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Physical training and hypertension have opposite effects on endothelial brain-derived neurotrophic factor expression

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Aims Changes in circulating brain-derived neurotrophic factor (BDNF) levels were reported in patients with or at risk for cardiovascular diseases associated with endothelial dysfunction, suggesting a link between BDNF and endothelial functionality. However, little is known on cardiovascular BDNF. Our aim was to investigate levels/localization, function, and relevance of cardiovascular BDNF.

Methods and results BDNF levels (western blotting) and localization (immunostaining) were assessed in the heart and aorta from rats with impaired (spontaneously hypertensive rats [SHR]), normal (Wistar Kyoto rats [WKY]), and improved (SHR and WKY subjected to physical training) endothelial function. BDNF levels were also measured in cultured endothelial cells (CECs) subjected to low and high shear stress. The cardiovascular effects of BDNF were investigated in isolated aortic rings and hearts. The results showed high BDNF levels in the heart and aorta, the expression being prominent in endothelial cells as compared with other cell types. Exogenous BDNF vasodilated aortic rings but changed neither coronary flow nor cardiac contractility. Hypertension was associated with decreased expression of BDNF in the endothelium, whereas physical training led to endothelial BDNF up-regulation not only in WKY but also in SHR. Exposure of CECs to high shear stress stimulated BDNF production and secretion.

Conclusion Cardiovascular BDNF is mainly localized within endothelial cells in which its expression is dependent on endothelial function. These results open new perspectives on the role of endothelial BDNF in cardiovascular health.

Keywords BDNF • Endothelium • Hypertension • Physical training • Shear stress

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a neurotrophin initially synthesized as a precursor protein (pre-proBDNF) and subsequently cleaved into proBDNF (35 kDa) that is further processed to generate mature BDNF (mBDNF, 15 kDa). Mature BDNF is the biologically active form of BDNF that induces genomic effects consecutive to TrkB receptors' activation. Most of what is known on BDNF derives from studies conducted on the nervous system where the main cellular source of BDNF is neurons.[1–3] Less well known is that BDNF is also present in the cardiovascular system. Seminal works showed that BDNF mRNA expression increased from embryonic to adult stages in the heart and vessels[4] and recognized the involvement of BDNF in the *in utero* development of the heart and coronary vasculature.[5,6] In contrast, the cardiovascular actions of BDNF are still ill-defined in adults.[7] Increased BDNF expression was found in human atherosclerotic coronary arteries.[8] Decreased plasma BDNF levels were reported in patients with acute coronary syndrome,[9,10] metabolic syndrome,[11] or type 2

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diabetes.[12] In addition, a positive correlation was observed between plasma BDNF levels and multiple risk factors for metabolic syndrome and cardiovascular dysfunction.[13] Recently, plasma BDNF was detected as an independent predictor of 4-year major coronary event in patients with angina pectoris.[14] Overall, clinical data support a contributing role of BDNF in cardiovascular homeostasis and/or pathogenesis. Moreover, it is striking that the diseases associated with impaired circulating BDNF levels share endothelial dysfunction as a common pathophysiological feature and that circulating BDNF levels inversely correlate with vascular cell adhesion molecule-1,[15] a biomarker of endothelial dysfunction.[16] From these data, BDNF emerges as a potential link between endothelial function and cardiovascular health.

The present study was designed to investigate levels/localization, function, and relevance of cardiovascular BDNF in adult rats. For this purpose, BDNF protein levels and localization were first determined in the heart and peripheral arteries by using western blotting and immunohistochemical analyses, respectively. Then, we investigated whether the impairment of endothelial function by hypertension[17] or its improvement by physical training[18] induced changes in BDNF expression. Lastly, the effect of BDNF on the cardiovascular system was assessed by using the models of isolated perfused hearts and aortic rings exposed to exogenous BDNF.

2. Methods

See Supplementary material online.

2.1 Animals

Ten-week-old male Wistar Kyoto rats (WKY, $n = 44$) and spontaneously hypertensive rats (SHR, $n = 31$) were purchased from Charles River (L'Arbresle, France), housed five per cage and kept under a 12 h–12 h light–dark cycle with food and water *ad libitum*. WKY rats and SHR were randomized in sedentary (WKY, $n = 32$ and SHR, $n = 16$) and treadmill-trained groups (WKY-T, $n = 12$ and SHR-T, $n = 15$). All experimental procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) were approved by the local committee for ethic in animal experimentation. All surgery was performed under chloral hydrate anaesthesia (400 mg/kg, i.p.) and all efforts were made to minimize suffering and stress of animals. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured in conscious rats by using the indirect tail-cuff method as previously described.[19] After anaesthesia, the heart, aorta, brain, and skeletal soleus muscle were collected and processed according to further analyses. Physiological parameters and all the organs including the soleus muscle were collected for 24 h after the last physical training boot or at corresponding times in sedentary rats. Rats assigned to physical training were trained 30 min/day in the morning at 18 m/min for 7 consecutive days using a level motorized rodent treadmill according to a protocol routinely used in our laboratory.[20] The impact of physical training on muscle metabolism was assessed by the measurement of citrate synthase activity[21] of the soleus muscle.

