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Genetic knockdown of brain-derived neurotrophic factor in the nervous system attenuates angiotensin II-induced hypertension in mice

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Abstract

Introduction: Brain-derived neurotropic factor (BDNF) is expressed throughout the central nervous system and peripheral organs involved in the regulation of blood pressure, but the systemic effects of BDNF in the control of blood pressure are not well elucidated.

Materials and methods: We utilized loxP flanked BDNF male mice to cross with nestin-Cre female mice to generate nerve system BDNF knockdown mice, nestin-BDNF (+/–), or injected Cre adenovirus into the subfornical organ to create subfornical organ BDNF knockdown mice. Histochemistry was used to verify injection location. Radiotelemetry was employed to determine baseline blood pressure and pressor response to angiotensin II (1000 ng/kg/min). Real-time polymerase chain reaction was used to measure the expression of renin–angiotensin system components in the laminal terminalis and peripheral organs.

Results: Nestin-BDNF (+/–) mice had lower renin–angiotensin system expression in the laminal terminalis and peripheral organs including the gonadal fat pad, and a lower basal blood pressure. They exhibited an attenuated hypertensive response and a weak or similar modification of renin–angiotensin system component expression to angiotensin II infusion. Subfornical organ BDNF knockdown was sufficient for the attenuation of angiotensin II-induced hypertension.

Conclusion: Central BDNF, especially subfornical organ BDNF is involved in the maintenance of basal blood pressure and in augmentation of hypertensive response to angiotensin II through systemic regulation of the expression of renin–angiotensin system molecules.

Keywords

BDNF, hypotension, RAS, targeting knockdown

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Introduction

Brain-derived neurotropic factor (BDNF) is enriched in the central nervous system (CNS) and some peripheral organs such as the liver and the kidney.[1] It is well recognized as a negative regulator of appetitive behavior and body weight through its neurotrophic and non-neurotrophic effects.[2–8] The renin–angiotensin system (RAS) is pivotal for blood pressure (BP) regulation by maintaining sodium and water homeostasis and sympathetic tones.[9–11] BDNF can regulate the RAS activity that is involved in the progression of hypertension and sensitization of hypertension.[8,9,12–15] For example, BDNF modulates angiotensin

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signaling in the hypothalamus to increase BP,[15,16] and BDNF/TrkB is a mediator of the long-term BP and sympathetic nerve activity responses to central angiotensin II (Ang II) activity.[8] In addition, local adipose RAS plays auto or/and paracrinal roles on adipogenesis and BP, therefore RAS components from adipocytes are an important contributor to systemic RAS activity.[17–20] Central BDNF has recently been found to regulate metabolism through brain–liver connection,[5] which supports its central synchronous effect on regulating energy homeostasis with the liver. However, the role of central BDNF on RAS activity in both central and periphery is not well understood.

Forebrain structures along with the laminal terminalis (LT), including the subfornical organ (SFO), play important roles in the regulation of BP, body fluid, and energy homeostasis.[21,22] A circulating BDNF signal may stimulate the CNS through actions at the SFO, characterized by the lack of a normal blood–brain barrier.[22] Given that the SFO is enriched with both Ang II type 1 receptor (AT1R) and BDNF,[1,8,15,22] it is likely that besides the hypothalamic paraventricular nucleus (PVN) and rostral ventrolateral medulla,[9,15,16] the SFO may also be a critical site where BDNF can interact with the RAS to regulate cardiovascular functions.

The aim of the study

The main aim of this study was to investigate the effect of neuronal BDNF, especially SFO BDNF on hypertensive response to Ang II as well as on RAS component expression in the brain and peripheral organs.

Materials and methods

Ethics statement

All procedures involving live animals were approved by Nanyang Institute of Technology Animal Use Committee and the University of Iowa Animal Care and Use Committee, and were performed in accordance with the standards set by the National Institutes of Health. All protocols were designed to minimize animal discomfort.

Generation of nestin-BDNF (+/–) mice and genotype identification

To generate nervous system targeting knockdown BDNF mice (nestin-BDNF (+/–)), male Tg(Nescre) mice (B6.Cg-Tg(Nescre)1Kln/J; stock number 003771; Jackson Laboratory) were crossed with female $Bdnf^{tm1krj}$ (stock number 006579; Jackson Laboratory). Tail DNA was isolated, and genotyping was performed according to the standard protocol suggested by Jackson Laboratory, yielding a 437 bp band for wild type, and 487 bp for mutant, and both for heterozygote. Polymerase chain reaction (PCR) reagents were from Easy-DNA Kit (Invitrogen). The mice were maintained in a 12:12 hour light–dark cycle (06:00 am to 06:00 pm), fed normal mouse chow and had access to water ad libitum. Heterozygote mice were used for experiments when they were 15 weeks old. Control mice were littermate wild type or nestin Cre only.

BDNF knockdown in the SFO by injection of adeno-Cre viral vector

The experiment aimed to test if BDNF in the SFO plays a crucial role on BP regulation. The procedure mainly followed the protocol described by Xue et al.[10] Briefly, a replication-deficient adenovirus encoding Cre-recombinase (Ad-Cre, 1×10^{10} plaque-forming units/ml, 100 nl) generated by the University of Iowa gene transfer vector core or an empty vector (Ad-Con) as control was injected into the ventral hippocampal commissure and allowed to diffuse ventrally into the SFO of the BDNF floxed (+/+) mice. The knockdown of BDNF in the SFO and location of microinjections were verified after the mice were killed using quantitative PCR and immunohistochemistry, respectively.

