Activation of the Mineralocorticoid Receptor Increases Striatin Levels

Luminita H. Pojoga[1], Patricia Coutinho[1], Alicia Rivera[2], Tham M. Yao[1], Enrique R. Maldonado[1], Rodeler Youte[1], Gail K. Adler[1], Jonathan Williams[1], Alexander Turchin[1], Gordon H. Williams[1] and Jose R. Romero[1]

BACKGROUND
Aldosterone (ALDO), a critical regulator of sodium homeostasis, mediates its effects via activation of the mineralocorticoid receptor (MR) through mechanisms that are not entirely clear. Striatin, a membrane associated protein, interacts with estrogen receptors in endothelial cells.

METHODS
We studied the effects of MR activation *in vitro* and *in vivo* on striatin levels in vascular tissue.

RESULTS
We observed that dietary sodium restriction was associated with increased striatin levels in mouse heart and aorta and that striatin and MR are present in the human endothelial cell line, (EA.hy926), and in mouse aortic endothelial cells (MAEC). Further, we show that MR co-precipitates with striatin in vascular tissue. Incubation of EA.hy926 cells with ALDO (10^{-8} mol/l for 5–24 h) increases striatin protein and mRNA expression, an effect that was inhibited by canrenoic acid, an MR antagonist. Consistent with these observations, incubation of MAEC with ALDO increased striatin levels that were likewise blocked by canrenoic acid. To test the *in vivo* relevance of these findings, we studied two previously described mouse models of increased ALDO levels. Intraperitoneal ALDO administration augmented the abundance of striatin protein in mouse heart. We also observed that in a murine model of chronic ALDO-mediated cardiovascular damage following treatment with N^G-nitro-L-arginine methyl ester plus angiotensin II an increased abundance of striatin protein in heart and kidney tissue.

CONCLUSION
Our results provide evidence that increased striatin levels is a component of MR activation in the vasculature and suggest that regulation of striatin by ALDO may modulate estrogen's nongenomic effects.

Keywords: *aldosterone; angiotensin; animal physiology; antagonists; blood pressure; endothelial cells; heart tissue; hypertension; inflammation; L-NAME; mineralocorticoid receptor; RAAS*

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Aldosterone (ALDO) is regulated by sodium intake and has direct adverse effects on the vasculature.[1] Administration of mineralocorticoids leads to heart lesions.[2] Impaired left ventricular diastolic function and prolonged PQ intervals are observed in patients with primary hyperaldosteronism.[3,4] In addition, circulating ALDO levels correlate with cardiac damage[5] and clinical studies with mineralocorticoid receptor (MR) antagonists show decreased morbidity and mortality in patients with heart failure.[6] Consistent with these observations, animal studies demonstrated that mineralocorticoid excess may lead to myocardial inflammation and fibrosis.[7,8] *In vitro* studies demonstrate direct effects of ALDO on plasminogen activator inhibitor-1 expression in cardiac cells[9] and cardiac fibrosis and heart failure have been observed in a transgenic mouse model overexpressing 11β-hydroxysteroid dehydrogenase type 2 in cardiomyocytes.[10] Moreover, MR interacts specifically with the cardiac myosin binding protein, suggesting a role of MR in cardiac remodeling.[11]

Increased ALDO levels lead to numerous signaling events.[12] ALDO's classical epithelial effect is to increase the transport of sodium via the effects of MR as a ligand-dependent transcription factor. These effects of ALDO are mediated in part by vesicular trafficking to the plasma membrane of proteins such as vacuolar H^+-ATPase, Na^+/H^+ exchange isoform 3 and the epithelial sodium channel.[13,14] There is evidence that ALDO mediates epithelial sodium channel trafficking to lipid rafts in the plasma membrane of A6 cells. However the mechanisms by which ALDO alters vascular tissue are not clear.