2.2 Cell culture and shear stress treatment

Cultured endothelial cells (CECs, EA-Hy926)[22] were exposed to no (static conditions), low (2 dynes/cm^2, LSS), or high shear stress (14 dynes/cm^2, HSS) for 24 h in a Streamer laminar flow chamber. Cells and medium were stored at -80°C until measurement of BDNF levels.

2.3 Western blots

Pro- (35 kDa) and mBDNF (15 kDa) levels were measured according to our previous study.[20] For cultured cell experiments, both pro- and mBDNF levels were assessed in cells, while only mBDNF levels were measured in the medium that was previously concentrated 10 times in a Speed-Vac concentrator system. For rat experiments, pro- and mBDNF levels were measured in the aorta, heart (left ventricle), and brain (hippocampus). Organs were collected after transcardiac perfusion with saline and then stored at -80°C.

2.4 Immunostaining and fluorescence microscopy

Immunofluorescence experiments were performed on aortic and cardiac sections exposed to anti-BDNF antibody that recognizes both pro- and mBDNF, to anti-von Willebrand factor (vWF, a marker of endothelial cells) antibody, and/or to anti-alpha smooth muscle actin (a marker of vascular smooth muscle cells, VSMCs) antibody. BDNF staining was graded by two persons blinded to the experimental conditions as follows: grade 0 = no or mild expression; grade 1 = moderate expression; grade 2 = high expression; grade 3 = intense expression.

2.5 Aortic vascular reactivity and isolated hearts

Rings collected from the thoracic aorta were suspended in a Krebs solution maintained at 37°C and continuously aerated with 95% O_2, 5% CO_2 for isometric tension recording in organ chambers as previously described.[19] After a 90-min equilibration period under a resting tension of 2 g, the presence of functional endothelium was verified by the ability of acetylcholine (10^{-6} mol/L) to induce more than 80% relaxation in rings preconstricted with phenylephrine (10^{-6} mol/L). The vascular effect of mBDNF was assessed in rings previously constricted with phenylephrine (10^{-6} mol/L). The concentration of mBDNF in the chambers was 10^{-8} mol/L. Besides heart rate, left ventricular systolic pressure and coronary flow were measured in Langendorff-perfused hearts exposed for 30 min to the perfusion buffer with or without mBDNF (15×10^{-11} mol/L).

2.6 Statistical analysis

All values were expressed as mean ± standard deviation (SD) except the grade of cellular BDNF staining intensity that was expressed as median affected by minimum and maximum values. Differences between groups of rats and between conditions of cell culture were assessed using the non-parametric Kruskal–Wallis one-way analysis of variance (ANOVA) followed by the Mann–Whitney *U* test to analyse the specific sample pairs for significant differences. We compared WKY with SHR to assess the effect of hypertension, sedentary to trained rats to assess the effect of physical training in WKY or SHR, cells exposed to shear stress to cells cultured in static conditions to assess the effect of shear stress. BDNF levels in the heart and aorta were compared with those found in the brain of the same rats using Friedman's test followed by the Wilcoxon's test. The effect of BDNF vs. vehicle on isolated aortic rings was compared by the Mann–Whitney *U* test. Time-response to BDNF in isolated hearts was analysed using one-way ANOVA for repeated measures. Statistical significance was set as $P < 0.05$.

3. Results

3.1 Basic characteristics

The results are summarized in *Table 1*. As expected, SBP, DBP, and HR were higher in SHR than in WKY. In these two groups, physical training did not change any of these parameters, while it significantly increased the weight and citrate synthase activity of the soleus muscle.

3.2 BDNF is present in high amounts in the cardiovascular system with a strong expression in endothelial cells

Experiments were performed in sedentary WKY. Levels of mBDNF (*Figure 1A*) and proBDNF (*Figure 1B*) in the heart and aorta were in the

Table 1 Basic characteristics

	WKY	SHR	WKY-T	SHR-T
Body weight (g)	305 ± 9	255 ± 10**	299 ± 11	252 ± 14
SBP (mmHg)	112 ± 3	180 ± 8**	114 ± 6	182 ± 10
DBP (mmHg)	92 ± 5	150 ± 12**	96 ± 9	150 ± 13
HR (bpm)	296 ± 16	404 ± 30**	291 ± 11	407 ± 15
Soleus muscle weight (mg)	127 ± 7	99 ± 6**	145 ± 14*	122 ± 9††
Citrate synthase activity (nmol/min/mg of proteins)	265 ± 14	284 ± 12	284 ± 19*	332 ± 12††

Body weight, SBP, DBP, HR, weight, and citrate synthase activity of the soleus muscle were measured in sedentary and trained (T) WKY and SHR. Values are expressed as means ± SD ($n = 14–16$ rats per group). Comparisons were done by using the Kruskal–Wallis test followed by the Mann–Whitney *U* test.
*$P < 0.05$ vs. WKY.
**$P < 0.001$ vs. SHR.
†$P < 0.01$.
††$P < 0.001$ vs. SHR.

same range than those observed in the brain. The spatial pattern of BDNF staining in cardiac and aortic sections was consistent with an endothelial localization of the neurotrophin as confirmed by the co-localization of BDNF with vWF (*Figure 1C*). No BDNF expression was observed in other tissue, except a slight expression in the media of both the aorta and coronary artery. Consistent with the presence of BDNF in VSMCs, BDNF co-localized with alpha smooth muscle actin (*Figure 2*). Regarding the heart, BDNF staining was intense in endothelial cells of the endocardium and absent in other tissue. In the vessels, BDNF staining was greatly higher in endothelial cells than in VSMCs in both the coronary artery and the aorta.

