Table 3 | Multiple regression models of ΔSBP2 in specified populations

	Model 1				Model 2				Model 3			
N	1,094				1,711				848			
Adjust R^2	0.366				0.366				0.553			
Independent variables	***B***	**95% CI**	***β***	***P***	***B***	**95% CI**	***β***	***P***	***B***	**95% CI**	***β***	***P***
Physical variables												
Gender	−2.808	−3.963 to −1.654	−0.170	<0.001	−2.687	−3.571 to −1.803	−0.169	<0.001	−4.539	−5.645 to −3.432	−0.292	<0.001
Age	−0.002	−0.040 to 0.036	−0.003	0.921	−0.005	−0.035 to 0.026	−0.007	0.764	0.176	0.151 to 0.201	0.378	<0.001
Height	−0.186	−0.251 to −0.120	−0.212	<0.001	−0.170	−0.221 to −0.119	−0.199	<0.001	−0.119	−0.181 to −0.057	−0.147	<0.001
BMI	−0.293	−0.410 to −0.175	−0.120	<0.001	−0.210	−0.301 to −0.120	−0.090	<0.001	−0.469	−0.598 to −0.340	−0.174	<0.001
Hemodynamic variables												
PR	−0.342	−0.375 to −0.308	−0.492	<0.001	−0.336	−0.362 to −0.310	−0.508	<0.001	−0.238	−0.273 to −0.203	−0.318	<0.001
DBP	0.149	0.114 to 0.184	0.212	<0.001	0.141	0.114 to 0.168	0.210	<0.001	0.257	0.214 to 0.300	0.295	<0.001
Group of drugs												
Nitro	−3.393	−5.058 to −1.728	−0.098	<0.001	−3.631	−4.913 to −2.350	−0.109	<0.001				
Drug group	2.678	0.984 to 4.372	0.075	0.002	2.709	1.073 to 4.345	0.064	0.001				
Mixed					0.448	−0.209 to 1.105	0.027	0.181				

Drug group: code "1" = treated with non-VD only; code "0" = other treatments with VD only and mixed combination with VD and non-VD. Mixed: code "1" = mixed combination with VD and non-VD; code "0" = all other treatments (with VD only or non-VD only). VD (vasodilating antihypertensive drugs) includes angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, and α-blockers; non-VD (nonvasodilating antihypertensive drugs) includes diuretics and β-blockers. Subject populations for Models 1 through 3 were hypertensives (HT) without mixed (VD + non-VD) combination, HT including mixed combination except Nitro only and non-HT, respectively. Sixteen HT patients taking Nitro alone were excluded from Models 1 and 2. ΔSBP2 is defined in **Figure 1**. 95% CI, 95% confidence interval of *B*; *B*, nonstandardized partial regression coefficient; BMI, body mass index; DBP, diastolic blood pressure; PR, pulse rate; *β*, standardized partial regression coefficient.

In contrast, with non-VD alone, it was even higher than in non-HT.

To enable the characterization of each individual class, we constructed a model including all classes of antihypertensive agents and a significant interactive term as independent variables (**Table 4**). Only "CCB × Diur" was significant among all interactive terms that could have been assessed previously (**Supplementary Table S2a–d** online). Among antihypertensive classes, ARB, CCB, and α-blockers had significant associations with lower ΔSBP2.

Using this model, we performed adjusted interclass comparisons of antihypertensive drugs to characterize each individual class in terms of ΔSBP2 (**Figure 3a**). Treatments with VD antihypertensive classes showed a lower ΔSBP2 than nonvasodilators comparably to VD and non-VD in **Figure 2**. Most importantly, no significant difference in ΔSBP2 was detected among any VD classes. The mean level of ΔSBP2 (−9.7 mm Hg) was 3.3 and 2.0 mm Hg lower than with nonvasodilators and in non-HT. In contrast, with βBL or diuretics, the averaged ΔSBP2 value was 1.3 mm Hg higher than in non-HT. When pulse rate adjustment was added (**Figure 3b**), the higher level of ΔSBP2 associated with βBL was reduced, which abolished the significant difference from ACEI.

