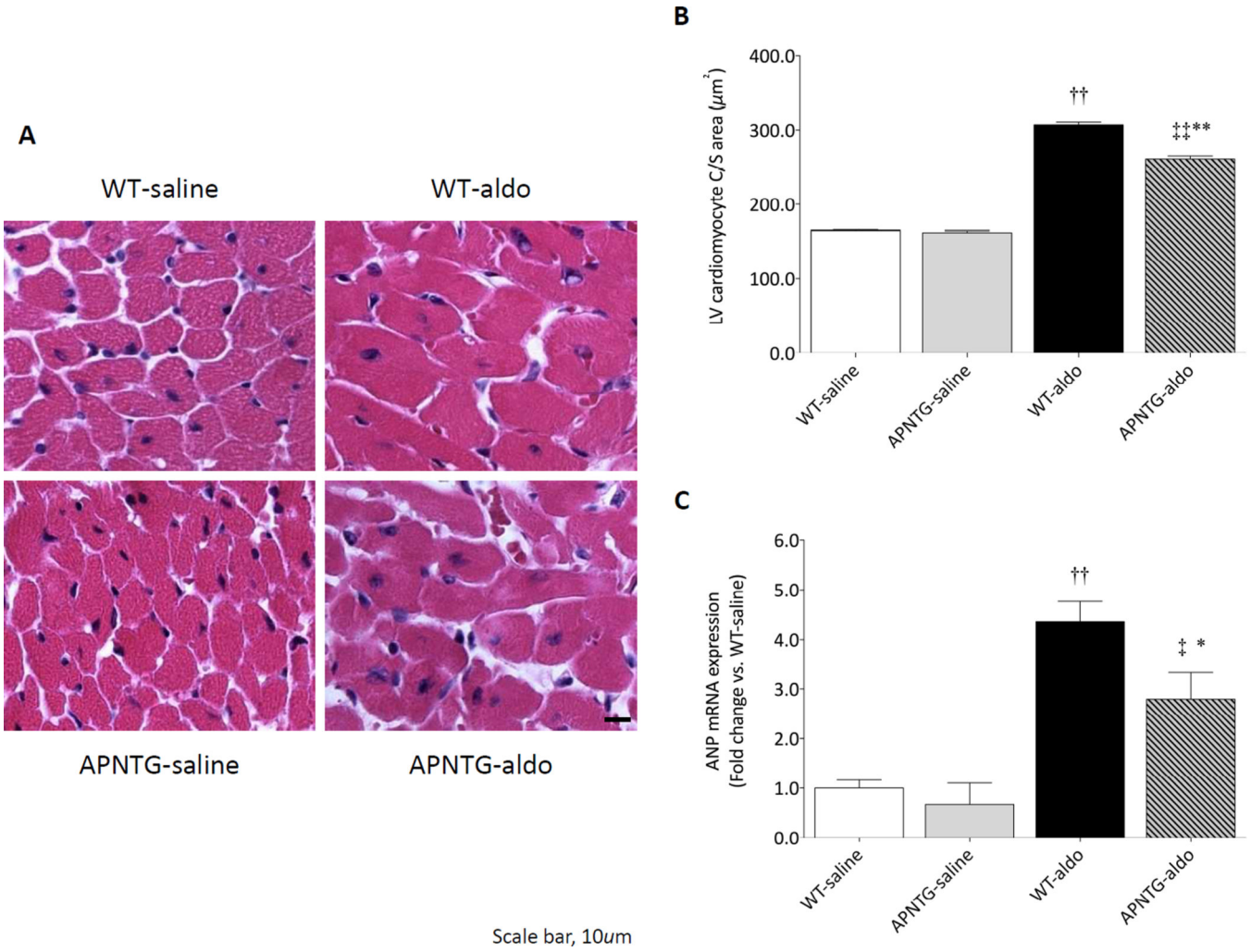


Figure 2.

LV cardiomyocyte hypertrophy in WT and APNTG mice 4 weeks after saline or aldosterone infusion. A) Representative H&E staining. Scale bar is 10μm; B) LV cardiomyocyte C/S area; C) ANP mRNA expression (n=4–9 per group). ††P<0.01 vs. WT-saline, ‡P<0.05 vs. APNTG-saline, ‡‡P<0.01 vs. APNTG-saline, *P<0.05 vs. WT-aldosterone, **P<0.01 vs. WT-aldosterone.