3.3 BDNF endothelial staining is dependent on endothelial function

Evidence of BDNF expression by the healthy endothelium led us to investigate whether conditions recognized to decrease (essential hypertension) or to improve (physical training) endothelial function can change endothelial BDNF staining. Results are summarized in *Figure 3* (representative figures of BDNF staining in *Figure 3A* and grade of cellular BDNF staining in *Figure 3B*). Hypertension resulted in decreased staining both in the endocardium and in the vascular endothelium (coronary artery and aorta), even though the difference did not reach significance for coronary arteries. Opposite effects were induced by physical training that resulted in WKY in a significant increase in endothelial BDNF staining whatever its localization. A trend towards an increase in BDNF staining was also noticed in VSMCs from WKY-T when compared with WKY. Notably, the effects of physical training on BDNF staining reported in WKY were still observed, when physical training was induced in SHR.

As the antibody used to localize BDNF in the aorta and the heart does not discriminate between pro- and mBDNF, whether changes in endothelial BDNF staining were due to changes in the expression of proBDNF, mBDNF or both was not known. Therefore, using western blot analysis, we measured the two forms of BDNF in the whole heart and aorta. The results are shown in *Figure 4*. It is noteworthy that the significant changes in endothelial BDNF staining induced by hypertension and physical training (in WKY) in the heart or in the aorta (*Figure 3B*) were associated with significant changes in the same direction of pro- and/or mBDNF levels in the corresponding whole organs. In detail, decreased endothelial BDNF staining in the aorta and the endocardium induced by hypertension was associated with reduced proBDNF levels in the aorta (−52%, $P < 0.01$, *Figure 4B*), yet without a decrease in any of the forms of BDNF in the heart. An explanation for this may be the lack of effect of hypertension on BDNF expression in the coronary arteries. Regarding the effect of physical training in WKY, its positive impact on endothelial BDNF staining (endocardium, coronary artery, and aorta, *Figure 3B*) was associated with a selective increase by ∼50% ($P < 0.05$) in mBDNF levels in the aorta (*Figure 4A*) and in proBDNF levels in the heart (*Figure 4D*). In contrast, when induced in SHR physical training increased mBDNF levels in the heart (*Figure 4C*, $P < 0.05$) and had no effect on the aorta (*Figure 4A* and *B*), suggesting a complex interaction between physical training and hypertension on BDNF metabolism. The above data indicate that hypertension and physical training not only target BDNF synthesis in an opposite way but also that the induced changes likely rely on different mechanisms.

3.4 Exposure of CECs to high shear stress mimics the effect of physical training on aortic endothelium

To gain insight into the mechanisms by which physical training induced endothelial BDNF up-regulation, pro- and mBDNF levels were measured in CECs subjected to no (static conditions), low (2 dynes/cm^2), or high shear stress (14 dynes/cm^2). As shown in *Figure 5A*, mBDNF levels in cells exposed to LSS and HSS were 135% (NS) and 457% ($P < 0.01$) of values obtained in static conditions, respectively, while proBDNF levels were not modified by shear stress. Consistent with a secretion of mBDNF by the cells, mBDNF was detected in the medium in which the levels were 175 and 320% of values obtained in static conditions after LSS and HSS, respectively (*Figure 5B*). These data suggest that the secretion of mBDNF by cells was proportional to shear stress intensity.

3.5 BDNF induces vasodilation in aorta but does not change cardiac function

As BDNF originating from endothelial cells may act as an autocrine/paracrine factor, we examined the effect of exogenous mBDNF on isolated aortic rings and perfused hearts from sedentary WKY. Exposure of aortic rings to mBDNF (10^{-8} mol/L) induced a relaxation that gradually increased from 2 to 10 min (*Figure 5C*). In contrast, perfusion of hearts with mBDNF (15×10^{-11} mol/L for 30 min) changed neither coronary flow nor heart rate and contractility (*Figure 6*).

4. Discussion

Our study provides important new findings on BDNF in the adult cardiovascular system. First, the heart and aorta contain high BDNF protein levels, the expression being prominent in endothelial cells when compared with other cell types. Second, unlike the heart, the aorta appears to be a target organ of BDNF. Third, essential hypertension is associated with decreased expression of endothelial BDNF, whereas physical training leads to endothelial BDNF up-regulation not only in WKY but also in SHR. Fourth, exposure of CECs to high shear stress mimics the vascular effect of physical training on BDNF.