Telemetry probe implantation and osmotic pump implantation

The mice were anesthetized with a ketamine–xylazine mixture (100 mg/kg and 10 mg/kg). Osmotic pumps, containing Ang II (Sigma Chemicals) at a concentration sufficient to allow an infusion rate of 1000 ng/kg/min, were implanted subcutaneously on the back as described in our previous work.[10]

Measurement of BP and heart rate

Nestin-BDNF (+/–) mice and BDNF floxed (+/+) mice were employed. Basal BP and heart rate (HR) were telemetrically recorded 5 minutes every hour for 5 days, after which osmotic pumps filled with Ang II (1000 ng/kg/min) were implanted in the back of nestin-BDNF (+/–) mice for 14 days. In the BDNF floxed (+/+) mice, a replication-deficient adenovirus encoding Cre-recombinase or an empty vector (Ad-Con) as control was injected into the SFO. Seven days later, the animals were infused with Ang II (1000 ng/kg/min) for 14 days by osmotic pumps. BP and HR were collected 5 days baseline and following 14 consecutive days during chronic infusion of Ang II and stored with the Dataquest ART data acquisition system for further analysis.

Immunohistochemistry and histological verification

On completion of the in vivo protocols, mice were anesthetized and perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were removed, postfixed in 4% paraformaldehyde for one hour and then cryoprotected for 2 days in 30% sucrose at 4°C. Frozen 20 μm coronal sections were cut with a cryostat.

Table 1. Primers used in real time polymerase chain reaction.

Gene	Forward primer	Reverse primer	Accession No.
β-actin	CATCCTCTTCCTCCCTGGAGAAGA	ACAGGATTCCATACCCAAGAAGGAAGG	XM_021163894.1
Renin	ACGGATCAGGGAGAGTCAAA	GAAAGCCCATGCCTAGAACA	NM_031192.3
AGT	ACAGACACCGAGATGCGTT	CCACGCTCTCTGGATTTATC	AH005858.1
AT1R	TGCCCATAACCATCTGCATAG	TTTCAGGAGCTGGAGGAAATAC	NM_177322.3
ACE1	ACCCTAGGACCTGCCAATCT	CTCCCAGGCAAACAACAACT	NM_207624.3
MR	TCACATTTTTAACATGTGACGGC	GCTTTTCATCCAGAGGAACG	NT_078575
BDNF	TACCTGGATGCCGCAAACAT	AGTTGGCCTTTGGATACCGG	AY057908.1

AGT: angiotensinogen; AT1R: angiotensin receptor 1; ACE: angiotensin-converting enzyme; BDNF: brain-derived neurotropic factor; MR: aldosterone receptor.

The tissue was washed with PBS and then blocked with 10% donkey serum (Jackson Laboratory) in PBS containing 0.2% Triton X-100 for one hour. Sections were then incubated with a mouse Cre-specific antibody (1:100, Covance; Berkeley, CA, USA) for 48 hours at 4°C in the dark. After washing with PBS, sections were incubated with rhodamine-conjugated donkey anti-mouse antibody to detect Cre (red) expression. Fluorescence was then identified using a confocal microscope. The animals with missed injections were excluded from analysis.

Measurement of mRNA expression

Total RNA was isolated from the LT or peripheral organs as indicated in figure legends, mRNA was reverse transcribed, and target genes cDNA levels were measured by quantitative PCR as described in our previous work.[10] Changes in mRNA expression levels were normalized to beta-actin levels and calculated using the $\Delta\Delta C_t$ method. Results are expressed as relative fold change or mean fold change ± SEM. The sequences of the primers (Coralville, IA, USA) are shown in Table 1.

Statistics

Data are presented as means ± SEM. Analysis of difference was performed using one or two-way analysis of variance (ANOVA), or repeated measures ANOVA. When ANOVA reached significance, the Fisher test or repeated measures *t*-test was used to compare the mean values among the different levels of mouse groups and treatments. A value of $P<0.05$ was considered to be significant. Repeated measures ANOVA was used, followed by repeated measures *t*-test ($n=5$).

Results

Characteristics of nestin-BDNF (+/–) mice regarding the expression of BDNF and RAS components

The breeding of homozygote BDNF loxP (+/+) mice with nestin-cre mice gave a genotype with both loxP-flanked BDNF allele and nestin-cre (nestin-BDNF (+/–)) and other three genotypes such as wild type, nestin-cre, and heterozygote BDNF mice (BDNF (+/–)). Mice were genotyped after they were weaned. Nestin-BDNF (+/–) mice were further characterized by organ genotyping for BDNF-specific expression. The brain and the LT in double transgenic mice had the wild type BDNF allele (flox-) only, and non-neuronal organs such as the kidney, the liver, the aorta and the lungs had both wild type (flox-) and the loxP-flanked BDNF allele (flox+), as predicted (Figure 1(a)). In contrast, single transgenic mice, BDNF (+/–), had both alleles in all organs checked (Figure 1(b)). The other two genotypes only had the wild type allele (flox-) (data not shown).