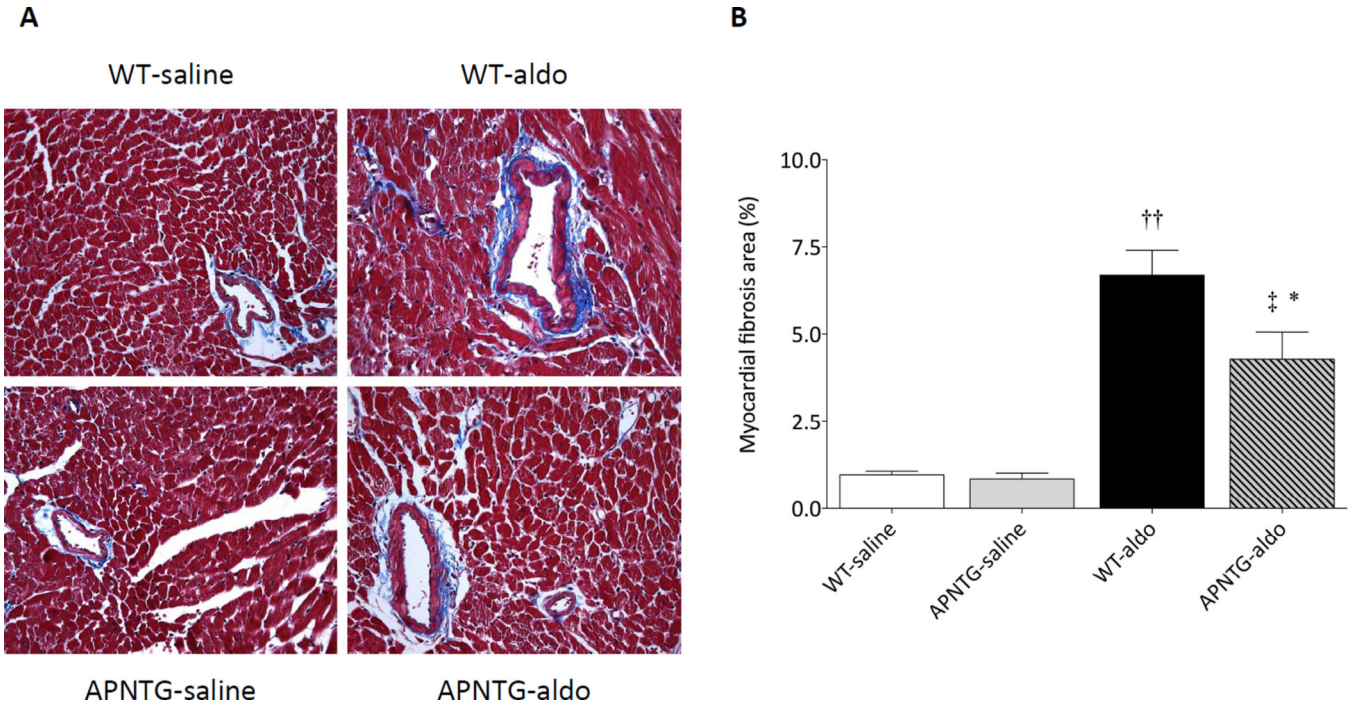


Figure 3.

Myocardial fibrosis in WT and APNTG mice 4 weeks after saline or aldosterone infusion. A) Representative Masson trichrome staining. Original magnification x400; B) Myocardial fibrosis area (n=4–9/group); ††P<0.01 vs. WT-saline, ‡P<0.05 vs. APNTG-saline, *P<0.05 vs. WT-aldosterone.

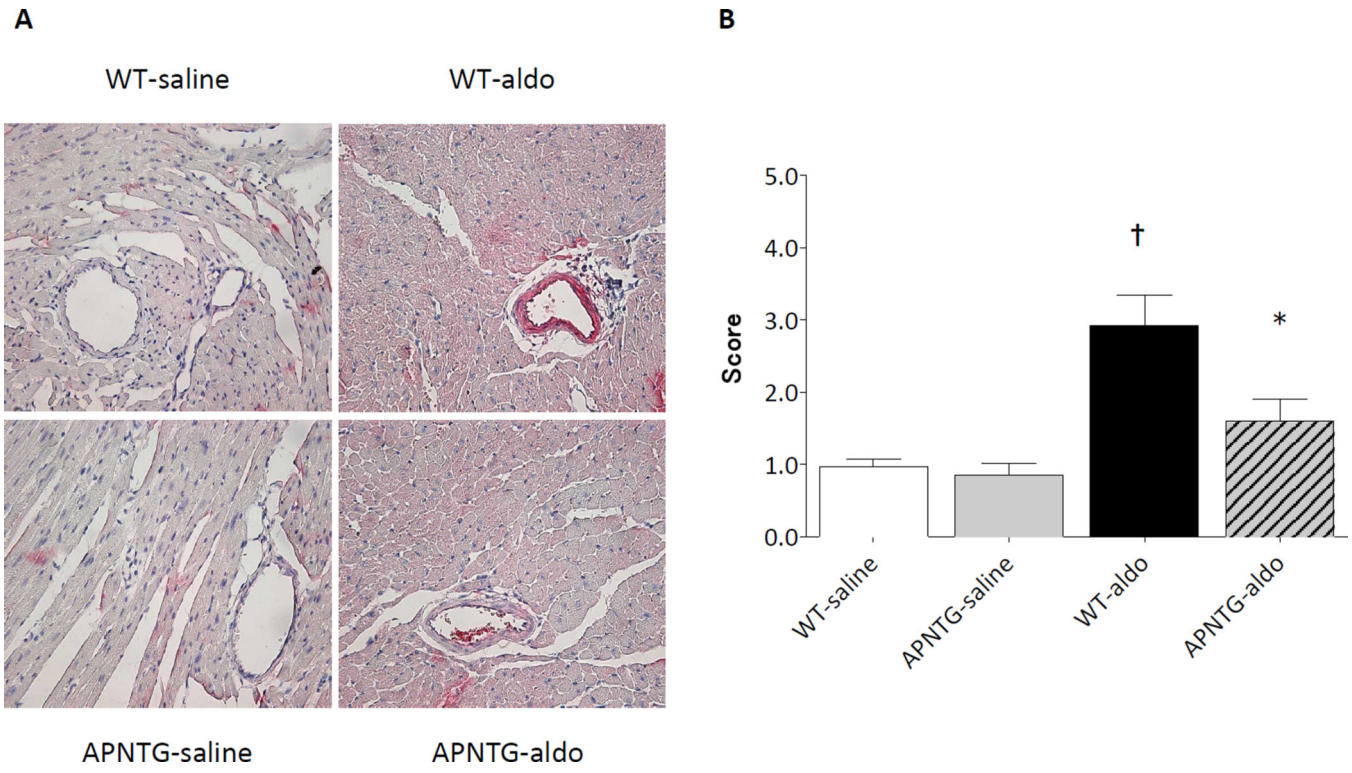
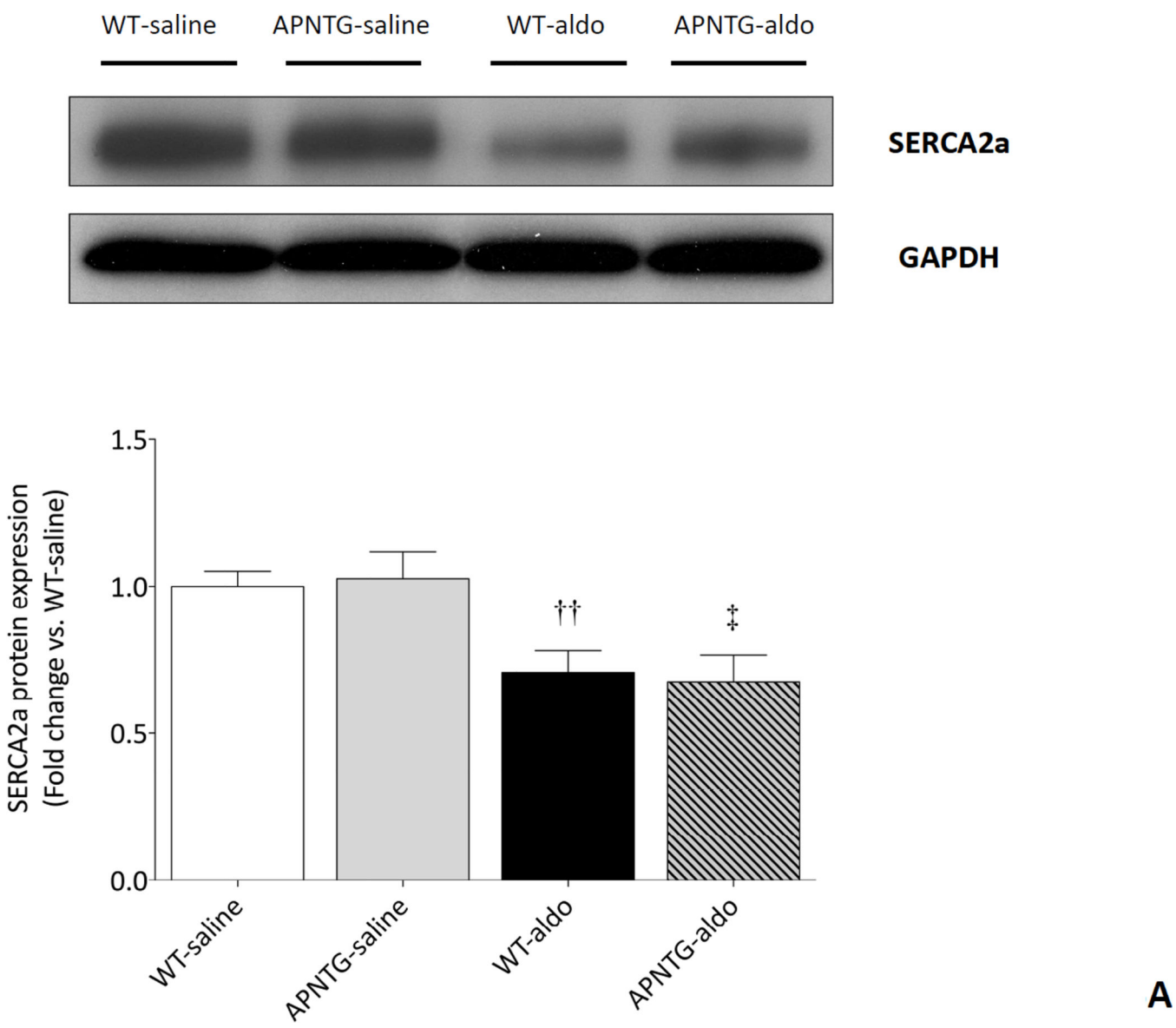