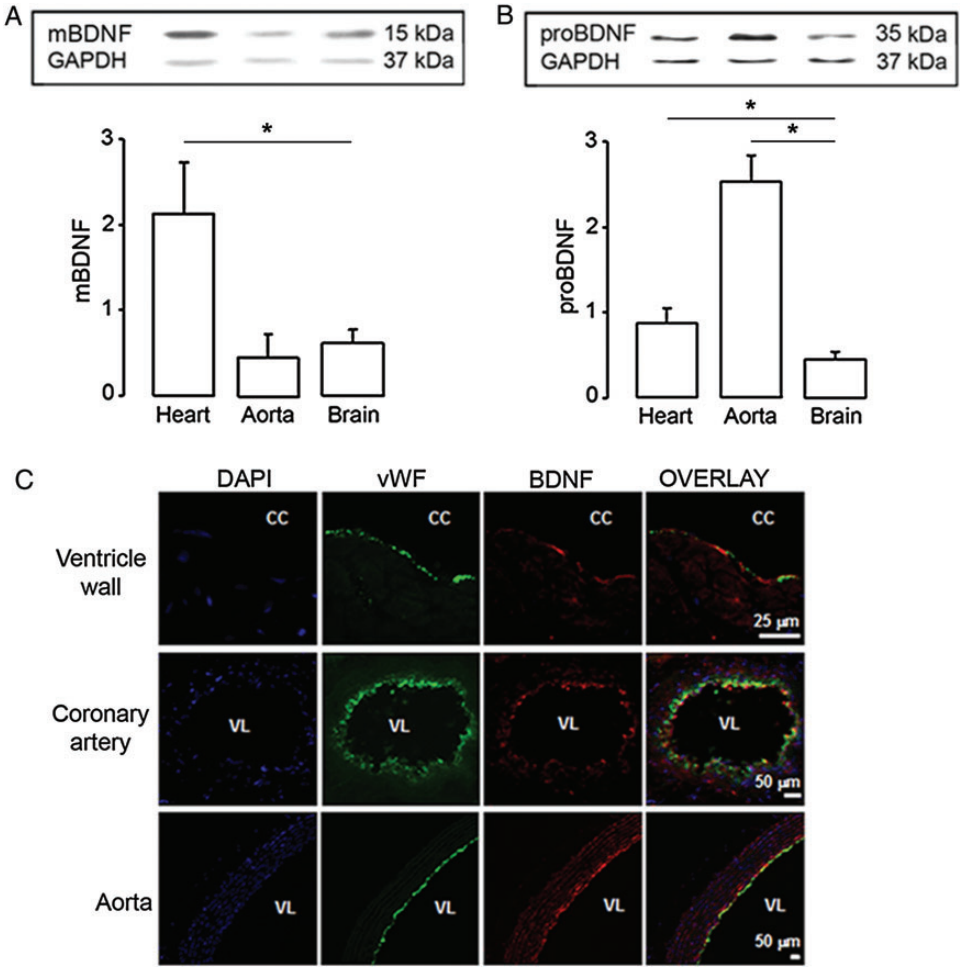


Figure 1 BDNF expressions in heart and vessels. (*A*) mBDNF and (*B*) proBDNF levels (means ± SD, $n = 7$ sedentary WKY) in the heart and aorta were compared with those of the brain (hippocampus) by using Friedman's test followed by Wilcoxon's test. *$P < 0.01$ vs. brain. (*C*) Representative figures of BDNF staining in the cardiac ventricle wall, coronary artery, and aorta from WKY. DAPI (blue) and von Willebrand factor (vWF, green) were used as nuclear and endothelial markers, respectively. Red-labelled immunofluorescence represents BDNF. Overlay shows BDNF staining in endothelial cells. Similar results were observed in all examinations ($n = 5–6$ rats per organ). CC, cardiac cavity; VL, vascular lumen. Scale bar25–50 μm.

The present study is the first to measure pro- and mature BDNF (mBDNF) levels in the cardiovascular system. Indeed, available data are scarce and essentially relate to BDNF mRNAs or ELISA-measured BDNF protein levels in the heart, aorta, pulmonary artery and cultured brain-derived endothelial cells.[23–26] However, BDNF mRNA is not obligatorily processed into the mature form of BDNF and ELISA tests do not distinguish between mature and premature forms of BDNF. Our results on comparative BDNF levels in the cardiovascular system vs. the brain are in opposition to the well-accepted idea that the nervous system contains most of the BDNF that is present in the body. Indeed, cardiovascular tissue levels of mature and proBDNF were in the same range as those measured in the hippocampus, which is the brain region with the highest BDNF levels.[20] Moreover, regarding BDNF production, our results suggest that the endothelial cell is to the cardiovascular system, what the neuron is to the nervous system. Indeed, the greatest BDNF expression was found in endothelial cells both in the heart and vessels, the expression being faint in VSMCs and absent in cardiomyocytes.