Striatin is abundant in neurons where it is proposed to function as a scaffolding protein that interacts with mediators of vesicular trafficking.[15–17] Striatin contains several protein association domains: a caveolin (cav)-binding motif, a coiled-coil structure, a Ca^{2+}-calmodulin-binding site and a large WD-repeat domain.[15,18] There is evidence that striatin's WD-repeat domain interacts with GαI protein and Protein

[1]Division of Endocrinology, Diabetes and Hypertension, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School; Boston, Massachusetts, USA; [2]Department of Laboratory Medicine and Pathology, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts, USA. Correspondence: José R. Romero (jrromero@rics.bwh.harvard.edu)

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Phosphatase 2A (PP2A) allowing for rapid activation of several transduction molecules (e.g., endothelial nitric oxide synthase) and mitogen-activated protein kinase [19]

More recently, striatin has been shown to be a key intermediary of the effects of estrogen receptor-α (ERα) activation.[20] Lu *et al.* provided evidence that striatin's N-terminal segment interacts with the DNA binding domain of ERα in the immortalized human endothelial cell line (EA.hy926) cells. This interaction organizes ERα-endothelial nitric oxide synthase membrane signaling leading to rapid nongenomic activation of endothelial nitric oxide synthase in EA.hy926 cells. Thus, the association between ERα and striatin raise the possibility that striatin may also interact with the MR.

We determined the effects of salt restriction on striatin levels in vascular tissue. We studied the *in vivo* effects of dietary sodium restriction in mice and observed marked increases in the levels of striatin in the heart. Our results also show that vascular tissue express striatin and that striatin co-precipitates with MR. We then studied the direct effects of ALDO on striatin levels in human and mouse endothelial cells and tested the *in vivo* relevance of our findings in two mouse models of MR activation. Our results suggest that MR interacts with striatin and are consistent with a direct effect of ALDO on striatin levels *in vivo*.

METHODS

Mouse aortic endothelial cells. Aortic endothelial cells were isolated as previously described.[21] Briefly, thoracic aortas were perfused with PBS containing 1,000 U/ml heparin and dissected out and placed in 20% Fetal bovine serum-Dulbecco's Modified Eagle Medium with 1,000 U/ml heparin. Aortas were filled with 2 mg/ml collagenase II (Sigma-Aldrich, St Louis, MO). Endothelial cells were cultured in type I collagen-coated T25 flasks in 20% Fetal bovine serum-Dulbecco's Modified Eagle Medium containing: 100 U/ml penicillin, 100 µg streptomycin, 2 mmol/l L-glutamine, 1% MEM amino acids, 1% sodium pyruvate, 100 µg/ml heparin (Invitrogen, Carlsbad, CA), 100 µg/ml endothelial cell growth supplements (Sigma-Aldrich) and incubated in 5% CO_2 at 37 °C in a humidified atmosphere. The cells were used at passages 2–3. The purity of the cultures was confirmed by testing specific monoclonal antibodies against vWF and PECAM-1.

Electrophoresis, immunoblot, and immunoprecipitation. Analyses were performed as previously described.[22] Tissues were homogenized in radioimmunoprecipitation assay buffer (Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were analyzed using enhanced chemiluminescence (Perkin-Elmer Life Sciences, Boston, MA). Quantitative data are presented as fold change relative to controls. Primary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA): rabbit anti-MR (cat. no. sc11412, 1:1000), BD Transduction Laboratories (San Diego, CA): mouse monoclonal anti-striatin (cat. no. 610838, 1:1000), Sigma-Aldrich: mouse monoclonal anti-β-actin (cat. no. A5316, 1:1000) and AbCam (Cambridge, MA): rabbit anti-β-tubulin (cat. no. ab6046, 1:1000). For immunoprecipitation experiments, protein extracts (500 µg) were mixed with 1 µg of corresponding primary antibody and 50 µl µMACS protein A or G MicroBeads (Miltenyi Biotec, Auburn, CA) and then incubated at 4 °C for 1–2 h. The mixture was then loaded on top of Miltenyi MACS separation columns and eluted according to manufacturer's protocol. The immunoprecipitates were then analyzed as described.[22]