Quantitative PCR revealed that BDNF mRNA levels in the brain and the LT of double transgenic mice were only about 0.35±0.02 and 0.55±0.06 of these in control mice, respectively ($n=7$). Meanwhile, BDNF expression had no significant difference in the kidney (1.05±0.28 vs. 1±0.39), the liver (0.84±0.21 vs. 1±0.10) ($P>0.05$) and the lungs (0.96±0.18 vs. 0.83±0.17) ($P>0.05$) (Figure 1(c)) and other peripheral organs checked (data not shown). Nestin-BDNF (+/–) mice were also overweight compared to control littermates ($P<0.05$, $n=13$) (Figure 1(d)).

Nestin-BDNF (+/–) mice exhibited altered expression of RAS components in the LT and some peripheral organs. In the LT, angiotensinogen (AGT), renin, and AT1R were only 0.59±0.08, 0.67±0.05, 0.68±0.12 of littermate controls, respectively ($n=5$, $P<0.05$). MR and ACE1 expression remained approximately comparable as in the littermate controls (Figure 2(a)) ($n=5$, $P>0.05$). In the liver, however, AGT, AT1R and MR were 0.59±0.13, 0.67±0.11 and 0.49.3±0.16 of these in the control littermates, respectively ($P<0.05$) (Figure 2(b)). In the kidney, renin mRNA was 1.51±0.16-fold increased compared with control mice, AT1R was significantly decreased (0.70±0.08 fold) ($n=5$, $P<0.05$). MR was similar in both genotypes (Figure 2(c)). In the lungs, ACE1 (0.63±0.09) were significantly lower in nestin-BDNF (+/–) mice than that in control littermates (Figure 2(d)). Nestin-BDNF (+/–) mice had significantly higher gonadal fat mass (Figure 2(e)). The expression of RAS components in the gonadal fad pad was also modulated by central BDNF deficiency compared with littermate controls. AGT, AT1R and ACE1 were dramatically

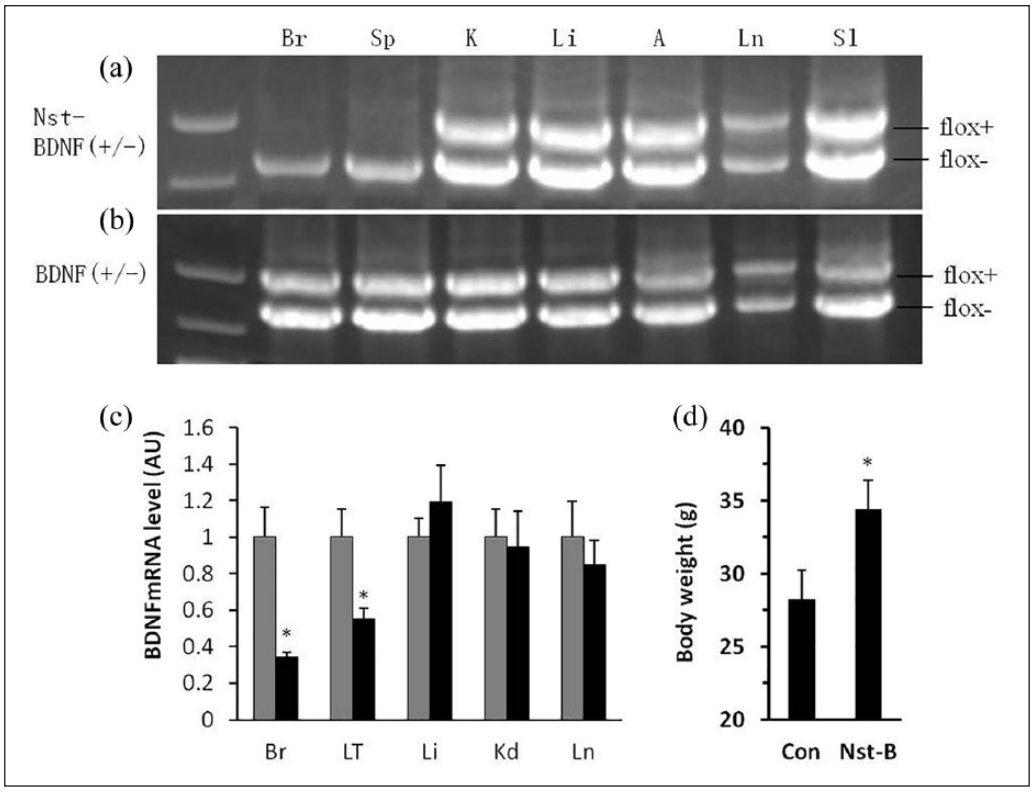

Figure 1. Neuronal BDNF knockdown in nestin-BDNF (+/–) mice. (a) Organ genotyping of nestin-BDNF (+/–) mice; (b) BDNF (+/–) mice uniformly have both alleles. (c) Relative BDNF mRNA levels in the whole brain, the LT and peripheral organs of nestin-BDNF (+/–) mice. (d) Nestin-BDNF (+/–) mice have higher body weight mass than control littermates. Bars represent mean ± SEM of the difference in fold change compared with littermate controls. *$P<0.05$ versus control littermates, $n=3–7$. A: aorta; ACE: angiotensin-converting enzyme; AGT: angiotensinogen; AT1R: angiotensin receptor 1; BDNF: brain-derived neurotropic factor; Br: brain; LT: laminal terminalis; Con: control; Li: liver; Ln: lung; nst: nestin; Sl: spleen; Sp: spinal cord.

decreased in nestin-BDNF (+/–) mice ($n=5$, $P<0.05$), whereas the renin mRNA level in both groups was comparable ($n=5$, $P>0.05$) (Figure 2(f)).

