HHS Public Access

Author manuscript

Hypertension. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Hypertension. 2015 September ; 66(3): 674–680. doi:10.1161/HYPERTENSIONAHA.115.05600.

Critical Role of Striatin in Blood Pressure and Vascular Responses to Dietary Sodium Intake

Amanda E. Garza[1], **Luminita H. Pojoga**[1], **Burhanuddin Moize**[1], **Wan M. Hafiz**[1], **Lauren A. Opsasnick**[2], **Waleed T. Siddiqui**[2], **Michael Horenstein**[2], **Gail K. Adler**[1], **Gordon H. Williams**[1], and **Raouf A. Khalil**[2]

[1]Cardiovascular Endocrine Section, Division of Endocrinology, Diabetes and Hypertension, Boston, Massachusetts 02115

[2]Division of Vascular Surgery, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts 02115

Abstract

Striatin is a protein regulator of vesicular trafficking in neurons that also binds caveolin-1 and Ca^{2+}-calmodulin, and could activate eNOS. We have shown that striatin colocalizes with the mineralocorticoid receptor, and that mineralocorticoid receptor activation increases striatin levels in vascular cells. To test if striatin is a regulator of vascular function, wild-type and heterozygous striatin-deficient mice ($Strn^{+/-}$) were randomized in crossover intervention to restricted (0.03%) and liberal sodium (1.6%) diets for 7 days on each diet, and blood pressure and aortic vascular function were measured. Compared to wild-type, sodium restriction significantly reduced blood pressure in $Strn^{+/-}$. On liberal salt intake, phenylephrine and high KCl caused a greater vascular contraction in $Strn^{+/-}$ than wild-type, and endothelium removal, NOS inhibitor L-NAME and guanylate cyclase inhibitor ODQ enhanced phenylephrine contraction to a smaller extent in $Strn^{+/-}$ than WT. On liberal salt, acetylcholine relaxation was less in $Strn^{+/-}$ than wild-type, and endothelium removal, L-NAME and ODQ blocked acetylcholine relaxation, suggesting changes in endothelial NO-cGMP. On liberal salt, eNOS mRNA expression and the ratio of eNOS activator pAkt/total Akt were decreased in $Strn^{+/-}$ versus wild-type. Vascular relaxation to NO donor sodium nitroprusside was not different among groups. Thus striatin deficiency is associated with salt sensitivity of blood pressure, enhanced vasoconstriction and decreased vascular relaxation, suggesting a critical role for striatin, through modulation of endothelial NO-cGMP, in regulation of vascular function and BP during changes in sodium intake.

Keywords

striatin; endothelium; nitric oxide; smooth muscle; contraction; blood pressure

Correspondence and Reprints: Raouf A. Khalil, MD, PhD, Brigham and Women's Hospital, Division of Vascular Surgery, 75 Francis Street, Boston, MA 02115, Tel: (617)525-8530, Fax: (617)264-5124, raouf_khalil@hms.harvard.edu.

Conflict of Interest/Disclosures: None.

Introduction

Striatin is a 780-amino acid protein that was identified and cloned in the late 1990s.[1] At the molecular level, striatin has four protein-protein interaction domains: a caveolin-1 (cav-1)-binding domain; a coiled-coil structure; a Ca^{2+}-calmodulin binding domain and a large WD-repeat domain at the C-terminal region.[2] Striatin plays a role in vesicular trafficking in neurons.[1,2] Striatin also binds regulatory proteins such as cav-1, Ca^{2+}-calmodulin,[3] $G_{\alpha i}$ and phosphatase 2A, and could regulate transduction molecules such as endothelial nitric oxide synthase (eNOS) and mitogen-activated protein kinase (MAPK).[2]

In 2004, Lu and coworkers showed that striatin could bind estrogen receptor-α (ERα) and mediate rapid non-genomic activation of eNOS upon activation of that ER subtype,[4] and these actions may contribute to the protective effects of estrogen against vascular injury.[5] We have recently reported a similar interaction between striatin and the mineralocorticoid receptor (MR), and demonstrated that MR co-localizes with cav-1 and striatin in endothelial cells and the heart,[6] and that activation of MR increases striatin levels in endothelial cells.[7] Also, we have reported that a polymorphic variant in the striatin gene is associated with salt-sensitive blood pressure (BP) in hypertensive human subjects, and that a striatin heterozygote knock-out mouse ($Strn^{+/-}$) shows salt sensitivity of BP.[8] Importantly, in contrast to the usual association of salt sensitivity of BP with hypertension, the $Strn^{+/-}$ mouse has similar BP to the wild-type (WT) mouse on a liberal salt intake but shows a significant reduction in BP when salt is removed from the diet compared to its WT littermates. These observations suggest that striatin may be involved in regulating factors that maintain normal volume homeostasis. In support of this possibility, we have reported that aldosterone responses to salt manipulations differ between $Strn^{+/-}$ and WT mice.[8] In this report, we assess the hypothesis that the salt sensitivity of BP in striatin-deficient states reflects defects in the vasoconstrictor/vasodilator responses of the vasculature to changes in dietary salt intake.