Real-time polymerase chain reaction. Quantitative real-time polymerase chain reaction was performed as previously described.[23] Briefly, total mRNA was extracted using the RNeasy mini kit (Qiagen, CA, USA). The ABI PRISM 7000 Sequence Detection System real-time quantitative PCR (Applied Biosystems, Foster City, CA) was used to perform the real-time PCR using TaqMan Gene Expression Assays for both mouse or human striatin (Applied Biosystems). Reactions were analyzed with the ABI software using the ΔΔCT method. Target gene expression was normalized to 18S rRNA levels.

siRNA knockdown of striatin. EA.hy926 cells were transfected with ON-TARGETplus siRNA predesigned duplex specific for striatin obtained from Dharmacon RNAi Technologies, Thermo Scientific (Chicago, IL). Control/blank siRNA was transfected in parallel with striatin siRNA following the manufacturer's protocols and using the Dharmafect 1 siRNA Transfection Reagent from Dharmacon RNAi Technologies. The cells were harvested for western blot analyses 48 h post-transfection.

EA.hy926. EA.hy926 cells were grown as originally described in 10% Fetal bovine serum-Dulbecco's Modified Eagle Medium and split 1:16 at confluence.[24]

Animals. Our studies followed guidelines approved by the Institutional Animal Care and Use Committee at Harvard Medical School that conforms with the *Guide for the Care and Use of Laboratory Animals* (US National Institutes of Health Publication No. 85–23). We studied male mice (C57Bl/6J) from Jackson Laboratory (Bar Harbor, ME). Animals were housed in a room lighted 12 h/day at an ambient temperature of 22 ± 1 °C and were allowed 1–3 weeks to recover after arrival with free access to Purina Lab Chow 5001 (Ralston Purina, St Louis, MO) and tap water unless stated otherwise.

Dietary sodium restriction model. Twelve-week-old male mice were randomized to either high-salt (HS) (4% NaCl) or low-salt (LS) (0.08% NaCl) diets (Purina, St Louis, MO) for 11 days to achieve sodium balance as previously described.[23] Animals were euthanized under isoflurane anesthesia, the thoracic and abdominal cavities were opened, and the aortas and hearts were rapidly excised. Tissues were placed in liquid nitrogen after collection.[22,23]

In vivo ALDO administration model. Male C57BL/6 mice (Charles River, Wilmington, MA) were uninephrectomized at 11 week of age and allowed 7 day recovery time. Starting at 12 week of age, all animals were fed 3% NaCl diet (Purina Test Diet Salt Series based on Basal Diet 5755, Pharmaserv,

Framingham, MA) for 1 week. Mice were administered via intraperitoneal injection of 125 µl mouse serum as control or 250 ng (~10 µg/kg) ALDO (Acros Organics, Geel, Belgium) diluted in 250 nl ethanol as described previously.[25] Five animals in each control and experimental groups were euthanized as described above, at the following intervals after the injection: 0 h (no injection), 0.5, 1, 2, and 3 h.

AngII/L-NAME mouse model. Animals received placebo or a combination treatment of N^G-nitro-L-arginine methyl ester (L-NAME) (0.2 mg m/l drinking fluid) and angiotensin II (AngII) (2.8 mg/kg/day), unless otherwise stated. L-NAME (Sigma-Aldrich) was administered in drinking water from days 1 to 11. Vehicle or Ang II (American Peptide, Sunnyvale, CA) was administered on days 8 through 11 via Alzet osmotic subcutaneous minipumps (Model 1007D, DURECT, Cupertino, CA). All mice consumed a high-sodium diet, 4% NaCl-supplemented rodent chow. Animals were euthanized on day 11 as described above.

RESULTS

EA.hy926 cells and early cultures of mouse aortic endothelial cells express striatin and the MR

We performed western blot analyses and observed the presence of striatin in EA.hy926 cells (**Figure 1a**).[24] We examined the specificity of our striatin antibody by reducing striatin levels in EA.hy926 cells with striatin-specific siRNA. As shown in **Figure 1b**, the striatin band intensity at 110 kDa was reduced following striatin siRNA in these cells. These results provide evidence for the specificity of this antibody to recognize striatin.