Figure 4.

Myocardial oxidative stress in WT and APNTG mice 4 weeks after saline or aldosterone infusion. A) Representative 3-nitrotyrosine staining. Original magnification ×400. B) Semiquantitative analysis of nitrotyrosine staining of C/S of murine myocardium. Nitrotyrosine staining was scored using an arbitrary grade from 1 to 4. (n=4–9/group). †P<0.01 vs. WT-saline, *P<0.05 vs. WT-aldosterone.



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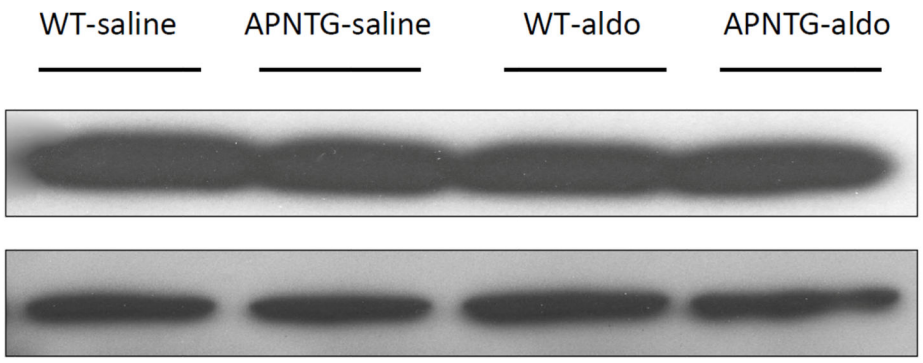