Methods

Animals

Male (12 weeks of age) heterozygous striatin-deficient mice lacking one allele for striatin ($Strn^{+/-}$, CSD26933) genetically-engineered by the trans-NIH 23 Knock-Out Mouse Project (KOMP), and WT littermates were randomized in a crossover intervention to restricted (ResS, 0.03%) and liberal sodium (LibS, 1.6%) diets for 7 days on each diet to achieve sodium balance as previously described.[8-10] All experiments followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the guidelines of Harvard Medical Area Standing Committee on Animals.

Urinary Sodium

To confirm sodium balance, 24 h urine was collected and stored at -80°C until assayed. Urinary sodium was measured according to the manufacturer's Stanbio Sodium Kit (Stanbio Laboratory) (Online Fig. S1).

Blood pressure (BP)

After reaching sodium balance at the end of 7 days on each of the specific sodium diet, systolic BP was measured in conscious mice using tail-cuff plethysmography and the CODA noninvasive BP system (KENT Scientific). We have previously demonstrated excellent correlation between systolic BPs assessed simultaneously by tail cuff and telemetry in mice.[11]

Tissue preparation

In euthanized mice, the thoracic aorta was excised, cleaned of connective tissue, and cut into 2 mm-wide rings for vascular function experiments. Other segments of the aorta were placed in liquid nitrogen for mRNA and protein analysis.

Isometric contraction and relaxation

Aortic vascular function was measured as previously described [12] and detailed in Data Supplement.

Western blot analysis

Protein amounts of striatin, eNOS and Akt were measured as previously described [8,12] and detailed in Data Supplement.

Real time RT-PCR

Expression of eNOS mRNA was measured as previously described [13] and detailed in Data Supplement.

Statistical analysis

Data were presented as means±SEM, with "n" = number of mice. Data were first analyzed using ANOVA for comparison of different groups (striatin status vs. salt treatment). When a statistical difference was observed, the data were further analyzed using Student-Newman-Keuls *post-hoc* test for multiple comparisons. Pairwise comparisons were also performed using paired or unpaired Student's *t*-test. Differences were considered significant if $P < 0.05$. In all studies, experiments were performed blinded to animal genotype and treatment.

Results

Western blot analysis confirmed that striatin protein was reduced in aorta of $Strn^{+/-}$ vs. WT mice (Fig. 1A). BP was significantly different during changes from ResS to LibS diet in $Strn^{+/-}$ but not WT (Fig. 1B). Some of the BP data were partially presented in a previous report.[8]

In endothelium-intact aorta, phenyephrine (Phe) caused concentration-dependent contraction that was not different in WT-LibS vs. WT-ResS or $Strn^{+/-}$-ResS vs. WT-ResS, but was enhanced in $Strn^{+/-}$-LibS vs. $Strn^{+/-}$-ResS and WT-LibS (Fig. 2A, Online Table S1). To test if the enhanced contraction in $Strn^{+/-}$-LibS is due to changes in α-adrenergic receptor sensitivity, Phe contraction was presented as % of maximum and Phe ED_{50} was calculated. The Phe concentration-response curve was similar, and the Phe ED_{50} was not different

between Strn$^{+/-}$ and WT on ResS or LibS (Fig. 2B, Online Table S1). To test if the changes in aortic contraction in Strn$^{+/-}$ are specific to a particular agonist/receptor, the vascular contraction to 96 mM KCl, which causes membrane depolarization and stimulates Ca^{2+} entry into vascular smooth muscle (VSM), was assessed. KCl contraction was not different in WT-LibS vs. WT-ResS or Strn$^{+/-}$-ResS vs. WT-ResS, but was enhanced in Strn$^{+/-}$-LibS vs. both Strn$^{+/-}$-ResS and WT-LibS (Fig. 2C). Relative to KCl contraction, the Phe contraction as % of KCl was not different in Strn$^{+/-}$ or WT mice on ResS or LibS (Fig. 2D, Online Table S1).