To determine whether the presence of striatin was unique to EA.hy926 cells, we prepared early cultures of mouse aortic endothelial cells (MAEC) following established protocols.[21] We confirmed that EA.hy926 cells and MAEC were endothelial in origin by positive immunohistochemical staining for vWF and PECAM-1 (CD31), two endothelial cell markers, but lacked staining for α-smooth muscle actin, a smooth muscle cell marker (data not shown). MAEC and mouse heart likewise expressed striatin (**Figure 1c**) and MR (**Figure 1d**).

In EA.hy926 cells, striatin co-precipitates with the ERα subunit.[20] We studied whether a similar relationship would occur with MR. Our results show that immunoprecipitation of EA.hy926 cells, mouse heart, and MAEC with anti-striatin antibody allows detection of MR (**Figure 1e,f**).

Dietary sodium restriction increases striatin levels in mouse hearts and aorta

We studied a sodium restriction mouse model that we described leads to increased ALDO levels.[26] As shown previously, mice given a LS diet for 11 days had significantly higher plasma ALDO levels (64.18+/−14.30 ng/dl, (n = 6)) than those on a HS diet (34.26+/−4.99 ng/dl (n = 7)) for 11 days. We isolated heart tissue from these mice and observed that striatin levels were significantly higher in mice on LS when compared to mice on a HS diet as were their serum ALDO levels (**Figure 2a**). Consistent with these results, we also

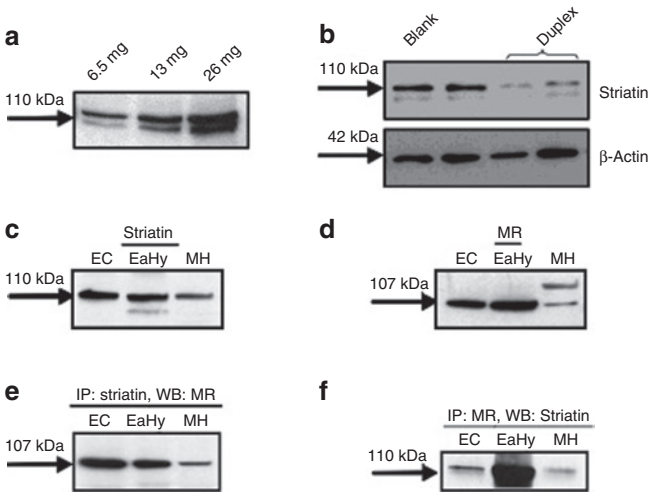

Figure 1 | Striatin and the mineralocorticoid receptor are expressed and co-immunoprecipitate in early cultures of mouse aortic endothelial cells, EA.hy926 cells, and mouse heart tissue. (**a**) EA.hy926 cells were lysed and protein levels quantified as described in the Methods. Cell lysates (6.5–26.0 µg) were analyzed by western blotting for striatin levels. (**b**) Prior to cell lysis and western blotting for striatin, EA.hy926 cells were either untransfected (blank, lane 1) or transfected with scrambled siRNA (lane 2) or anti-striatin duplex (lanes 3–4), as described under Methods. Early cultures of mouse aortic endothelial cells (MAEC), EA.hy926 cells and mouse heart tissue were processed for western blotting for striatin (**c**) or (**d**) mineralocorticoid receptor (MR). (**e**) Immunoprecipitation with striatin flowed by western blot for MR. (**f**) Immunoprecipitation with MR followed by western blot for striatin as described under Methods. EA.hy926, human endothelial cell line.