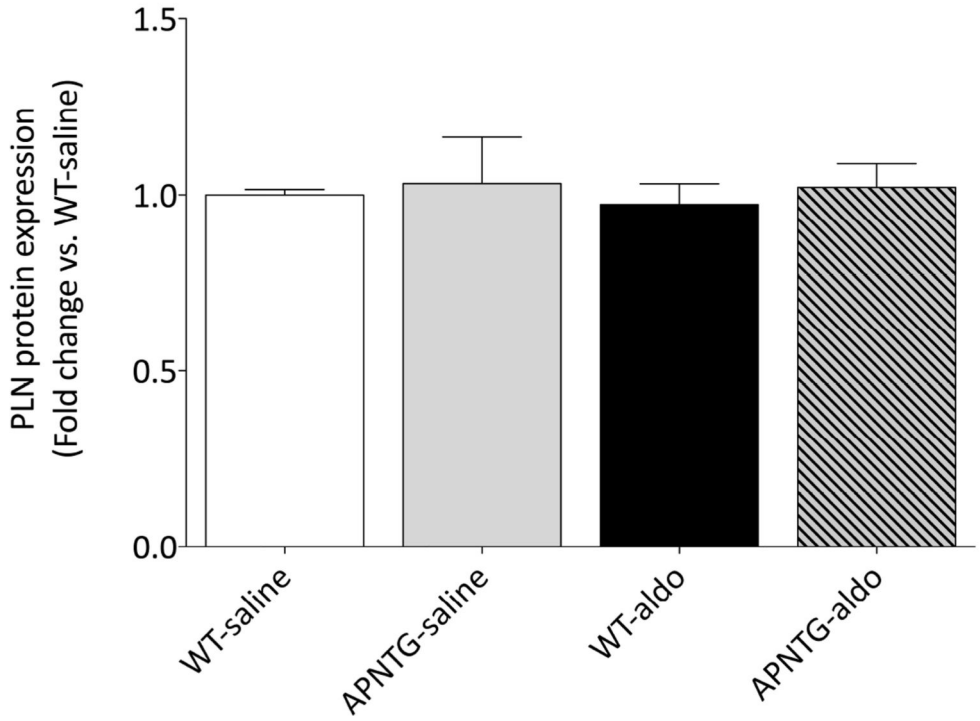

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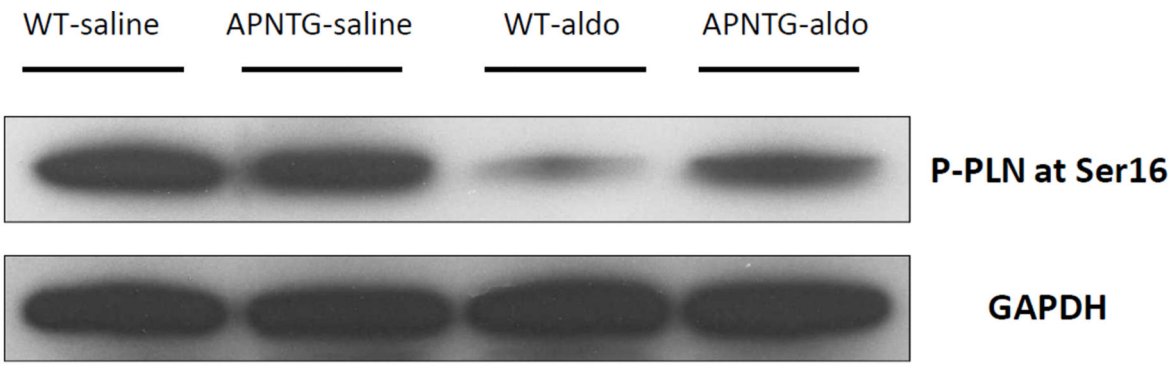