Effect of neuronal BDNF knockdown on basal BP and pressor response to Ang Ⅱ

Under normal conditions, nestin-BDNF (+/–) mice had mild but significantly lower basal mean arterial pressure (MAP) compared with littermates (96.1±1.08 vs. 99.4±1.11 mmHg) ($n=7–10$, $P<0.05$).

Ang II increased MAP progressively over time with the rise in pressure reaching significance on days 1 and to the end of the experiment (Figure 3(a)). Infusion of the same dose of Ang II (1000 ng/kg/min) into nestin-BDNF (+/–) mice also increased MAP on the same day, but the amplitude was significantly lower than in control mice. Their difference became larger during the rest period of the experiment (Figure 3(a)). BDNF deficiency also decreased mean HR in nestin-BDNF (+/–) mice compared with the littermate controls ($n=5$, Figure 3(b)).

Nestin-BDNF (+/–) mice exhibited an attenuated or a similar response of the expression of RAS components in response to Ang II infusion compared to control mice in the LT, except AGT which was significantly increased (Figure 4(a)). ACE1 in the lungs (Figure 4(b)) and AGT in the liver (Figure 4(c)) were significantly decreased ($P<0.05$). These results were similar to our previous work.[10]

Effect of knockdown of BDNF in the SFO on pressor response to Ang Ⅱ

BDNF knockdown in the SFO is confirmed by immunohistochemistry (Figure 5(a)), and real time PCR (Figure 5(b)), showing the BDNF knockdown effect in the SFO. The BDNF knockdown specific in the SFO resulted in an attenuated increase in BP during infusion of Ang II compared to the control group (BDNF (+/+) + SCM control vector) (Figure 5(c)), indicating that BDNF in the SFO plays the key role in the hypertensive response to Ang II.