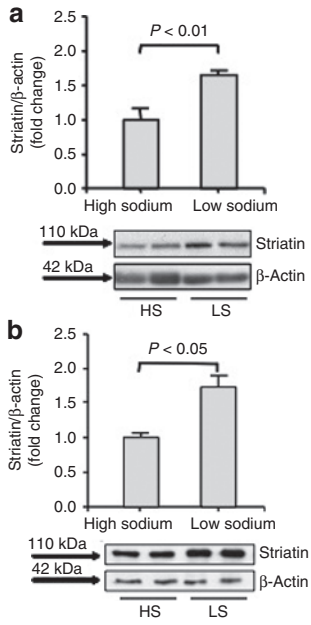

Figure 2 | Dietary sodium restriction increases striatin levels in mouse hearts. (**a**) Mouse heart tissues were obtained from mice maintained on a high-sodium (HS) (n = 7) or low-sodium (LS) diet (n = 6) for 11 days. (**b**) Mouse aorta were obtained from mice maintained on a high- (n = 3) or low-sodium diet (n = 4) for 11 days. Tissue homogenates were analyzed for striatin and β-actin protein levels as described in Methods.

observed that striatin levels were significantly higher in aortas from LS mice when compared to HS mice (Figure 2b). We also measured blood pressure in these mice by tail cuff.[23] As shown previously under these conditions, LS was associated with lower blood pressure than HS (108.8 ± 2.8 mm Hg vs 114.7 ± 3.1 mm Hg, LS vs. HS respectively, mean ± standard deviation, $P < 0.03$).[23]

ALDO increases the expression of striatin in endothelial cells via activation of the MR

We characterize the direct *in vitro* effects of ALDO on striatin levels in EA.hy926 cells incubated with 10 nmol/l ALDO for 1–24 h at 37 °C. Our results show that incubation of cells with ALDO increased striatin levels by 5 h that returned to baseline by 12 h (Figure 3a). We then studied the effect of cells incubated with 10 nmol/l ALDO in the presence or absence of canrenoic acid, a competitive ALDO antagonist to MR, on striatin levels. We confirmed that ALDO increased striatin protein levels and that preincubation with 1 μmol/l canrenoic acid prevented this increase (Figure 3b). The effect of ALDO on striatin mRNA expression levels using quantitative real-time polymerase chain reaction with specific Taqman Assays for striatin was analyzed. We observed that 10 nmol/l ALDO for 5 h at 37 °C led to increased expression of striatin mRNA in these cells (Figure 3c). These results suggest a genomic effect of ALDO that is specific for striatin expression. Consistent with this proposal, we have identified several potential MR response elements in the 5′ region of the striatin gene (data not shown). Furthermore, treatment for 5 h with 100 nmol/l dexamethasone had no significant effect on striatin mRNA levels in EA.hy926 cells, suggesting specificity of ALDO-mediated MR activation (data not shown). We also studied the effect of ALDO on striatin levels in MAEC, and observed that ALDO stimulated an increase in striatin protein levels that was likewise sensitive to canrenoic acid (Figure 4a).

Acute *in vivo* administration of ALDO increases striatin levels in mouse hearts

To characterize the *in vivo* relevance of these findings, we studied the effects of ALDO on striatin levels in mouse heart by using additional mouse models of MR activation. We analyzed the effects of an acute *in vivo* ALDO administration on striatin levels in the heart. We included at each time point a response to vehicle injection, thus allowing us to distinguish the effects of experimental method from those of ALDO administration. Male C57BL/6 mice on a 3% NaCl diet were injected IP with either 10 μg/kg of ALDO or vehicle and sacrificed at the following time points after the injection: 0 h (no injection), 1, 2, and 3 h (five mice in each group at each time point). We observed that in isolated heart tissue from ALDO-treated mice, striatin levels increased when compared to vehicle injected mice (Figure 4b). Plasma ALDO concentrations were determined in each animal as previously reported.[25] Plasma ALDO levels increased only in the ALDO injected group where the highest levels reached 280 ng/dl at 30 min.