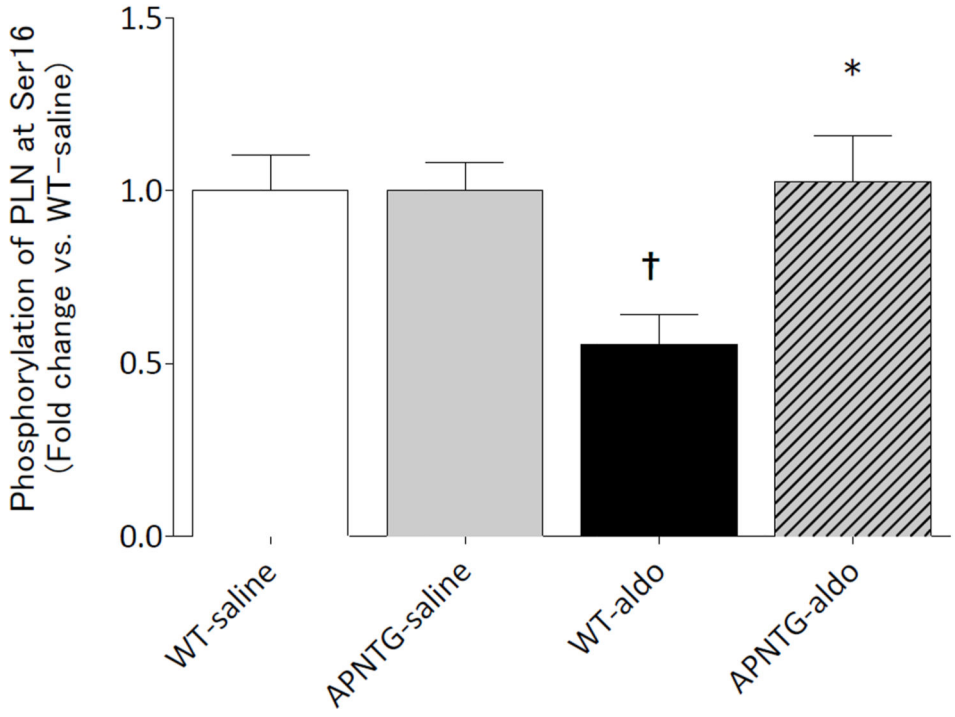

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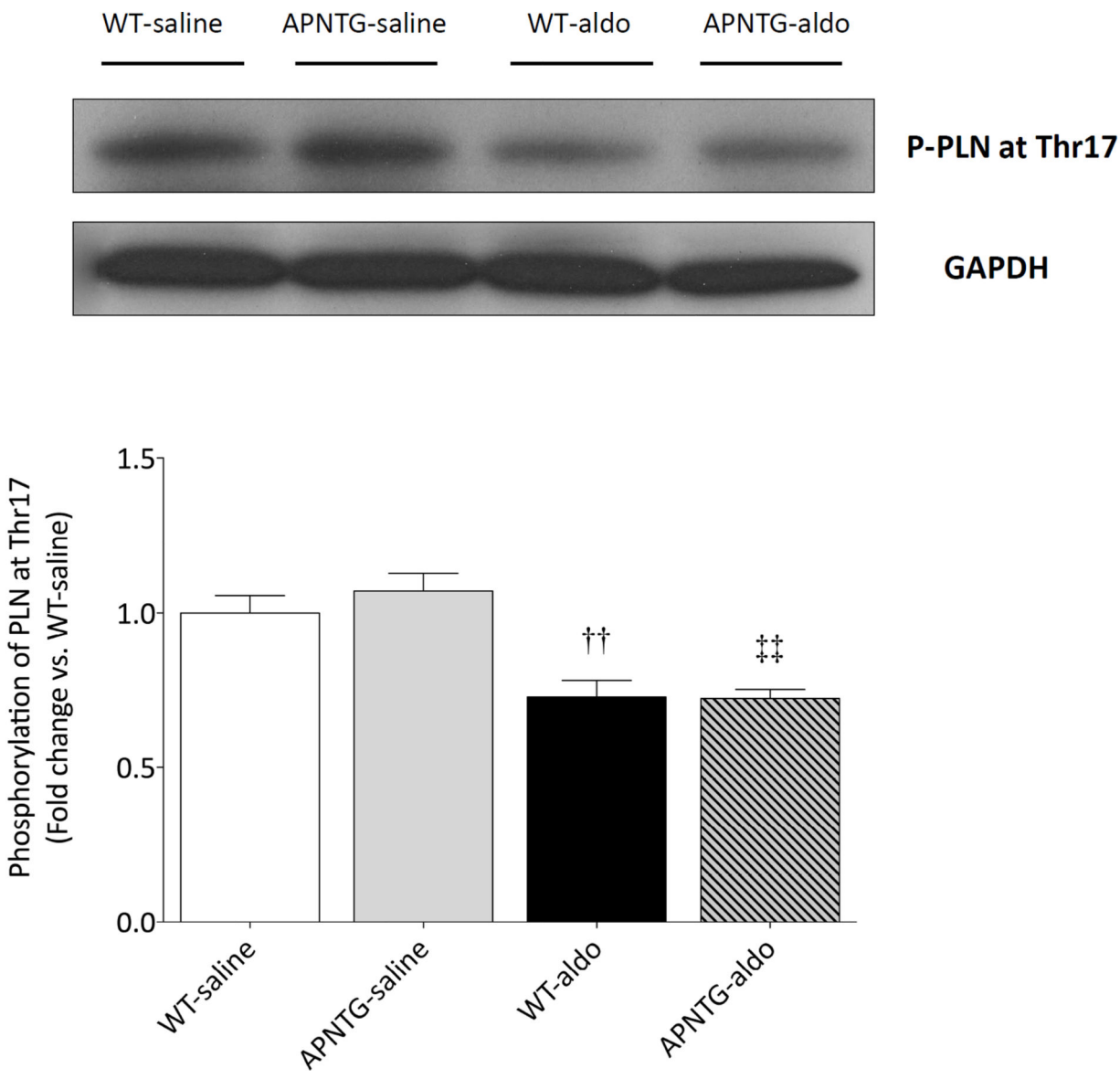


Figure 5.

Expression of calcium handling regulatory proteins in WT and APNTG mice 4 weeks after saline or aldosterone infusion. A) *(Upper)* Representative blots of SERCA2a and GAPDH. *(Lower)* Quantitative analysis of SERCA2a protein expression; B) *(Upper)* Representative blots of PLN and GAPDH. *(Lower)* Quantitative analysis of PLN protein expression; C) *(Upper)* Representative blots of PLN phosphorylation at Ser16 and GAPDH. *(Lower)* Quantitative analysis of PLN phosphorylation at Ser16; D) *(Upper)* Representative blots of PLN phosphorylation at Thr17 and GAPDH. *(Lower)* Quantitative analysis of PLN phosphorylation at Thr17. ††P<0.01 vs. WT-saline, ‡P<0.05 vs. APNTG-saline, ‡‡P<0.01 vs. APNTG-saline, *P<0.05 vs. WT-aldosterone; (n=3–8/group).