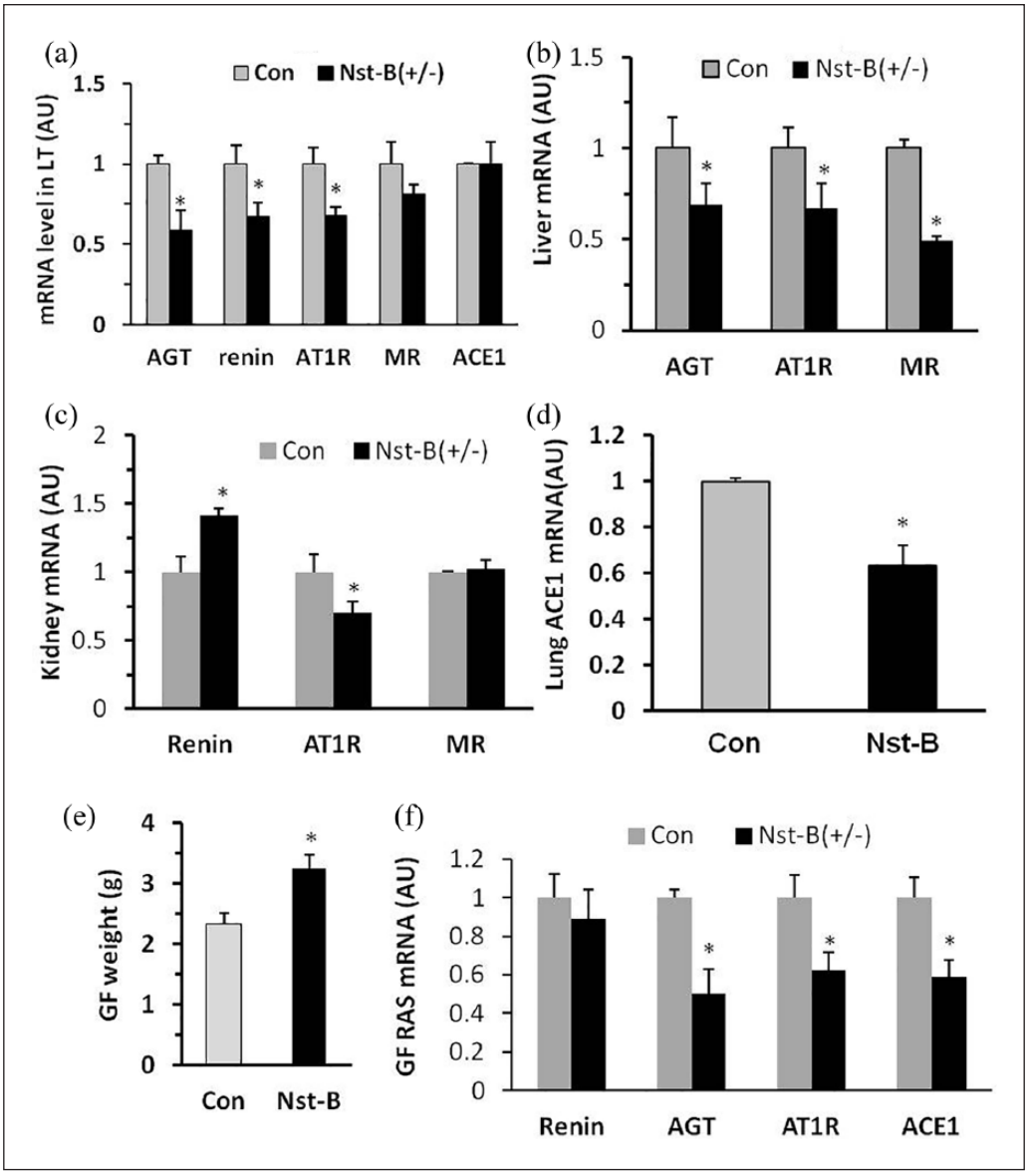

Figure 2. Central BDNF deficiency leads to alternation of RAS components in the LT (a) and peripheral organs including the liver (b), kidney (c), and lung (d). Nestin-BDNF (+/–) mice also have higher gonadal fat (GF) pad mass (e) and altered RAS expression (f). *$P<0.05$ versus control littermates, $n=3–10$. BDNF: brain-derived neurotropic factor; LT: laminal terminalis; MR: aldosterone receptor; RAS: renin–angiotensin system.

Discussion

The nestin-BDNF (+/–) mice have about 50% down expression of BDNF in the nervous system and about the same BDNF expression level in their peripheral organs. The mice exhibited less expression of most RAS components in the brain and the peripheral organs including thr liver, lung and gonadal fat pad. With this system, we found: (a) the nervous system BDNF is required for tonic maintenance of basal BP and hypertensive response to systemic administration of Ang II; (b) the SFO is a key site where BDNF interacts with blood-borne Ang II; and (c) the reciprocal regulation of gene expression between BDNF and the RAS in central and periphery may account for the alternations of BP responses. The above results are consistent with previous observations showing that either overexpression of BDNF in the PVN[15] or ICV administration of TrkB blocker[8] or BDNF augmented hypertensive response to Ang II.[9] Furthermore, the present study extended these previous studies by showing that SFO BDNF is also involved in BP regulation and is sufficient to alter BP response to pressor agent, and gonadal fat pad may remarkably contribute to the vascular phenotype of nestin-BDNF (+/–) mice.

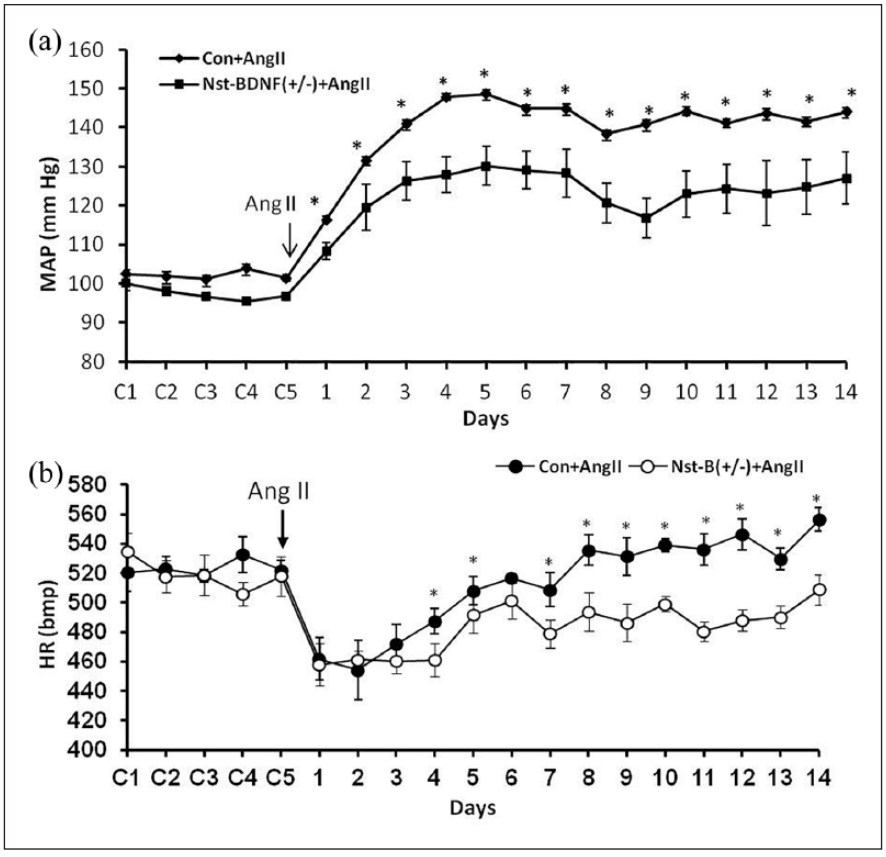

Figure 3. MAP (a) and HR (b) time course of nestin-BDNF (+/–) mice and controls with Ang II treatment. Data are represented as mean ± SE (n=5). Repeated measures t-test is used. (a) Nestin-BDNF (+/–) mice have lower MAP compared with control littermates. Nestin-BDNF (+/–) + saline serves as a control. (b) HR change of nestin-BDNF (+/–) mice and control littermates during the treatment of Ang II. *$P<0.05$ Con. + Ang II versus nestin-BDNF (+/–) + Ang II. Ang II: angiotensin II; BDNF: brain-derived neurotropic factor; HR: heart rate; MAP: mean arterial pressure.