Treatment of AngII/L-NAME increases striatin levels in mouse hearts and kidneys

We previously studied an animal model of acute, generalized, multiple organ injury that is secondary to vascular inflammation.[27,28] In this model, we treat mice with the nitric oxide synthase inhibitor, L-NAME, in combination with AngII. This treatment induces myocardial damage initiated at the vascular level that can be prevented by

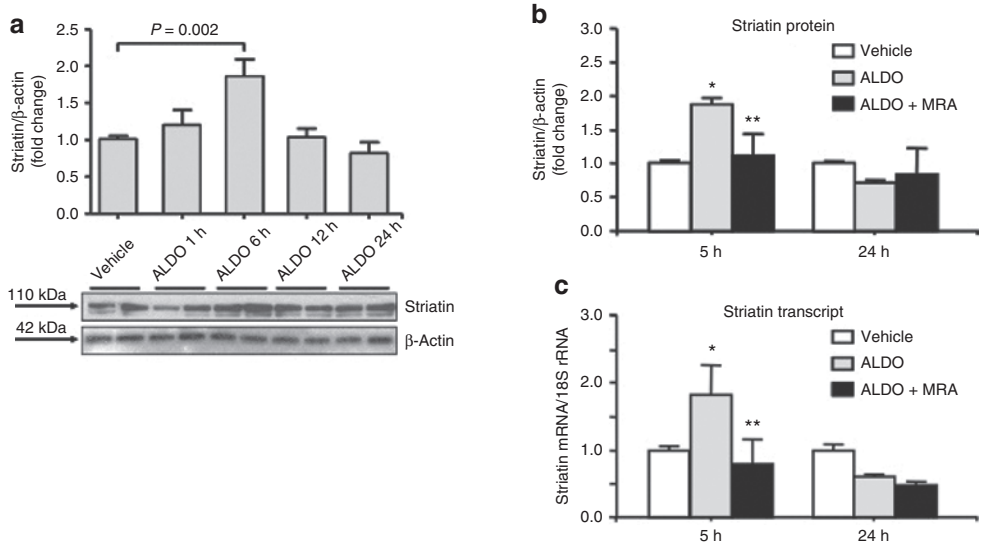

Figure 3 | The aldosterone-induced increases in striatin expression are mediated via activation of the mineralocorticoid receptor (MR) in EA.hy926 cells. (**a**) EA.hy926 cells were incubated with 10 nmol/l ALDO for 1, 5, 12, 24 h. Cell lysates were analyzed for striatin protein levels as described in Methods. Data were normalized to β-actin. (**b**) EA.hy926 cells were incubated with vehicle (white bars) or 10 nmol/l ALDO (gray bars) for 5 h and 24 h in the absence or presence (black bars) of the MR antagonist, canrenoic acid (MRA) and analyzed for striatin protein normalized to β-actin (**b**) and mRNA normalized to (**c**) 18S rRNA. *$P < 0.05$ vs. vehicle-treated cells. **$P < 0.05$ vs. ALDO-treated cells. ALDO, aldosterone; EA.hy926, human endothelial cell line.