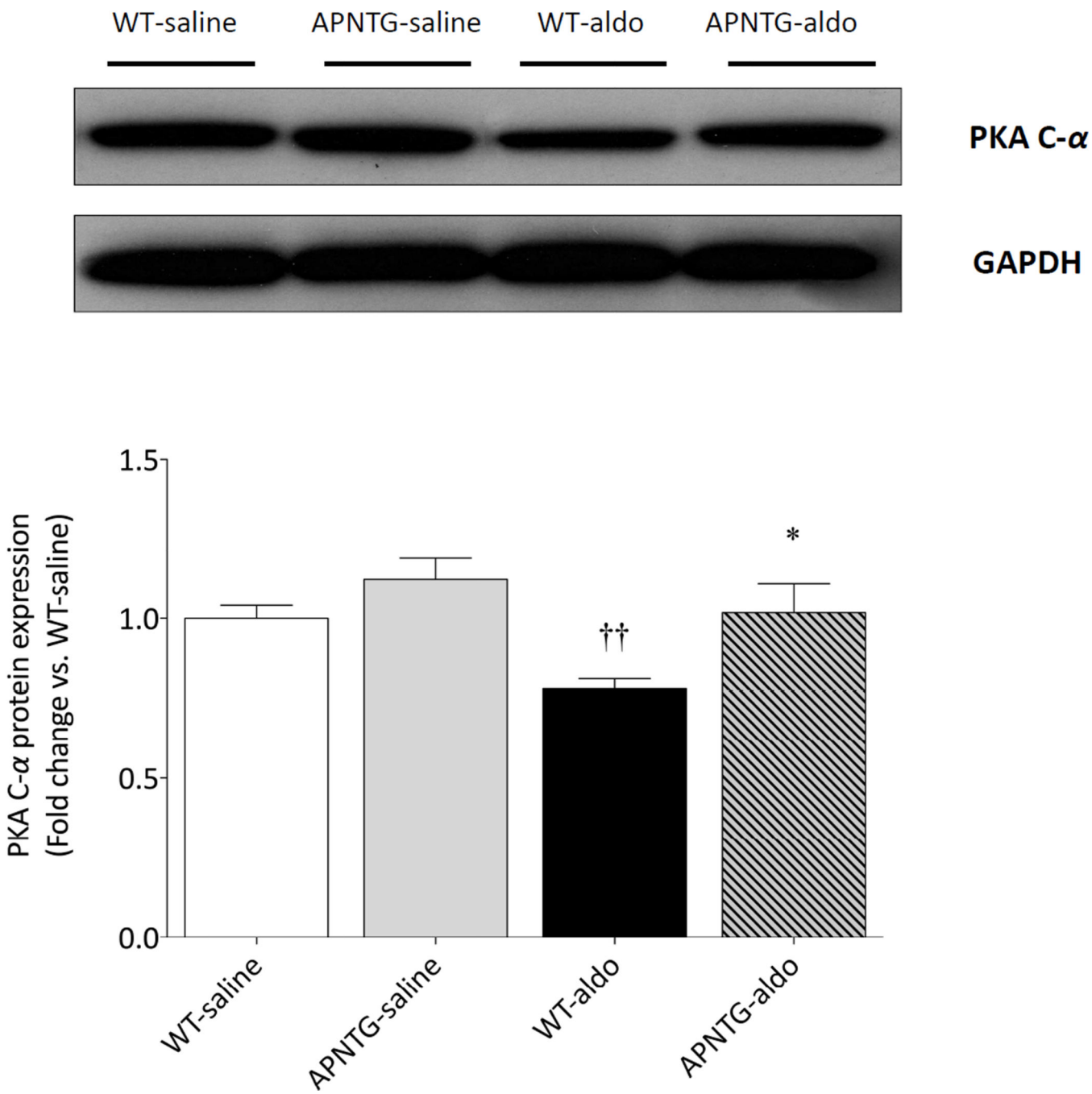
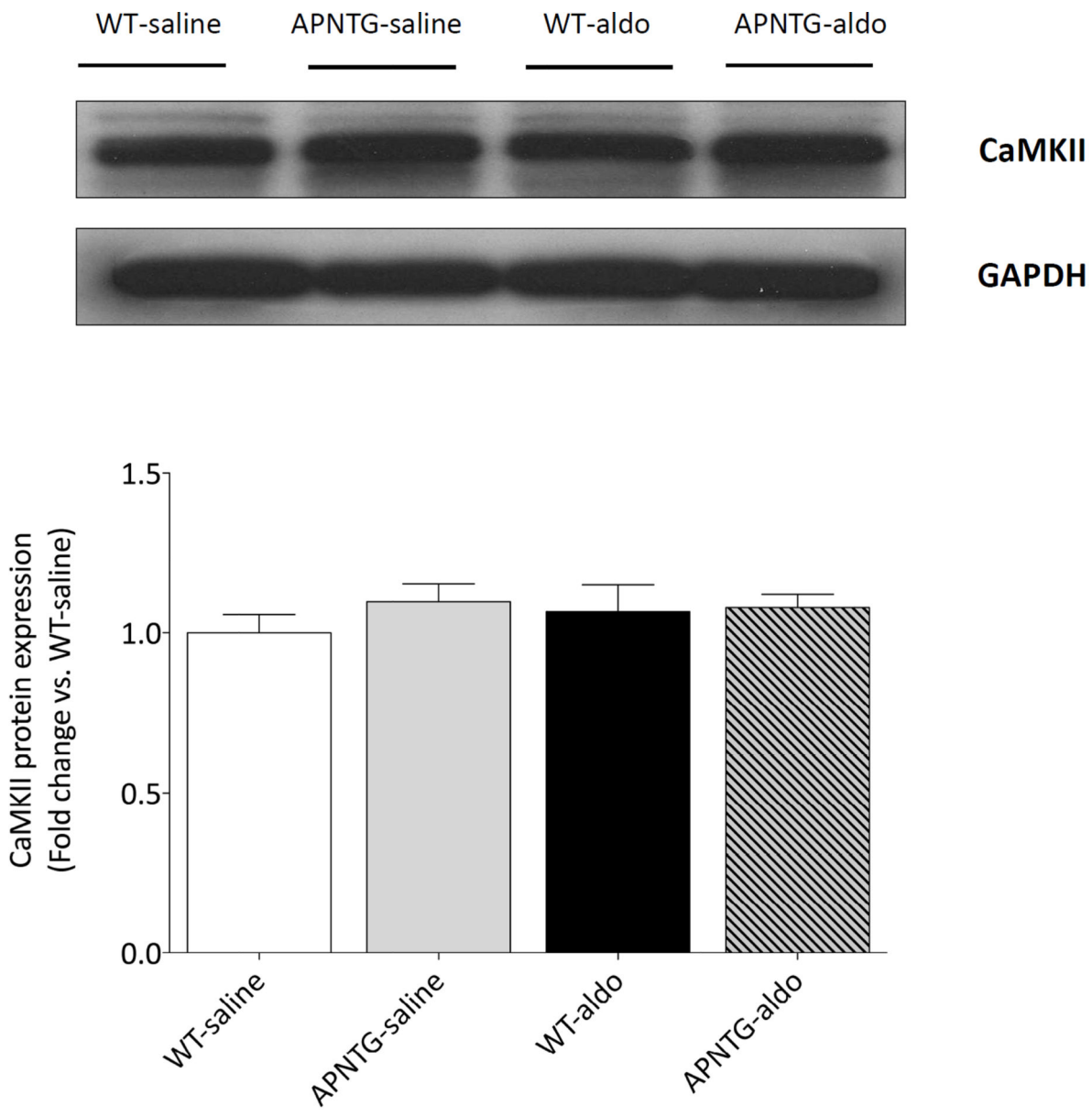


Figure 6.

PKA C-α protein expression in WT and APNTG mice 4 weeks after saline or aldosterone infusion. *(Upper)* Representative blots of PKA C-α and GAPDH. *(Lower)* Quantitative analysis of PKA C-α expression (n=3–8/group); ††P<0.01 vs. WT-saline, *P<0.05 vs. WT-aldosterone.