We next examined the role of endothelium in the altered vascular responses in Strn$^{+/-}$-LibS mice, Phe contraction was enhanced in endothelium-denuded vs. intact aorta of WT-ResS (164%), WT-LibS (210%), and Strn$^{+/-}$-ResS (173%); however, this enhancement was blunted in Strn$^{+/-}$-LibS (140%) (Online Fig. S2, A-D). Importantly, Phe or KCl contraction in endothelium-denuded vessels was not different between groups (Online Table S1). To test the role of endothelial NO, the aortic rings were pretreated with the NOS inhibitor L-NAME. L-NAME enhanced Phe contraction in WT-ResS (196%), WT-LibS (238%), and Strn$^{+/-}$-ResS (192%); however, this enhancement was blunted in Strn$^{+/-}$-LibS (181%) (Online Fig. S2, A-D). Also, the guanylate cyclase inhibitor ODQ enhanced Phe contraction significantly in WT-ResS (192%), WT-LibS (210%), and Strn$^{+/-}$-ResS (204%), but insignificantly in Strn$^{+/-}$-LibS (175%) (Online Fig. S2, A-D). Phe contraction among L-NAME- or ODQ-treated vessels was not different between groups (Online Table S1).

In all groups, when Phe contraction was presented as % of maximum and the Phe ED_{50} was calculated, the Phe concentration-response curve was shifted to the left and Phe was more potent in endothelium-denuded and L-NAME or ODQ-treated vs. control intact non-treated aorta (Online Fig. S2, E-H, Online Table S1), suggesting a role of endothelial NO-cGMP in dampening the vascular sensitivity to Phe. Also, when Phe contraction was measured relative to KCl, which stimulates Ca^{2+} influx,[14] Phe contraction was enhanced in endothelium-denuded, and L-NAME or ODQ-treated vs. control intact non-treated aorta of all groups (Online Fig. S2, I-L, Online Table S1), suggesting a role of endothelial NO-cGMP in reducing Phe-induced Ca^{2+} sensitization mechanisms of VSM contraction.

Acetylcholine (ACh) caused aortic relaxation that was not different in WT-LibS vs. WT-ResS or Strn$^{+/-}$-ResS vs. WT-ResS, but was reduced in Strn$^{+/-}$-LibS vs. Strn$^{+/-}$-ResS and WT-LibS mice (Fig. 3A). Analysis of ACh ED_{50} showed that ACh was equally potent in inducing relaxation in WT-Res, WT-LibS and Strn$^{+/-}$-ResS, but less potent in Strn$^{+/-}$-LibS vs. Strn$^{+/-}$-ResS (Online Table S1). RT-PCR revealed that eNOS mRNA was not different in aorta of WT-LibS vs. WT-ResS or Strn$^{+/-}$-ResS vs. WT-ResS, but was reduced in Strn$^{+/-}$-LibS vs. WT-LibS (Fig. 3B). Western blots revealed that the amount of total eNOS (Online Fig. S3A), activated peNOS and peNOS/total eNOS ratio were not different between groups (Fig. 3C). Also, total Akt was not different between groups (Fig. 3D). While the amount of the eNOS activator pAkt was not different in aorta of WT-LibS vs. WT-ResS or Strn$^{+/-}$-ResS vs. WT-ResS, the pAkt/total Akt ratio was reduced in Strn$^{+/-}$-LibS vs. WT-LibS (Fig. 3D).

In all groups, ACh relaxation was abolished by endothelium removal, the NOS blocker L-NAME or guanylate cyclase inhibitor ODQ (Fig. 4), supporting a role of endothelial NO-cGMP. The endothelium-dependent and L-NAME- and ODQ-sensitive component of ACh relaxation was less in $Strn^{+/-}$-LibS vs. $Strn^{+/-}$-ResS and WT-LibS (Fig. 4).

Relaxation to the exogenous NO donor sodium nitroprusside (SNP) was not different in aorta of WT and $Strn^{+/-}$ on ResS and LibS (Online Fig. S3B, Online Table S1).

Discussion

We investigated the role of striatin in the regulation of BP and vascular function using a genetically modified mouse lacking one copy of the striatin gene. Striatin protein was reduced in the aorta of these $Strn^{+/-}$ vs. WT mice. Further, striatin deficiency was associated with salt sensitivity of BP and on a liberal salt diet enhanced vasoconstriction and decreased vascular relaxation. We propose that striatin regulates vascular function and BP during changes in dietary sodium intake, through modulation of the endothelial NO-cGMP pathway