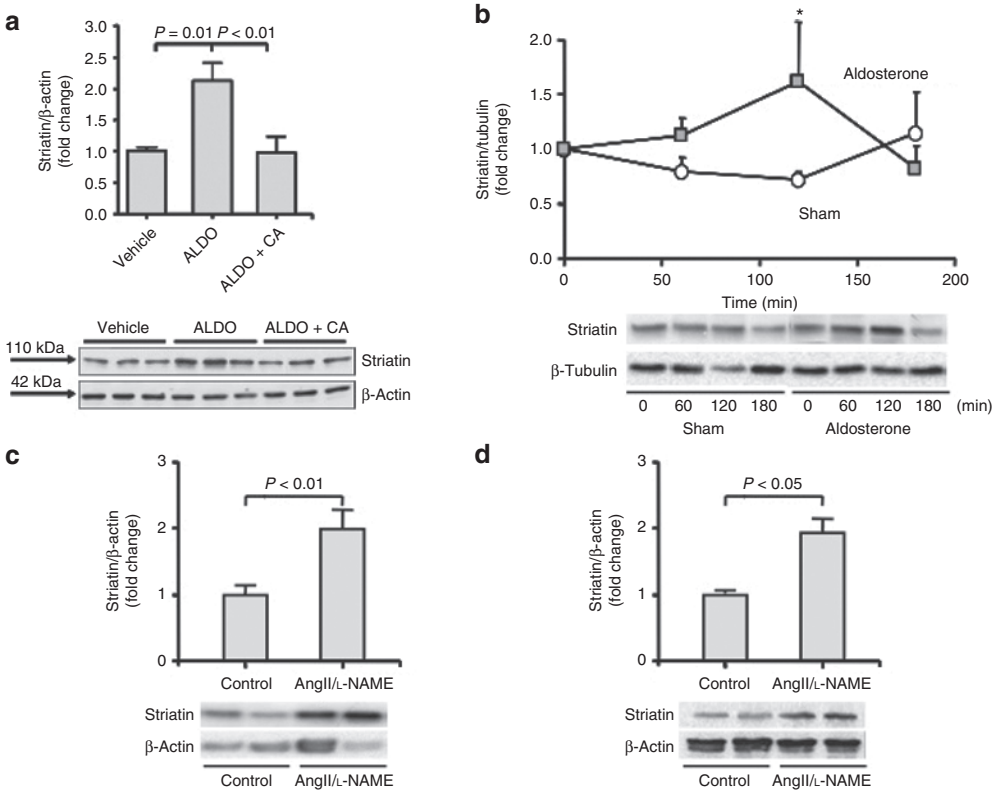

Figure 4 | The *in vivo* and *in vitro* effects of increased aldosterone on striatin levels in mice. (**a**) Aldosterone (ALDO) increases striatin expression via activation of the mineralocorticoid receptor (MR) in early cultures of mouse aortic endothelial cells. Mouse aortic endothelial cells (MAEC) were incubated with 10 nmol/l ALDO for 5 h, in the presence or absence of the MR antagonist canrenoic acid. Cell lysates were analyzed for striatin and β-actin protein levels as described in Methods. (**b**) Acute *in vivo* administration of aldosterone increases striatin levels in mouse heart. Heart tissues were obtained from mice at 0, 1, 2, or 3 h after acute intraperitoneal administration of ALDO (10 µg/kg, gray squares) or vehicle (Sham, white circles). Tissue homogenates were analyzed for striatin and β-tubulin protein levels as described in Methods. **P* < 0.05 vs. Sham. (**c**,**d**) Treatment of mice with Ang II/L-NAME increases striatin levels in mouse (**c**) heart and (**d**) kidney. Heart and kidney tissues were obtained from mice treated with placebo (control) or L-NAME/AngII for 11 days. Tissue homogenates were analyzed for striatin and β-actin protein levels as described in Methods. EA.hy926, human endothelial cell line. AngII, angiotensin II; L-NAME, N^G-nitro-L-arginine methyl ester.

MR blockade or by adrenalectomy.[27,29] Thus we used this AngII/L-NAME model as a "*stressor*" to assess the interaction of MR activation on striatin. Our results show that AngII/L-NAME treatment is associated with increased striatin levels in heart (**Figure 4c**) and kidney tissues (**Figure 4d**) when compared to vehicle treated control. In these studies, AngII/L-NAME treatment increased circulating ALDO levels (122.1+/−34.1 ng/dl (n = 6)) when compared to vehicle treatment (34.26+/−4.99 ng/dl (n = 7); P = 0.008). In addition, we measured blood pressure in these mice. As shown previously, AngII/L-NAME was associated with increased blood pressure vs. vehicle treated mice (120 ± 3.8 mm Hg vs 161 ± 23 mm Hg, vehicle vs. AngII/L-NAME respectively, mean ± standard deviation, $P < 0.04$).[22]

DISCUSSION

The studies presented herein characterized the effects of MR activation on striatin levels, a protein that has been shown to be associated with steroid receptors. We provide evidence that MR activation leads to increased striatin levels in two cell lines and heart tissue from three *in vivo* mouse models of MR activation. Furthermore, our studies in both human and mouse endothelial cells demonstrate that ALDO increases striatin protein and mRNA levels via MR activation *in vitro* and suggest the existence of a striatin/MR complex.