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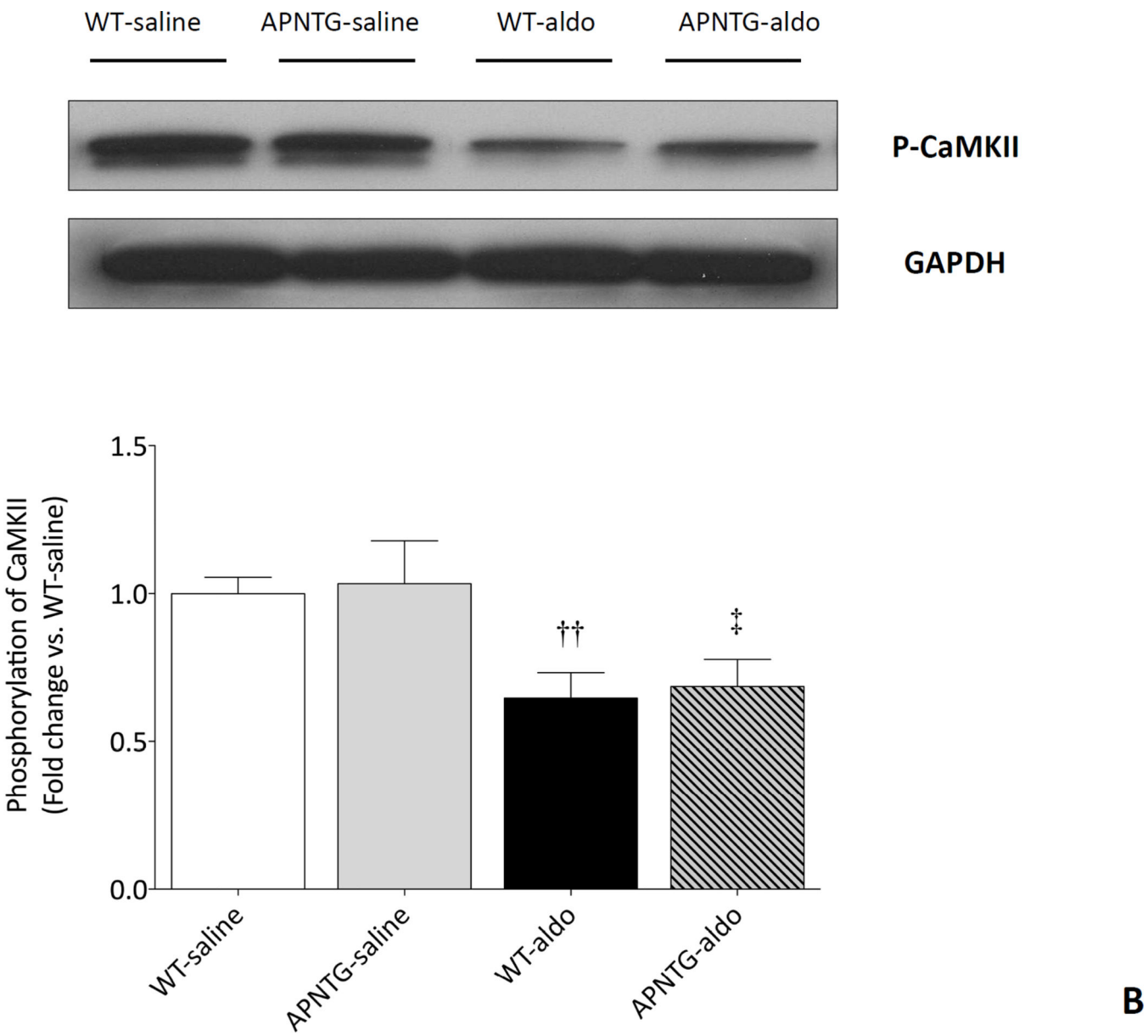

Figure 7.

CaMKII protein expression and phosphorylation of CaMKII in WT and APNTG mice 4 weeks after saline or aldosterone infusion. A) *(Upper)* Representative blots of CaMKII and GAPDH. *(Lower)* Quantitative analysis of CaMKII protein expression; B) *(Upper)* Representative blots of phosphorylation of CaMKII and GAPDH. *(Lower)* Quantitative analysis of phosphorylation of CaMKII (n=3–8/group); ††P<0.01 vs. WT-saline, ‡P<0.05 vs. APNTG-saline.