Although SFO BDNF has been demonstrated to be sufficient for BDNF and Ang II interaction in our and other investigations,[22] this does not exclude their interaction in other brain areas and peripheral organs. In the present study, we also demonstrated that neuronal BDNF globally affects the expression of RAS components, including the gonadal fat pad, liver and lung. A lower expression of the RAS components was evident in BDNF knockdown mice. The results indicate a synchronizing mechanism exists between the CNS and the peripheral organs through mutual interaction of systemic RAS and BDNF-TrkB signals. We observed hypotension and bradycardia in nestin-BDNF (+/–) mice, which may be the consequence of lower expression of RAS components in both nerve systems and peripheral organs caused by central BDNF deficiency. Furthermore, nestin-BDNF (+/–) mice demonstrated an attenuated response to Ang II hypertensive stimulus, which was also accompanied by fewer increases in the expression of RAS components in the LT and in the peripheral organs. In contrast, Ang II treatment induced an augmented hypertensive response and an upregulated expression of the RAS components and the BDNF in control mice. This is consistent with the results from the overexpression of BDNF in the PVN[15] and from our previous work that RAS expression is upregulated coupled with BDNF increase in response to Ang II treatment.[13] Collectively, it can be speculated that reduced RAS activity in the deficiency of the BDNF may account for an attenuated hypertensive response to Ang II, and that neuronal BDNF integrates the peripheral BP signals and confounds the output information for RAS expression.

Besides the RAS mechanisms, the mechanisms underlying regulatory effects of BDNF on BP may also involve some other pathways. First, BDNF-TrkB may interplay with other signaling pathways to regulate synaptic plasticity.[2,15] Second, central BDNF and some peripheral organs may be connected through specific circuits for homeostasis as it suppresses energy intake through a BAT neural circuit.[4] By this account, the deficiency of central BDNF may induce neuronal circuit dysfunction that leads to reduced sensitivity to peripheral stimulation

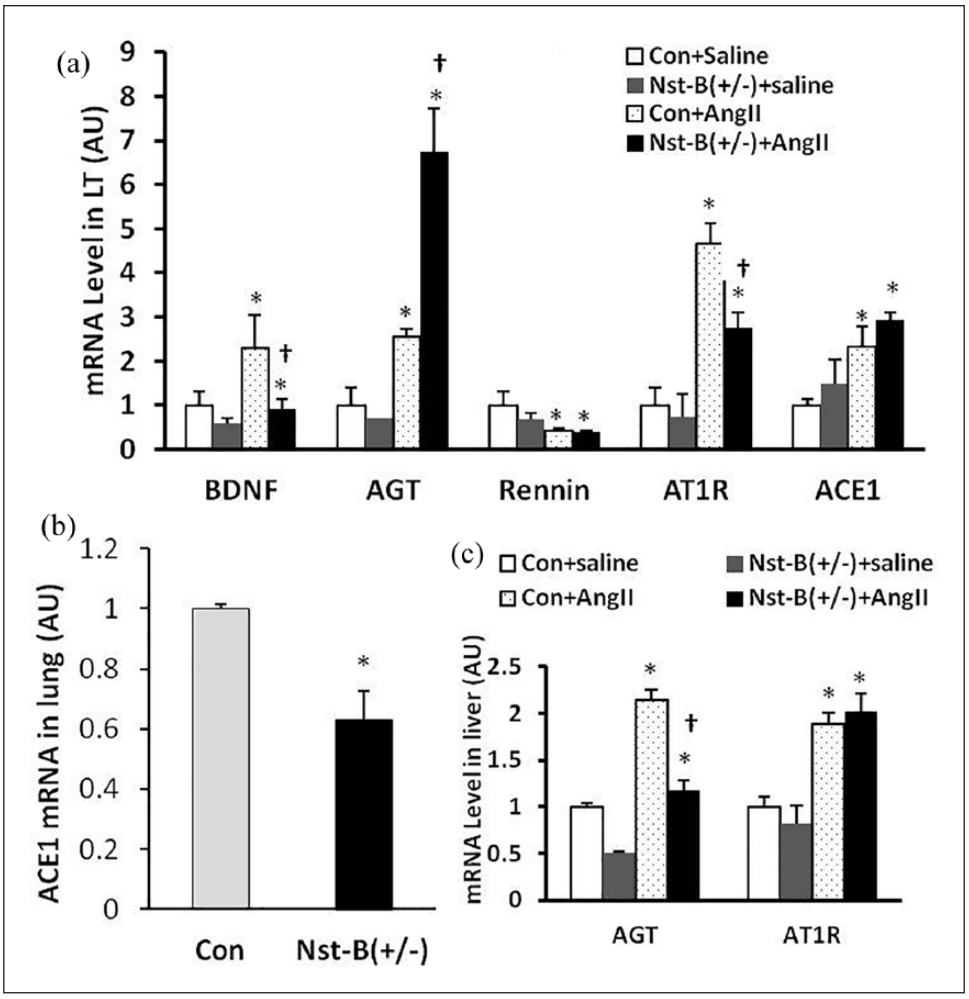

Figure 4. The expression of RAS components in response to Ang II infusion in the LT (a), the lungs (b) and the liver (c). Nestin-BDNF (+/–) mice have attenuated response regarding RAS expression compared to control mice except AGT in the LT (a). *$P<0.05$ versus saline; †$P<0.05$ nestin-BDNF (+/–) + Ang II versus control + Ang, n=3–10. AGT: angiotensinogen; Ang II: angiotensin II; BDNF: brain-derived neurotropic factor; LT: laminal terminalis; RAS: renin–angiotensin system.