Consistent with our previous report,[8] BP was modified significantly by salt intake in $Strn^{+/-}$ but not WT mice. However, the driving force for this difference was not a difference in BP on LibS diet, which was similar in the two genotypes, but rather a lower BP in $Strn^{+/-}$ on ResS diet, such that switching to LibS increased BP and salt restriction decreased BP in $Strn^{+/-}$ but not WT, supporting salt sensitivity of BP in $Strn^{+/-}$ mice. Previous studies in rats and mice have shown that LibS diet alone may not change BP or vascular reactivity,[15,16] likely because any LibS-induced increases in vascular volume and renal blood flow cause feedback inhibition of renin-angiotensin-aldosterone-system and decrease salt and water reabsorption.[17,18] Also, in the presence of intact endothelium, any LibS-induced vasoconstriction is normally counterbalanced by compensatory NO production and vascular relaxation.[15,16] However, in the presence of aberrant renal or vascular mechanisms, LibS diet could change BP. Hence, the observed change in BP in $Strn^{+/-}$-LibS mice could be due to alterations in renal and/or vascular control mechanisms of BP.

We recently demonstrated that aldosterone responses to salt manipulations do differ between $Strn^{+/-}$ and WT mice, and suggested that striatin may interact with factors that maintain normal volume homeostasis.[8] In the present study, we asked whether the salt sensitivity of BP associated with striatin deficiency involves underlying changes in the vascular control mechanisms of BP. We found that Phe contraction was not different in $Strn^{+/-}$ vs. WT on ResS, but was enhanced in $Strn^{+/-}$ vs. WT on LibS. The enhanced Phe contraction in $Strn^{+/-}$-LibS is not due to changes in α-adrenergic receptor sensitivity as Phe ED_{50} was similar in WT and $Strn^{+/-}$ on ResS or LibS. Also, KCl contraction, which mainly involves Ca^{2+} influx,[14] was not different in $Strn^{+/-}$ vs. WT on ResS, but was enhanced in $Strn^{+/-}$-LibS vs. $Strn^{+/-}$-ResS and WT-LibS, suggesting that the enhanced contraction is not specific to a particular agonist/receptor. Assuming that KCl contraction is mainly due to Ca^{2+} influx, the enhanced contraction in $Strn^{+/-}$-LibS is possibly due to activation of a common mechanism involving enhanced Ca^{2+} influx.

Compared with the enhancing effect of LibS on vascular contraction in $Strn^{+/-}$ mice the lack of effect of LibS in WT mice is likely because any vasoconstrictive effects of LibS are counterbalanced by compensatory endothelium-dependent vascular relaxation. The observation that Phe contraction was enhanced in endothelium-denuded vs. intact vessels of WT-LibS is consistent with activation of an endothelium-dependent vasodilator pathway that reduces vascular contraction. This endothelium-dependent vasodilator pathway appears to be less active in $Strn^{+/-}$-LibS mice because: 1) Phe contraction was enhanced to a less extent in endothelium-denuded vs. intact vessels of $Strn^{+/-}$-LibS vs. WT-LibS, 2) The enhancement of Phe or KCl contraction observed in endothelium-intact vessels of $Strn^{+/-}$-LibS vs. WT-LibS or $Strn^{+/-}$-ResS was absent in endothelium-denuded vessels, 3) ACh-induced relaxation was reduced in $Strn^{+/-}$-LibS vs. $Strn^{+/-}$-ResS or WT-LibS, and 4) ACh potency was reduced in $Strn^{+/-}$-LibS vs. $Strn^{+/-}$-ResS.

NO is a major endothelium-derived vasodilator. Under basal conditions eNOS is bound to cav-1 in endothelial cell caveolae. An increase in endothelial cell Ca^{2+} by ACh induces the release of eNOS from cav-1 to the cytosol [19,20] where it is phosphorylated and fully activated by MAPK and phosphatidylinositol 3-kinase-Akt.[21,22]. Activated eNOS converts L-arginine to L-citrulline and increases NO release. NO diffuses into VSM where it activates guanylate cyclase,[23] and increases cGMP production. cGMP activates cGMP-dependent protein kinase, which promotes vascular relaxation by decreasing $[Ca^{2+}]_i$, and inhibiting myosin light chain kinase or Ca^{2+} sensitization pathways of VSM contraction such as protein kinase C (PKC) and Rho-kinase.[24] The reduced vascular relaxation in $Strn^{+/-}$-LibS is likely due to decreased eNOS and the NO-cGMP pathway because: 1) Phe contraction was enhanced by the NOS inhibitor L-NAME or guanylate cyclase inhibitor ODQ in WT-LibS and this enhancement was smaller in $Strn^{+/-}$-LibS, 2) Any significant enhancement of Phe contraction observed in control non-treated vessels of $Strn^{+/-}$-LibS vs. WT-LibS or $Strn^{+/-}$-ResS was eliminated in L-NAME or ODQ-treated vessels. 3) ACh relaxation was blocked by endothelium removal, L-NAME or ODQ, 4) vascular eNOS mRNA expression was reduced in $Strn^{+/-}$-LibS, and 5) the amount of eNOS activator pAkt relative to total Akt was reduced in $Strn^{+/-}$-LibS. These observations suggest that striatin may be conducive of the increase in eNOS expression/activity during LibS diet and that, when striatin levels are reduced, mice on LibS diet are unable to mount the usual increase in eNOS mRNA expression and activation of the NO-cGMP relaxation pathway. Striatin deficiency did not affect the ability of blood vessels to relax or the sensitivity of VSM to NO-cGMP because the vascular relaxation to the NO donor and guanylate cyclase activator SNP was not different in WT and $Strn^{+/-}$ on ResS or LibS. We could not detect differences in the protein amount of eNOS and peNOS between groups, possibly because striatin deficiency during LibS may be associated with not only decreased eNOS mRNA expression but also decreased eNOS degradation, and therefore needs to be further examined.