Striatin, abundant in the brain, is detected in fibroblasts and cardiac tissue.[15,30,31] We now report its endogenous expression in both mouse and human endothelial cells. Striatin and its family members are characterized by caveolin binding sites[30,32,33] and are reported to function as molecular anchors and scaffolds that interact with cav-1[33] in caveolae mediating the downstream, nongenomic transduction effects of estrogen.[20] Striatin has also been shown to interact with PP2A to regulate vesicular trafficking.[17,31] Few studies have reported on the physiological functions of striatin. In one study, down-regulation of striatin *in vivo* using antisense oligonucleotides against striatin resulted in decreased locomotor activity and reduced dendritic growth *in vitro*.[34]

The cav-1 rich caveolae are docking sites for several steroid receptors, such as ERα and the androgen receptor.[35,36] Recent evidence shows MR present in vascular tissue caveolae and that cav-1 is critical for the cellular effects of ALDO.[22,37,38] Furthermore, ALDO mediates epithelial sodium channel and cav trafficking to lipid rafts in A6 cells.[39] Our results using

co-immunoprecipitation studies expand on these observations by documenting that striatin is associated with another steroid receptor, MR. The large number of receptors and proteins, such as striatin, that anchor at the caveolae strongly suggest that alterations in components of the caveolae may have major effects on signaling pathways. Thus, it is tempting to speculate that MR forms a complex with striatin and cav-1 in vascular tissue and that modulation of striatin levels may alter vascular function via modulation nitric oxide signaling and PP2A signaling. Indeed, ALDO has been reported to regulate endothelial nitric oxide synthase by PP2A activation in endothelial cells.[40]

We examined three animal models of MR activation to study the chronic and acute effects of ALDO on striatin levels in the heart. In one model, we take advantage of the fact that the renin-angiotensin-ALDO system is modified during changes in dietary sodium intake where chronic LS diet is associated with increased ALDO.[26] We have also used an animal model of generalized multiple organ injury that is secondary to vascular inflammation where we treat mice with the nitric oxide synthase inhibitor L-NAME and AngII to induce myocardial damage.[1,27,29] We reasoned that if striatin is ALDO sensitive, then changing dietary sodium intake or administrating L-NAME followed by AngII should likewise modulate striatin levels. We observed that in both chronic models of MR activation, the levels of striatin were increased in heart tissue. Consistent with these effects, in our acute model of MR activation by intraperitoneal ALDO injection when compared to placebo injection, similar results were observed. It is important to note that this latter experimental design allows us to separate the effects of the experimental method from those of ALDO. Indeed, we have previously reported that intraperitoneal injections with either ALDO or placebo were associated with increases in circulating corticosterone levels in this murine model.[25] However, only in mice that were injected with ALDO did circulating ALDO increase. These *in vivo* observations further support the contention that ALDO regulates striatin and that the MR/striatin complex may mediate some of the membrane-related effects of ALDO. However, the cellular effects of increasing striatin levels and how this may in turn modulate steroid receptor function (e.g., MR and ERα activation) are at the present unknown. Further studies are needed to determine the potential mechanisms involved and should include the *in vivo* use of an MR antagonist, to determine whether striatin plays a role in end-organ damage.

There are some limitations to our studies. Sodium restriction and AngII/L-NAME also lead to changes in AngII, renin levels, and blood pressure. However a potential role for AngII and renin on striatin is unlikely since (i) the *in vitro* studies confirm that ALDO has a direct effect on striatin and (ii) acute *in vivo* administration of ALDO leads to suppression of AngII and renin. Therefore, in all three *in vivo* studies both ALDO and striatin are increased while opposite effects on AngII levels are seen during acute ALDO administration when compared to the salt restriction. Regarding blood pressure, dietary sodium restriction was associated with lower blood pressure when compared to high salt while AngII/L-NAME showed opposite effects on blood pressure. Thus it seems that striatin increases in these *in vivo* mouse models of MR activation are independent of blood pressure changes.

Our findings are consistent with the hypothesis that MR activation is a key factor in regulating striatin expression in heart and endothelial cells. We posit that some of the chronic and acute effects of MR activation *in vivo* may be secondary to the effects of increased ALDO on striatin expression. Thus, our results together with those of others implicate striatin as a critical component of a protein complex that mediates the effects of steroid receptor activation.

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