Adjusted comparisons of ΔSBP2 among treatments with frequently used combinations of antihypertensives were also performed (**Figure 4**) based on the model (**Table 4**). The combination of two different VD antihypertensive classes, such as CCB plus ARB or ACEI, showed the lowest level of ΔSBP2 (−10.5 mm Hg; **Figure 4a**), which was lower than in any single VD antihypertensive class shown in **Figure 3a**. When the drug combined with ARB or CCB was a diuretic or βBL, the ΔSBP2 value increased in this order. The combination of diuretics and βBL showed the highest ΔSBP2 (−3.9 mm Hg). Additional pulse rate adjustment tended to reduce ΔSBP2 with βBL-including combinations, whereas its influence varied for Diur-including combinations. Differences between the combination of CCB plus ARB or ACEI and that of diuretics plus βBL remained significant even after pulse rate adjustment.

DISCUSSION

In the present study, all individual classes of antihypertensive agents commonly used in Japan and combinations of two different classes were tentatively characterized in terms of central

Table 4 | Multiple regression models of ΔSBP2 in treated hypertensives

N		1,727		
Adjusted R^2		0.379		
Independent variables	*B*	95% C.I.	*β*	*P*
Physical variables				
Gender	−2.358	−3.236 to −1.480	−0.148	<0.001
Age	−0.004	−0.034 to 0.027	−0.005	0.813
Height	−0.185	−0.235 to −0.134	−0.215	<0.001
BMI	−0.200	−0.290 to −0.109	−0.085	<0.001
Hemodynamic variables				
PR	−0.336	−0.362 to −0.310	−0.508	<0.001
DBP	0.140	0.113 to 0.167	0.207	<0.001
Class of drugs				
ARB	−1.012	−1.682 to −0.343	−0.062	0.003
ACEI	−0.516	−1.512 to 0.479	−0.021	0.309
CCB	−0.837	−1.619 to −0.056	−0.049	0.036
αBL	−2.122	−3.158 to −1.087	−0.077	<0.001
Diur	1.890	0.690 to 3.090	0.102	0.002
βBL	0.537	−0.218 to 1.292	0.028	0.163
Nitro	−3.675	−4.882 to −2.468	−0.118	<0.001
Interactive term				
CCB × Diur	−1.953	−3.414 to −0.493	−0.089	0.009

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; CCB, calcium channel blockers; DBP, diastolic blood pressure; Diur, diuretics; Nitro, nitrates or nicorandil; PR, pulse rate; αBL, α-blockers; βBL, β-blockers.

effects indexed by ΔSBP2. We found that treatment with any VD antihypertensive class showed lower CBP than any nonvasodilatory class when peripheral BP was lowered to the same level. CBP assessment was highly objective using a validated semiautomatic radial artery tonometry system,[15,19] which could minimize variance and errors related to observer or operator skill.

Feature of ΔSBP2 and its cross-sectional determinants

The augmentation index reportedly depends on age,[21] gender,[22] height,[23] heart rate,[24,25] and BP levels.[26] ΔSBP2 relates to radial augmentation index by definition as radial augmentation index is the ratio of (PP+ΔSBP2) to PP1 (**Figure 1**). The ΔSBP2 value is always negative and reflects the actual reduction in SBP and pulse pressure from peripheral to central sites. Comparing Models 2 and 3 (**Table 3**), significant associations between these variables and ΔSBP2 observed in the non-HT population were partially preserved even in treated HT except for age.

Interpretation of the results

In addition to adjustment for common confounders, model-based estimation of ΔSBP2 compensating for coadministered drug effects enabled interclass comparisons of central