Other observations/considerations are: 1) Endothelium-denudation and blockade of NO-cGMP with L-NAME or ODQ increased vascular sensitivity to Phe and Phe ED_{50} particularly during LibS, consistent with a role of endothelial NO-cGMP pathway in reducing the vascular α-adrenergic receptor sensitivity and reactivity to Phe and norepinephrine.[25] 2) Endothelium-denudation or blockade of NO-cGMP with L-NAME or ODQ was associated with an enhancement of Phe contraction relative to KCl. KCl

contraction is mainly due to Ca^{2+} influx through voltage-gated Ca^{2+} channels [14], while α-adrenergic receptor agonists such as Phe stimulate Ca^{2+} entry through voltage-gated, ligand-gated and store-operated Ca^{2+} channels [26,27]. Assuming that KCl contraction is mainly due to Ca^{2+} influx, then the enhanced Phe contraction during endothelium-removal or blockade of NO-cGMP pathway is likely due to activation of other Ca^{2+} channels or Ca^{2+}-sensitization by PKC or Rho-kinase.[24] The effects of Ca^{2+} sensitization are possibly obscured by increased NO-cGMP, and only after endothelium removal or blockade of NO-cGMP these enhanced contraction mechanisms could be manifested. This is supported by reports that NO-cGMP reduce $[Ca^{2+}]_i$,[28] and cGMP-dependent protein kinase causes phosphorylation and inhibition of myosin light chain kinase, PKC and Rho-kinase.[24] 3) The present study was conducted on the aorta, and while working with small vessels from small animals such as the mouse could pose a challenge, the effects of striatin deficiency on vascular function should be further examined in small resistance vessels. 4) The present study was performed on male mice, and as striatin is thought to be a critical intermediary in the nongenomic actions of estrogen/ERα,[4] future studies should examine potential gender differences in the vascular role of striatin in females. 5) We have shown that activation of MR increases striatin levels in vascular cells,[7] and examining the effects of modulation of MR activity using aldosterone and MR antagonists would further elucidate the role of striatin in mediating steroid signalling and the control of vascular function and BP. 6) The salt-intake-mediated changes in BP in $Strn^{+/-}$ mice were likely due to the decrease in BP with ResS intake rather than an increase in BP with LibS intake, and while BP did not significantly increase, vasoconstriction increased and vasodilation decreased in $Strn^{+/-}$-LibS. Although the present study has focused on the role of striatin in the vasculature, striatin is expressed in other tissues and cell types such as the kidney and central nervous system.[29] As the mechanisms of salt sensitivity of BP are not limited to modulation of the vasculature and BP is regulated by other renal and neuronal control mechanisms,[30] changes in striatin expression in renal and neuronal tissues and consequently their BP control mechanisms may contribute to the development of salt sensitivity in the striatin heterozygotes. This is supported by our recent findings of different aldosterone responses to salt manipulations in $Strn^{+/-}$ and WT mice, and potential role of striatin in maintaining normal volume homeostasis by modulating MR-mediated genomic and non-genomic responses in the kidney.[8]

Thus striatin deficiency during LibS diet is associated with salt sensitivity of BP, enhanced vasoconstriction and decreased vascular relaxation. The results suggest a critical role for striatin, possibly through modulation of endothelial NO-cGMP pathway, in regulation of vascular function and BP during changes in dietary sodium intake.