While BDNF appears essential for cardiovascular development,[4,5] little is known about the role of BDNF in regulating the adult cardiovascular system. Consistent with the modulation of vasoreactivity by BDNF, a relaxant effect of exogenous mBDNF was recently revealed in a model of pig pulmonary arterial rings.[27] In the present study, we reported a differential effect of mBDNF on aortic vs. coronary reactivity. Indeed, the vasorelaxation of aortic rings induced by BDNF contrasted with the failure of the neurotrophin to increase coronary flow in perfused hearts. Notably, perfusion of the heart with BDNF was without impact on contractile function, excluding changes in cardiomyocytes activity as a confounding factor. One possible explanation for the differential effect of BDNF on coronary vs. aortic vasculature might be that mBDNF concentration in heart perfusion buffer was not high enough (15×10^{-11} vs. 10^{-8} mol/L in the incubation medium of aortic rings). Another possibility relates to a differential expression of TrkB receptors among vascular beds as previously shown.[25] The present study did not aim to investigate the signalling pathways involved in BDNF-induced vasodilation. However, our results showing that the relaxant effect of

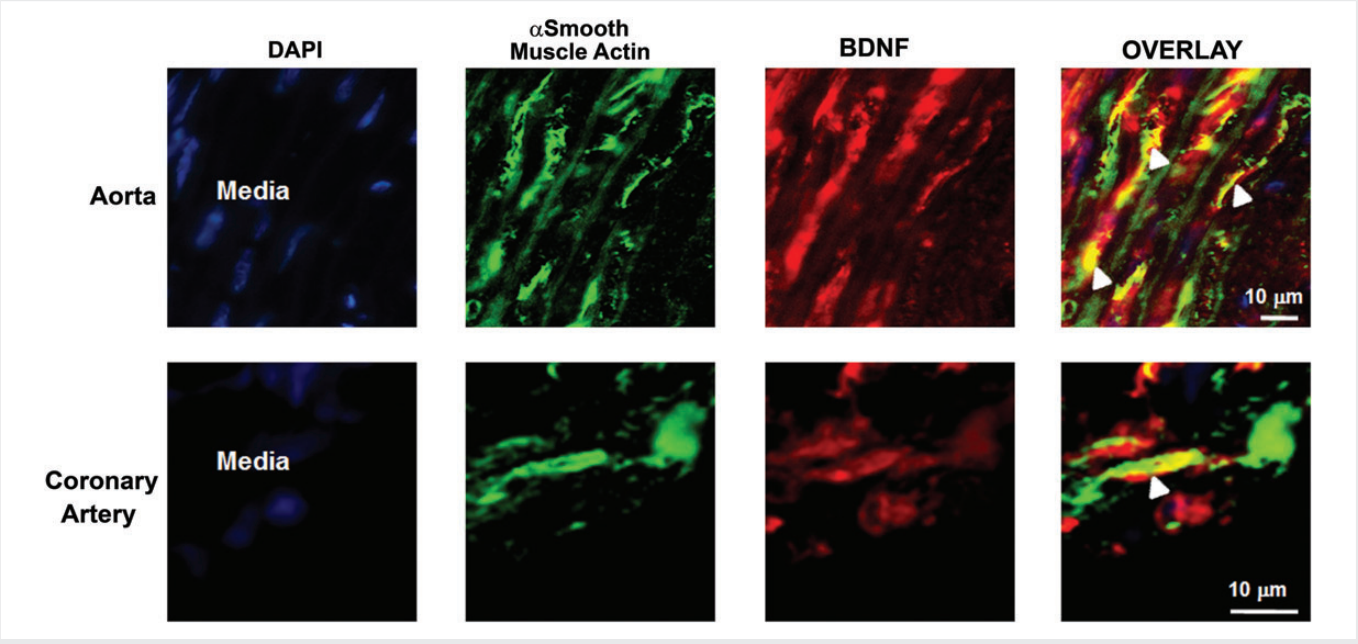
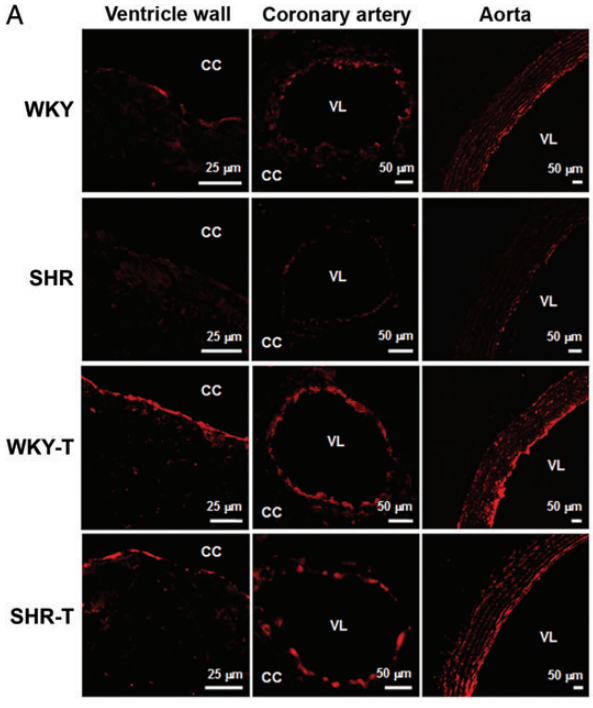


Figure 2 BDNF expression in vascular smooth muscle cells. Representative figures of the media of the aorta and the coronary artery collected from sedentary WKY show immunofluorescence staining for DAPI (blue), alpha smooth muscle actin (green), BDNF (red), and the overlay (yellow). Arrowheads represent cells staining for BDNF and alpha-actin. Scale bar 10 μm.