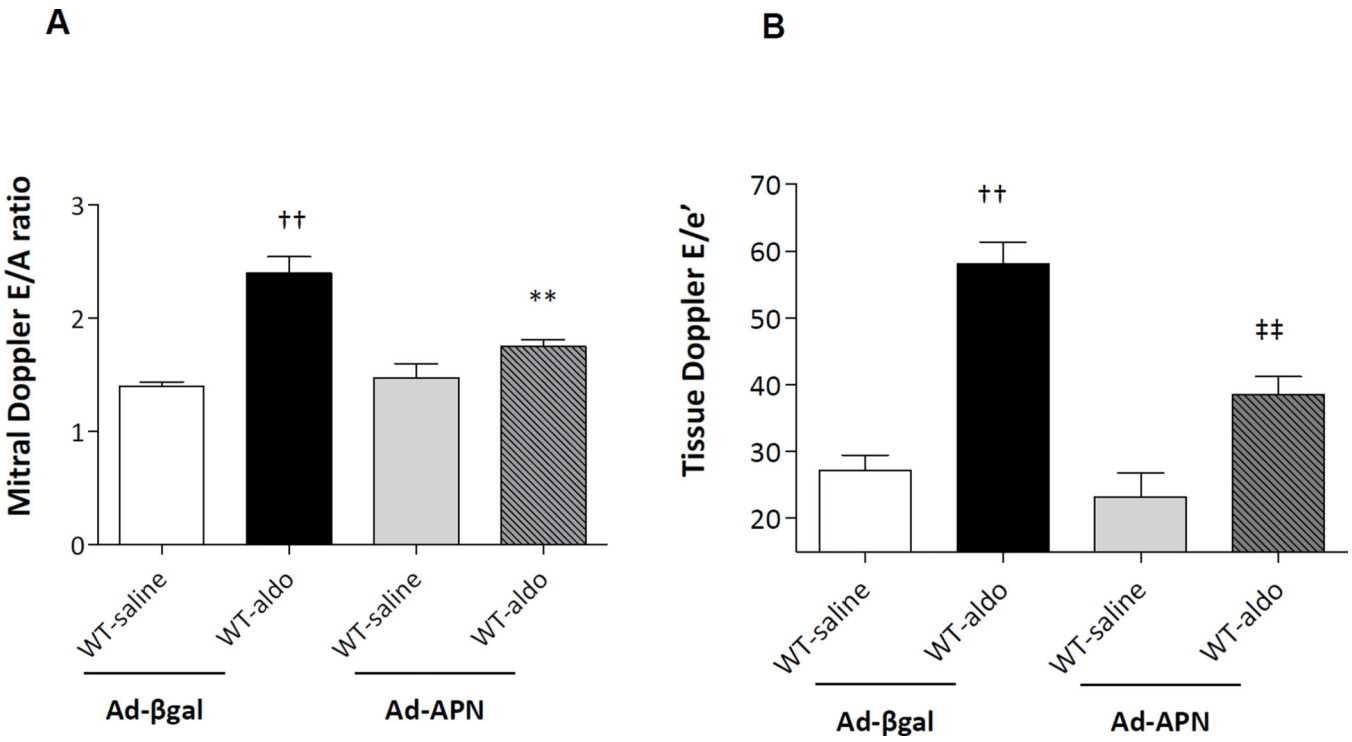

Figure 8.

Ad-APN supplementation modulates diastolic dysfunction in aldosterone-infused WT. A) Ad-APN attenuated the E/A ratio by 37% in WT-aldosterone mice treated with Ad-APN vs. WT-aldosterone mice treated with Ad-βgal (***P*<0.01). B) Ad-APN decreased E/e' by 34% in WT-aldosterone mice vs. WT-aldosterone mice treated with Ad-βgal (††*P*<0.01).

Table 1

Characteristics of WT/APNTG Mice 4 Weeks after Saline/Aldosterone Infusion

Groups	WT-saline *n* = 7	APNTG-saline *n* = 7	WT-aldo *n* = 15	APNTG-aldo *n* = 15
General				
Body Weight (g)	21.9±1.2	22.5±2.4	22.5±0.7	24.2±1.0
Heart Rate (beats/min)	676±13	679±18	654±13	675±12
Systolic Blood Pressure (mm Hg)	105±2	100±2	127±3††	125±4‡‡
Heart weight/Body Weight (mg/g)	4.35±0.78	4.42±0.21	5.69±0.15††	5.14±0.07‡‡, *
Wet-Lung Weight/Dry-Lung Weight	3.97±0.06	3.99±0.11	4.48±0.05††	4.25±0.06‡, *
Fibrosis area (%)	1.0±0.1	0.9±0.2	6.7±0.7††	4.3±0.8‡, *
Blood Chemical Analysis				
Serum aldosterone levels (pg/mL)	626.0±66.4	810.7±85.9	6542.6±324.2††	5317.4±551.7‡‡
Serum adiponectin levels (μg/mL)	14.5±1.3	27.0±1.0††	14.0±0.9	29.2±0.3‡‡, *

Data are expressed as mean ± SEM.

Abbreviations: WT; wild-type, APNTG; adiponectin transgenic, aldo; aldosterone

†† $P < 0.01$ vs. WT-saline,

‡ $P < 0.05$ vs. APNTG-saline,

‡‡ $P < 0.01$ vs. APNTG-saline,

* $P < 0.05$ vs. WT-aldo,

** $P < 0.01$ vs. WT-aldo.

WT-saline, ‡$P < 0.05$ vs. APNTG-saline, ‡‡$P < 0.01$ vs. APNTG-saline, *$P < 0.05$ vs. WT-aldo, **$P < 0.01$ vs. WT-aldo.

Table 2

Echocardiographic Parameters of WT/APNTG Mice 4 Weeks after Saline/Aldosterone Infusion

Group	WT-saline *n* = 7	APNTG-saline *n* = 7	WT-aldo *n* = 15	APNTG-aldo *n* = 15
LV Structure and Systolic Function				
TWT (mm)	0.75±0.01	0.73±0.01	1.04±0.02††	0.93±0.02‡‡, **
LVEDD (mm)	3.42±0.06	3.44±0.08	3.49±0.08	3.34±0.23
LVESD (mm)	1.95±0.06	2.04±0.12	2.03±0.07	1.95±0.09
LVEF (%)	75.12±1.59	73.83±1.65	73.96±0.93	75.69±1.62
LV mass (mg)	83.40±2.26	81.38±3.72	140.4±6.52 ††	110.9±12.69‡*
Diastolic Function				
Peak E (mm/s)	654.83±13.79	677.60±14.50	1008.73±27.19††	873.75±20.96‡‡, **
Peak A (mm/s)	454.04±9.37	449.61±15.34	490.50±40.89	591.24±34.94‡
E/A	1.45±0.04	1.51±0.04	2.17±0.17††	1.50±0.09**
DT (ms)	22.08±1.00	21.88±0.63	15.56±1.00††	20.42±0.77**
IVRT (ms)	19.58±0.77	20.63±1.88	19.72±0.77	20.00±1.12
e' (mm/s)	27.26±1.25	28.26±1.56	17.81±0.33††	20.55±0.69‡‡, **
E/e'	24.17±0.72	24.14±1.02	56.84±1.96††	42.67±1.28‡‡, **

Data are expressed as mean± SEM.

Abbreviations: WT; wild-type, APNTG; adiponectin transgenic, aldo; aldosterone, LV; left ventricular, LVEDD; LV end-diastolic diameter, LVESD; LV end-systolic diameter, TWT; total wall thickness, LVEF; LV ejection fraction, E; early, A; late, DT; early filling deceleration time, IVRT; isovolumic relaxation time, e'; peak early diastolic myocardial velocity.

†† $P < 0.01$ vs. WT-saline,

‡ $P < 0.05$ vs. APNTG-saline,

‡‡ $P < 0.01$ vs. APNTG-saline,

* $P < 0.05$ vs. WT-aldo,

** $P < 0.01$ vs. WT-aldo.