Perspectives

An important question is whether humans with the polymorphic variant in the striatin gene would show reduction of BP when salt intake is restricted. The answer is unclear at the present time since our previously observed salt-sensitive changes in BP in humans with the polymorphic variant in the striatin gene were performed in a hypertensive cohort.[8] Of note, while $Strn^{+/-}$ mice showed sensitivity of BP to changes in dietary sodium, they were not hypertensive. Nevertheless, the observation that BP was less in $Strn^{+/-}$-ResS vs. $Strn^{+/-}$-LibS

makes it tempting to postulate the presence of the same phenotype in humans, and to evaluate the effects of salt restriction in normotensive subjects. Because of the low frequency of the risk allele in our study with a hypertensive cohort, a similar study using a normotensive cohort would require a substantial number of normotensive subjects. However, the current results coupled with our previous studies in humans raise the intriguing possibility that the vascular changes reported herein may lead to an increased risk of cardio- and/or reno-vascular damage as the $Strn^{+/-}$ population ages. Such an association could provide one mechanistic answer to the general association between aging and increase in BP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Tham M. Yao and Paul Loutraris for technical assistance.

Sources of Funding: Supported by NIH grants HL-69208 and T32HL007609 to GHW, HL-104032 to LHP, and HL-65998, HL-98724, HL-111775 to RAK.

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Novelty and Significance

What Is New?

The present study provides new information on the role of striatin in regulation of vascular function and BP.

The data provide first evidence that striatin deficiency causes enhanced vascular contraction, decreased relaxation and salt-sensitive BP during high salt intake.

What Is Relevant?

Striatin deficiency could be an important factor in mediating vasoconstrictor and pressor effects during high salt diet.

Screening for striatin genotype could help to identify individuals whose blood pressure control and vascular function would benefit from reducing dietary salt intake.

Summary

Striatin deficiency is associated with salt sensitivity of blood pressure and enhanced vasoconstriction and decreased vascular relaxation on a liberal salt diet.

The data suggest a critical role for striatin, through modulation of endothelial NO-cGMP, in regulation of vascular function and blood pressure during high dietary sodium intake.

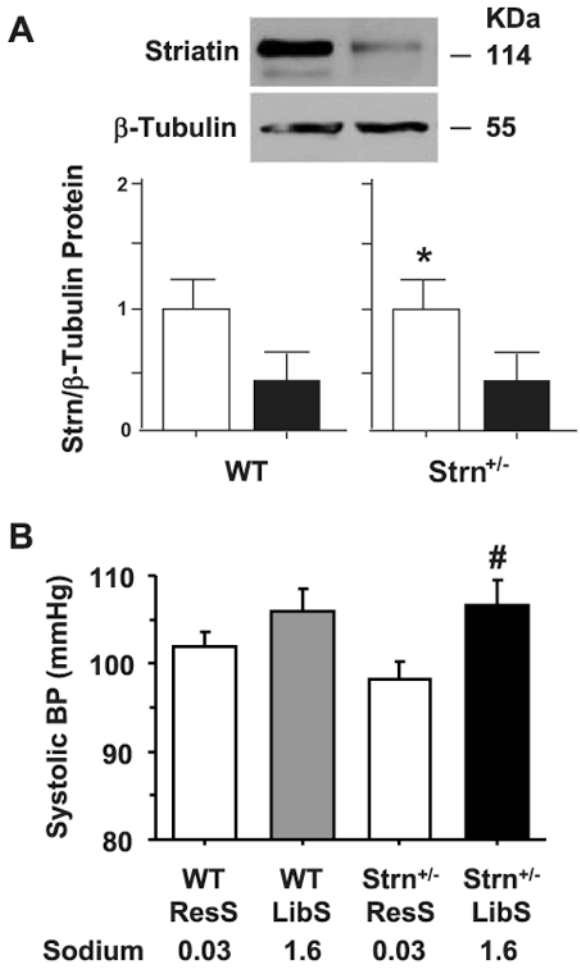

Fig 1.

Aortic striatin expression as measured by Western blots (A) and systolic BP in WT and $Strn^{+/-}$ mice (B) on ResS and LibS diets.

Data represent means±SEM (n=4 for Western blots, n=22-24 for BP).

* $p<0.05$, $Strn^{+/-}$ vs. WT.