B

	WKY	SHR	WKY-T	SHR-T
Endothelial cells				
Endocardium	1 (1-2)	1 (0-1)*	3 (2-3)**	2 (1-3)††
Coronary artery	1 (1-2)	1 (0-1)	3 (2-3)*	2 (1-3)†
Aorta	2 (1-2)	1 (0-1)*	3 (2-3)*	2 (1-2)†
Muscle cells				
Cardiomyocyte	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
VSMC	0 (0-1)	0 (0-1)	1 (0-2)	1 (0-1)

Figure 3 Endothelial BDNF staining is dependent on endothelial function. (*A*) Representative figures of BDNF staining (red) in sedentary and trained (T) WKY or SHR. Similar results were observed in all examinations ($n = 4–7$ rats per organ). CC, cardiac cavity; VL, vascular lumen. Scale bar 25–50 μm. (*B*) Intensity of BDNF staining was graded on a scale from 0 to 3 (0, no or mild expression; 1, moderate expression; 2, high expression; 3, intense expression). Values are expressed as median affected by minimum and maximum grade value (in brackets). Values were compared by using the Kruskal–Wallis test followed by the Mann–Whitney *U* test. *$P < 0.05$, **$P < 0.01$ vs. WKY, †$P < 0.05$, ††$P < 0.01$ vs. SHR.

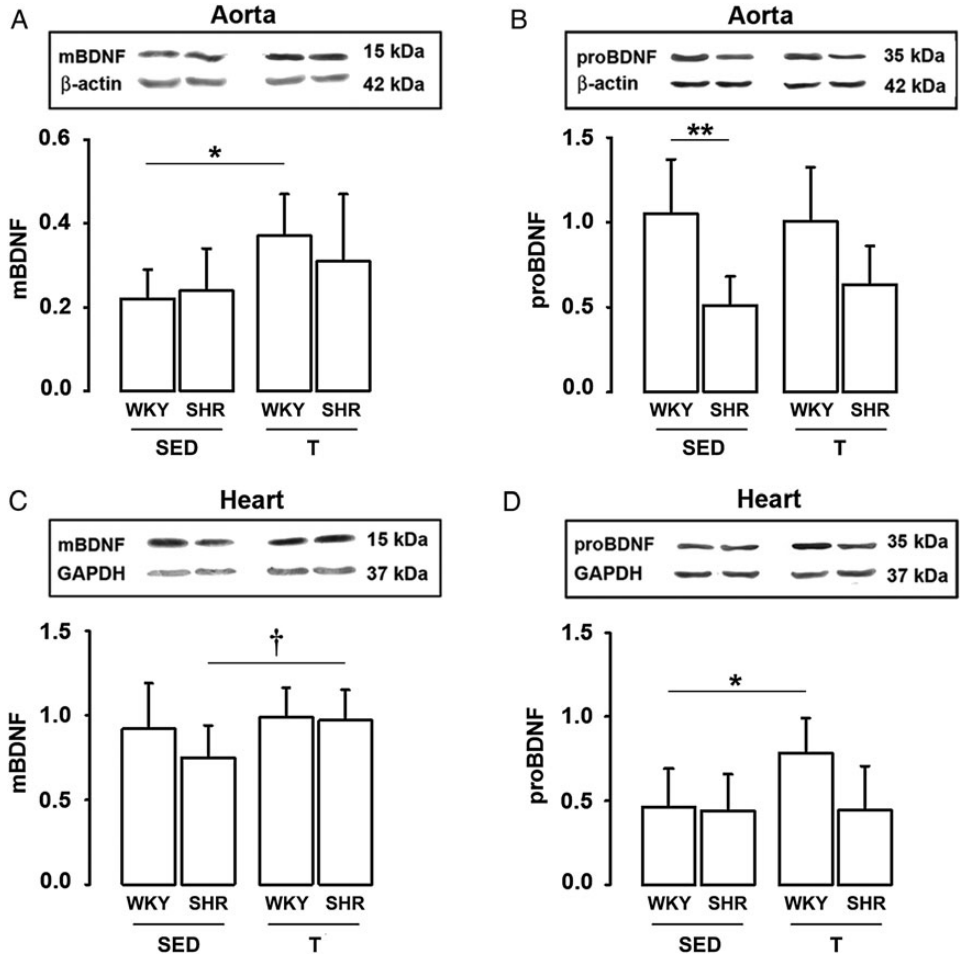


Figure 4 Effects of hypertension and physical training on cardiovascular BDNF levels. mBDNF (*A* and *C*) and proBDNF (*B* and *D*) levels were measured in the heart and aorta from sedentary (SED) and trained (T) WKY or SHR using western blot analysis. Values expressed as means ± SD ($n = 7–8$ rats) were compared by using Kruskal–Wallis test followed by the Mann–Whitney *U* test. *$P < 0.05$, **$P < 0.01$ vs. WKY, †$P < 0.05$ vs. SHR.

mBDNF occurs within the minute-time frame suggest the involvement of 'non genomics'-dependent mechanisms.