such as Ang II. In particular, the SFO has long been considered to be a critical peripheral–central interface for the cardiovascular actions of the circulating RAS and metabolic factors.[21,23]

The nestin-BDNF (+/–) mice exhibited increased body weight gain due to fat mass increase as shown in other BDNF-deficient mouse models.[6,24,25] Adipocytes express the whole set of RAS components, which serve as important paracrinal and autocrinal factors to regulate BP.[26] It is estimated that 24–28% of plasma AGT is generated by adipose tissue, and plasma AGT and Ang II are positively correlated with the level of adiposity.[11,19,27] In contrast, adipocyte AGT deficiency prevents high fat-induced elevation in blood pressure.[28] In the present study, BDNF knockdown in the nervous system elicited deceased expression of AGT, AT1R and ACE1 in the gonadal fat pad, suggesting that these downregulations of the RAS activity in fat pads may partially account for the low MAP and resistance to Ang II-induced hypertension. These results are consistent with clinical studies on Prader–Willi syndrome and severe obese patients characterized by severe hyperphagia with lower plasma BDNF content.[29]

In summary, the BDNF in the nervous system, especially in the SFO, is involved in basal BP and pressor response to Ang II. These effects are likely to be associated with its regulation of the RAS activity in the brain and peripheral organs including the gonadal fat pad, suggesting a strong central–peripheral organ connection for BP regulation. However, the mechanisms underlying the interactions between BDNF and RAS, and how the signals of BDNF and the RAS integrates and communicates to regulate homoeostatic function are far from being elucidated. Studies on these issues are warranted in the future.

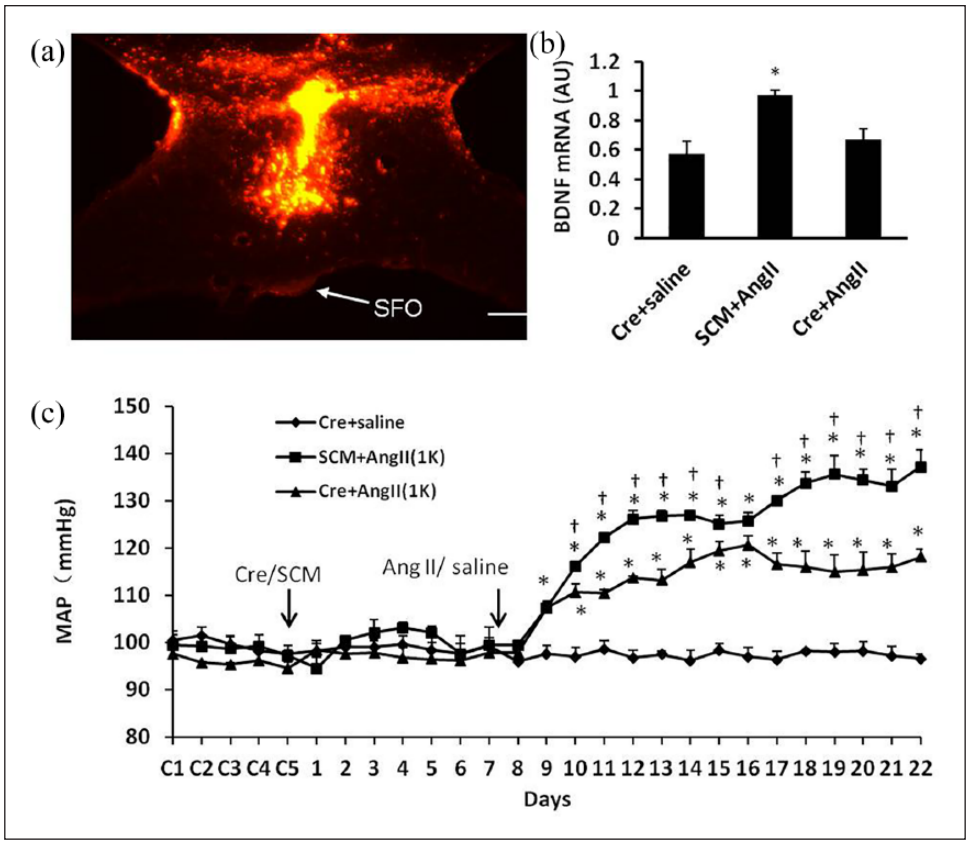

Figure 5. The function of the SFO BDNF on Ang II-induced hypertension. (a) Photomicrograph which illustrates the anatomical placement of the SFO cannula and the injection site (bar = 20 μm). (b) Real time PCR identification of Cre-viral injection into the SFO to confirm 50% BDNF knockdown effect (n=5). (c) Using SFO injection of cre – to demonstrate SFO BDNF effect on blood pressure regulation. Repeated measures ANOVA was used, followed by repeated measures t-test. BDNF SFO knockdown significantly decreased Ang II-induced hypertension (137.1±9.7 mmHg) when compared with cre + Ang II treatment (144.0±1.8 mmHg, data not shown). *P<0.05, SCM-Ang II or Cre-Ang II versus cre+saline; †P<0.05, SCM-Ang II versus cre+ang (1K), n=5. Ang II: angiogensin II; ANOVA: analysis of variance; BDNF: brain-derived neurotropic factor; SFO: subfornical organ.