# $p<0.05$, $Strn^{+/-}$-LibS vs. $Strn^{+/-}$-ResS.

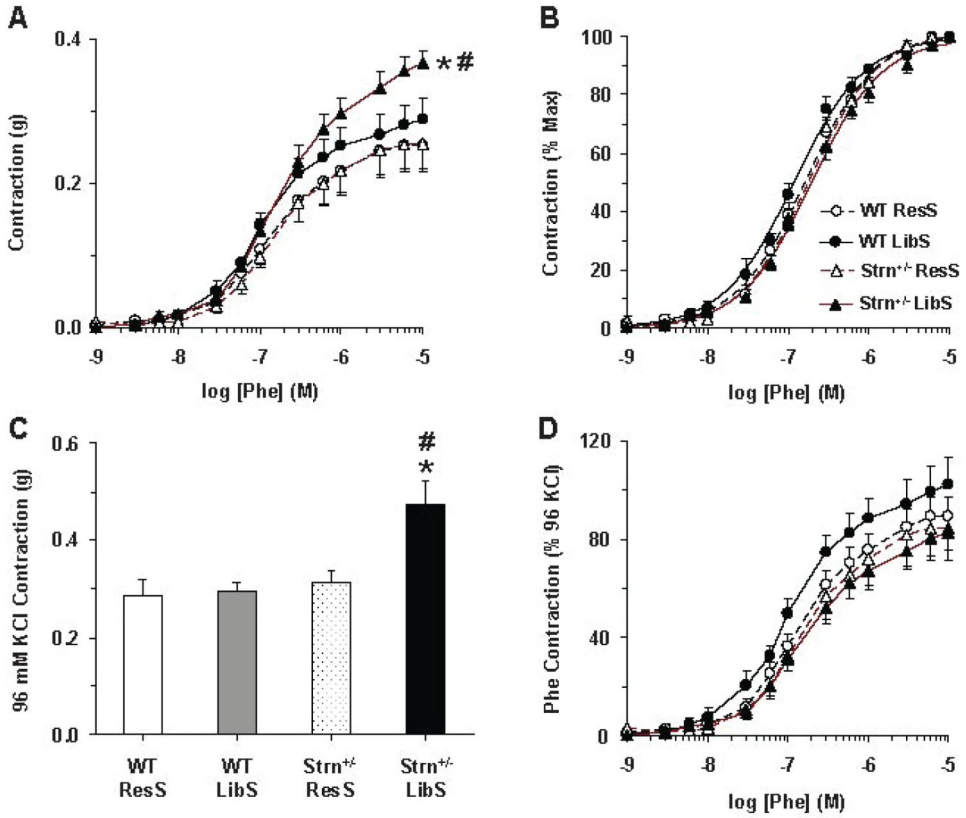

Fig 2.

Phe- and KCl-induced contraction in aorta of WT and Strn$^{+/-}$ mice on ResS and LibS diets. Endothelium-intact aortic rings were stimulated with increasing concentrations of Phe, the contractile response was measured and presented in grams (A) or as % of maximum Phe contraction (B). Aortic contraction to 96 mM KCl was also measured (C), and the contractile response to Phe was presented as % of KCl contraction (D). Data represent means±SEM, n=7-9 mice.

* $p<0.05$, Strn$^{+/-}$ vs. WT.

# $p<0.05$, LibS vs. ResS.

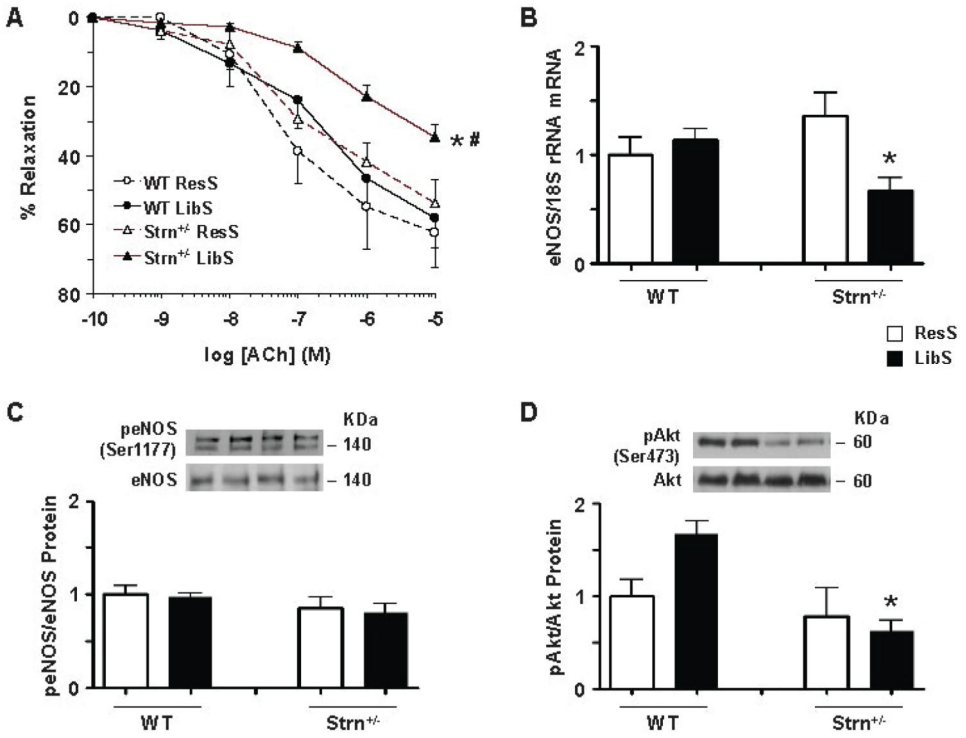

Fig 3.