It is well documented that physical training has a strong positive link with cardiovascular health.[28] The cardiovascular benefits of physical training are, at least in part, due to changes in the phenotype of endothelial cells as a result of increased shear stress.[29] Although shear stress-sensing mechanisms are ill-defined, DNA microarray experiments showed that more than 600 endothelial genes are shear stress responsive.[30] The present study reveals that physical training *in vivo*, like shear stress *in vitro*, increased mBDNF production by endothelial cells, suggesting that mBDNF up-regulation in response to physical training relies on shear stress-dependent mechanisms. These data apparently contrast with the sole study on the subject that reported decreased BDNF levels in the medium of HUVECs exposed to shear stress (24 dynes/cm^2 for 24 h).[31] However, in this study, BDNF levels were measured with ELISA tests. Evidence that proBDNF levels did not increase in response to physical training and shear stress is compatible with the notion that stimulation of the enzymatic cleavage of proBDNF into mBDNF contributes to physical training-induced increased production of mBDNF as recently reported for the brain.[32,33] To the best of our knowledge, it is also the first time that physical training is reported to stimulate BDNF production in organs other than the brain, the spinal cord, or the skeletal muscle.[34–36] Interestingly, using the same protocol of physical training, we recently reported the presence of BDNF in the endothelium of cerebral vessel in trained rats but not in sedentary rats.[20] Accordingly, physical training targets vascular BDNF irrespective of vessels localization (central vs. peripheral). Moreover, our results may help the interpretation of clinical studies reporting an elevation in plasma BDNF levels during and after physical training.[37–40] It was hypothesized that this increase was due to an enhanced release of BDNF from the brain[41,42] or from platelets.[43] Evidence that physical training up-regulates endothelial BDNF and that endothelial cells secrete BDNF in response to shear stress (our present results on CECs) supports endothelium as an alternative source of the BDNF excess found in plasma during physical training. There is a consensus on neuronal BDNF as the main chemical translator of the benefits of physical training on the brain.[33] Likewise from our data emerges the hypothesis that endothelial BDNF might be involved in the benefits of physical training on the cardiovascular system.

Endothelial dysfunction plays an important role in clinical expression of significant cardiovascular diseases. It has been consistently associated with cardiovascular risk factors including hypertension and was proposed to