Declaration of conflicting interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Nakagomi A, et al. Role of the central nervous system and adipose tissue BDNF/TrkB axes in metabolic regulation. *NPJ Aging Mech Dis* 2015; 1: 15009.
2. Xu B and Xie X. Neurotrophic factor control of satiety and body weight. *Nat Rev Neurosci* 2016; 17: 282–292.
3. Bochukova EG, et al. A transcriptomic signature of the hypothalamic response to fasting and BDNF deficiency in Prader–Willi syndrome. *Cell Rep* 2018; 22: 3401–3408.
4. An JJ, et al. Discrete BDNF neurons in the paraventricular hypothalamus control feeding and energy expenditure. *Cell Metab* 2015; 22: 175–188.
5. Meek TH, et al. BDNF action in the brain attenuates diabetic hyperglycemia via insulin-independent inhibition of hepatic glucose production. *Diabetes* 2013; 62: 1512–1518.
6. Lyons WE, et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci USA* 1999; 96: 15239–15244.
7. Waterhouse EG and Xu B. The skinny on brain-derived neurotrophic factor: evidence from animal models to GWAS. *J Mol Med (Berl)* 2013; 91: 1241–1247.
8. Becker BK, Wang H and Zucker IH. Central TrkB blockade attenuates ICV angiotensin II-hypertension and sympathetic

nerve activity in male Sprague–Dawley rats. *Auton Neurosci* 2017; 205: 77–86.
9. Schaich CL, et al. BDNF acting in the hypothalamus induces acute pressor responses under permissive control of angiotensin II. *Auton Neurosci* 2016; 197: 1–8.
10. Xue B, et al. Genetic knockdown of estrogen receptor-alpha in the subfornical organ augments ANG II-induced hypertension in female mice. *Am J Physiol Regul Integr Comp Physiol* 2015; 308: R507–R516.
11. Massiera F, et al. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J* 2001; 15: 2727–2729.
12. Johnson AK, et al. The roles of sensitization and neuroplasticity in the long-term regulation of blood pressure and hypertension. *Am J Physiol Regul Integr Comp Physiol* 2015; 309: R1309–R1325.
13. Clayton SC, et al. CNS neuroplasticity and salt-sensitive hypertension induced by prior treatment with subpressor doses of ANG II or aldosterone. *Am J Physiol Regul Integr Comp Physiol* 2014; 306: R908–R917.
14. Amare AT, et al. The genetic overlap between mood disorders and cardiometabolic diseases: a systematic review of genome wide and candidate gene studies. *Transl Psychiatry* 2017; 7: e1007.
15. Erdos B, et al. Brain-derived neurotrophic factor modulates angiotensin signaling in the hypothalamus to increase blood pressure in rats. *Am J Physiol Heart Circ Physiol* 2015; 308: H612–H622.
16. Wang H and Zhou XF. Injection of brain-derived neurotrophic factor in the rostral ventrolateral medulla increases arterial blood pressure in anaesthetized rats. *Neuroscience* 2002; 112: 967–975.
17. Favre GA, Esnault VL and Van Obberghen E. Modulation of glucose metabolism by the renin–angiotensin–aldosterone system. *Am J Physiol Endocrinol Metab* 2015; 308: E435–E449.
18. Koizumi M, et al. Adipocytes do not significantly contribute to plasma angiotensinogen. *J Renin Angiotensin Aldosterone Syst* 2016; 17: 1470320316672348.
19. Pahlavani M, et al. Regulation and functions of the renin–angiotensin system in white and brown adipose tissue. *Compr Physiol* 2017; 7: 1137–1150.
20. Thatcher S, et al. The adipose renin–angiotensin system: role in cardiovascular disease. *Mol Cell Endocrinol* 2009; 302: 111–117.
21. Johnson AK, Cunningham JT and Thunhorst RL. Integrative role of the lamina terminalis in the regulation of cardiovascular and body fluid homeostasis. *Clin Exp Pharmacol Physiol* 1996; 23: 183–191.
22. Black EAE, et al. Brain-derived neurotrophic factor acts at neurons of the subfornical organ to influence cardiovascular function. *Physiol Rep* 2018; 6: e13704.
23. Mimee A, Smith PM and Ferguson AV. Circumventricular organs: targets for integration of circulating fluid and energy balance signals? *Physiol Behav* 2013; 121: 96–102.
24. Carim-Todd L, et al. Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. *J Neurosci* 2009; 29: 678–685.
25. Toriya M, et al. Long-term infusion of brain-derived neurotrophic factor reduces food intake and body weight via a corticotrophin-releasing hormone pathway in the paraventricular nucleus of the hypothalamus. *J Neuroendocrinol* 2010; 22: 987–995.
26. Bruce EB and de Kloet AD. The intricacies of the renin–angiotensin–system in metabolic regulation. *Physiol Behav* 2017; 178: 157–165.
27. Yasue S, et al. Adipose tissue-specific regulation of angiotensinogen in obese humans and mice: impact of nutritional status and adipocyte hypertrophy. *Am J Hypertens* 2010; 23: 425–431.
28. Yiannikouris F, et al. Adipocyte deficiency of angiotensinogen prevents obesity-induced hypertension in male mice. *Hypertension* 2012; 60: 1524–1530.
29. Araki S, et al. Decreased plasma levels of brain-derived neurotrophic factor and its relationship with obesity and birth weight in obese Japanese children. *Obes Res Clin Pract* 2014; 8: e63–e69.