ACh-induced relaxation (A), eNOS mRNA expression (B), peNOS relative to total eNOS (C), and pAkt relative to total AKt (D) in aorta of WT and Strn$^{+/-}$ mice on ResS and LibS diets. Aortic rings were precontracted with Phe, increasing concentrations of ACh were added and the % relaxation of Phe contraction was measured. Aortic tissue homogenates were prepared for measurement of eNOS mRNA expression using RT-PCR, and the amount of eNOS, peNOS, Akt and pAkt using Western blots. Data represent means±SEM (n=7-8 for ACh relaxation, n=4 for RT-PCR and Western blots).

* $p<0.05$, Strn$^{+/-}$ vs. WT.

# $p<0.05$, LibS vs. ResS.

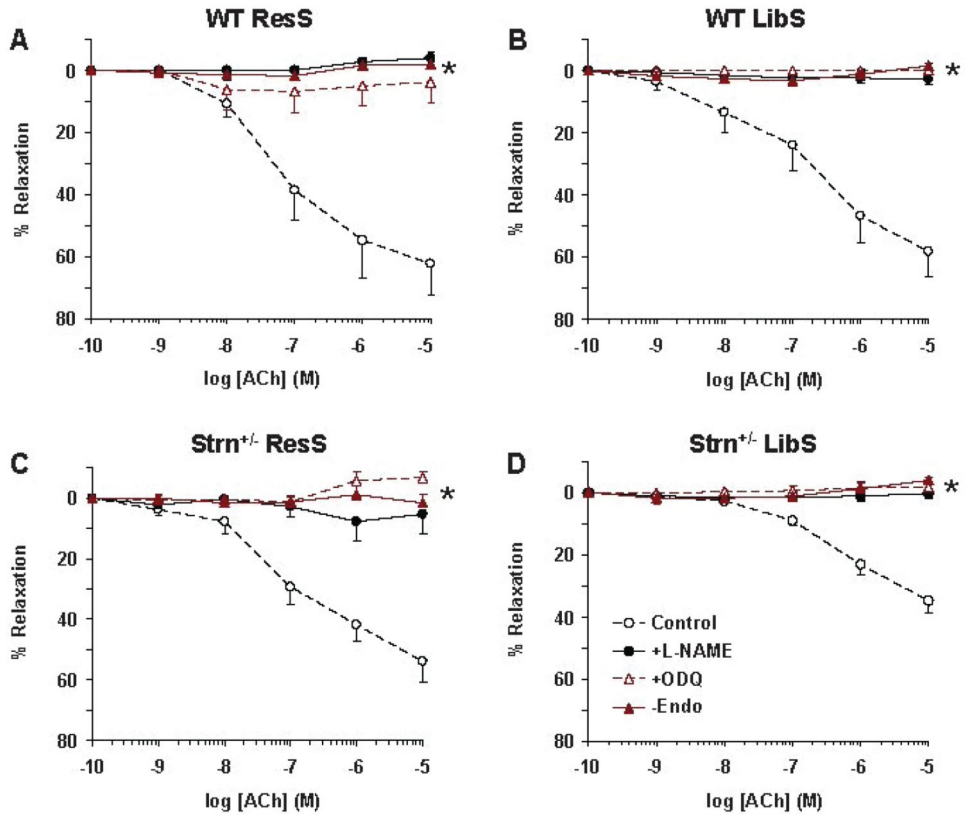

Fig 4.

Effect of endothelium removal or blockade of NO-cGMP pathway on ACh-induced relaxation in aorta of WT and $Strn^{+/-}$ mice on ResS and LibS diets. Aortic rings from WT-ResS (A), WT-LibS (B), $Strn^{+/-}$-ResS (C), and $Strn^{+/-}$-LibS mice (D) were either endothelium-intact, pretreated with L-NAME (3×10^{-4} M) or ODQ (10^{-5} M), or endothelium-denuded. The vessels were precontracted with Phe, increasing concentrations of ACh were added and the % relaxation of Phe contraction was measured. Data represent means±SEM (n=7-11 mice).

* $p<0.05$, L-NAME or ODQ-treated or endothelium-denuded arteries vs. control non-treated arteries.