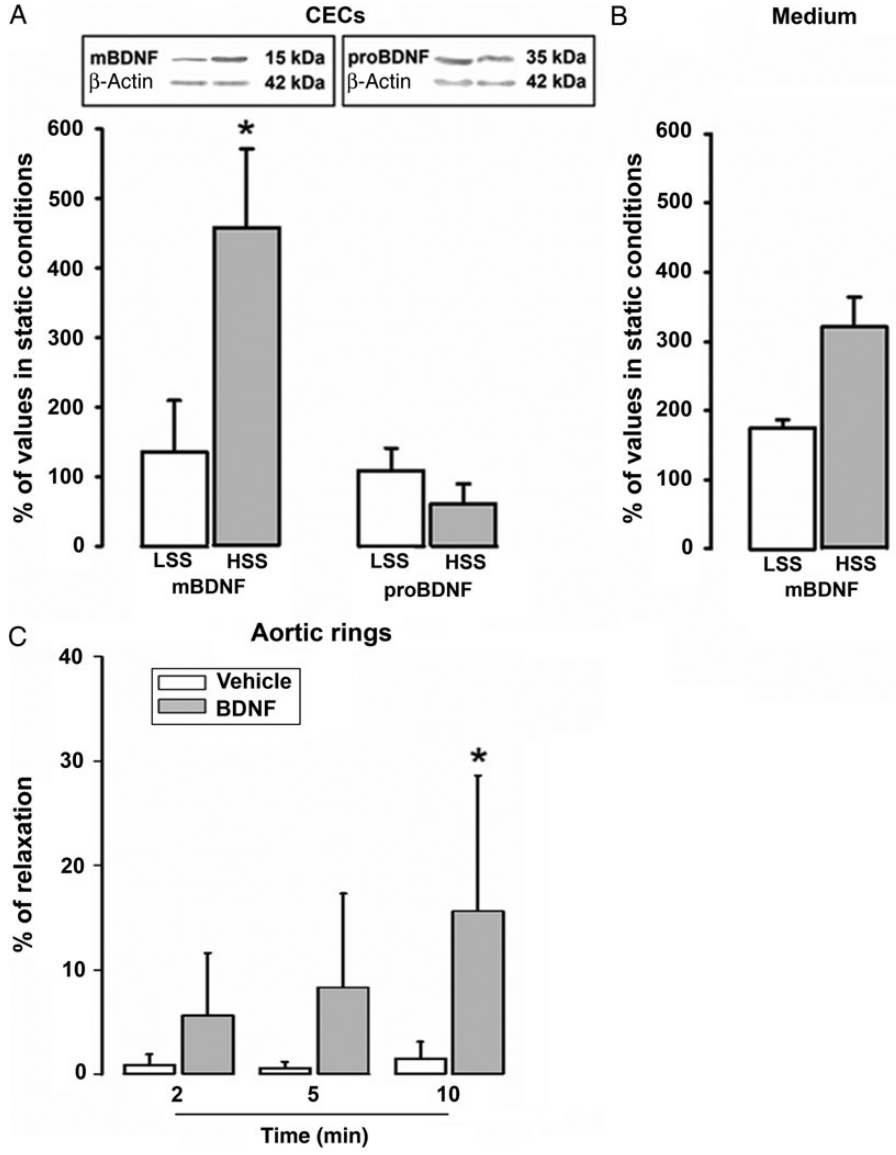


Figure 5 Effect of shear stress on BDNF levels in CECs (*A* and *B*) and vasorelaxant effect of BDNF on isolated aortic rings (*C*). BDNF levels were measured (*A*) in CECs and (*B*) in the culture medium after exposure of cells for 24 h to static conditions, LSS (2 dynes/cm^2) or HSS (14 dynes/cm^2). Values (means $\pm$ SD, $n = 4-5$ for each condition) are expressed as percentage of values obtained in static conditions and compared using the Kruskal–Wallis test followed by the Mann–Whitney U test. *$P < 0.01$ vs. static conditions. The vascular effect of BDNF was studied on isolated phenylephrine-preconstricted aortic rings collected from sedentary WKY (*C*). Rings were exposed to vehicle or recombinant mBDNF (10^{-8} mol/L) and relaxation was assessed from 2 to 10 min after injection. Values (means $\pm$ SD, $n = 14$ rings) are expressed as the percentage reduction of phenylephrine-induced tone. *$P < 0.05$ vs. vehicle after Mann–Whitney *U* test.

predict a high risk of cardiovascular events.[44] The present study reveals a lower endothelial BDNF expression in the intima of hypertensive SHR than in their normotensive controls. This new finding completes two previous studies in SHR that identified an interaction between hypertension and BDNF metabolism in the brain[45] and lungs.[46] The mechanisms involved in hypertension-induced BDNF hypostaining remain to be further explored. However, an involvement of a defect in endothelial transcription of the *bdnf* gene is suspected from our results showing decreased proBDNF levels in the aorta. Notably, as physical training did not exert a hypotensive effect in our conditions, mechanisms independent of blood pressure lowering are likely involved in hypertension-induced changes in vascular BDNF metabolism as recently reported for cerebral BDNF metabolism in stroke prone SHR.[47]

5. Conclusion

Our results report that BDNF is expressed in a great amount in peripheral vessels, where it can act as a local modulator of vasoreactivity and that endothelial BDNF expression mirrors endothelial function. These data are compatible with the exciting hypothesis that BDNF can be a new link between endothelial function and cardiovascular health and

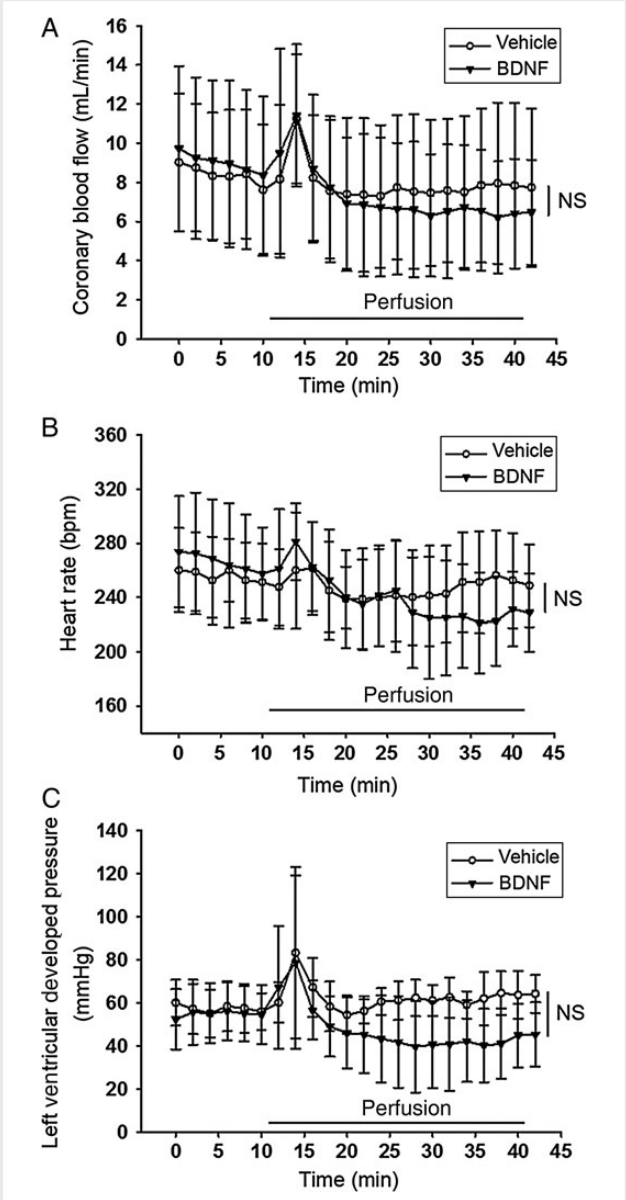


Figure 6 BDNF does not change heart parameters. (*A*) Coronary blood flow, (*B*) heart rate, (*C*) left ventricular developed pressure were measured on isolated hearts from sedentary WKY perfused for 30 min with vehicle or recombinant mBDNF (15×10^{-11} mol/L). Values are means $\pm$ SD ($n = 8$). Time-response to mBDNF in isolated hearts was analysed using one-way ANOVA for repeated measures. NS, non significant.

consequently open new therapeutic avenues in cardiovascular diseases associated with endothelial dysfunction.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Conflict of interest: none declared.

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