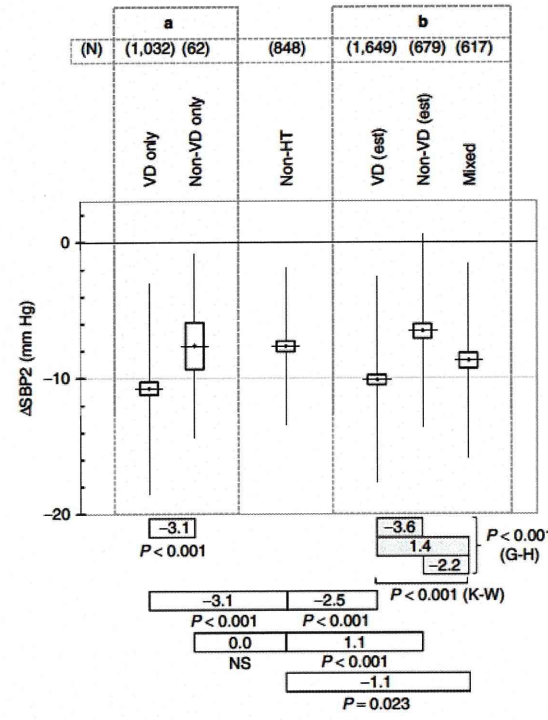

Figure 2 | Adjusted comparisons of ΔSBP2 between vasodilating (VD) and non-VD antihypertensive drugs. VD group includes angiotensin receptor blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, and α-blockers, and non-VD group includes β-blockers and diuretics. Data are shown as the mean level (horizontal line) and the 95% confidence interval (box height) as well as the range of ±1 s.d. by a vertical error bar. *P* value in the lower part of the figure indicates the result of the Mann–Whitney *U*-test of each specified intergroup comparison unless specified in the figure (K-W, Kruskal–Wallis test; G-H, Games–Howell multiple comparison test). The number in each box indicates the difference (mm Hg) of mean ΔSBP2 between compared groups. Gray area A shows the comparison between actual VD and non-VD only regimens irrespective of the number of drugs. ΔSBP2 data were adjusted for confounding factors (age, gender, height, BMI, DBP, and the use of nitrates = "0") based on Model 1 in **Table 3**. Cases with mixed combination (VD + non-VD) regimens were excluded. Gray area B shows the comparison among VD(est), non-VD(est) and Mixed combinations of VD and non-VD. "(est)" indicates including data derived from mixed combination, for which the effects of VD or non-VD alone on ΔSBP2 were estimated using Model 2 in **Table 3**. Data in the nonhypertensive (non-HT) population indicate the physiological reference value of ΔSBP2 estimated by adjusting confounding factors to the mean value of treated HT using Model 3 in **Table 3**.

effects of antihypertensives that were impossible to make directly with raw data, and played a "data-mining" role in this study.

Central effects of antihypertensive classes. The lower level of CBP, even lower than in non-HT, with VD antihypertensives administered alone (area B in **Figures 2** and **3a**) might lead to more effective unloading of pulsatile mechanical stress on the cardiovascular system than nonvasodilatory agents. The observed reduction of ΔSBP2 with βBL by additional pulse rate adjustment (**Figure 3b**) suggested that the CBP-raising feature of βBL might be attributable to its negative chronotropic effect.

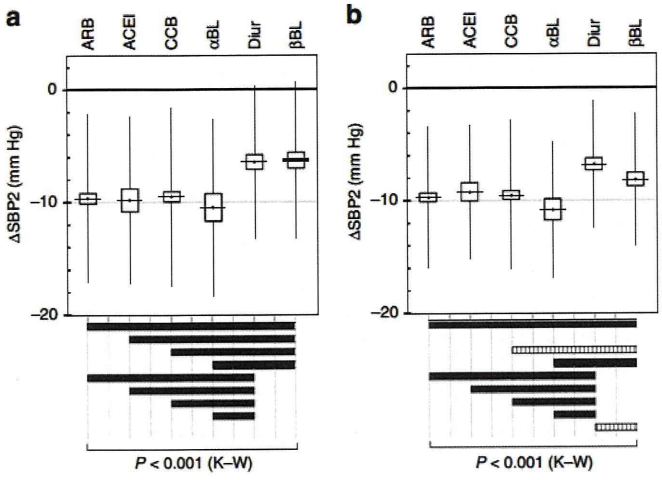

Figure 3 | Adjusted interclass comparisons of ΔSBP2. Data are shown in the same format as in **Figure 2**. A solid or striped horizontal bar in the lower part of the figure denotes a comparison with significant difference determined by Games–Howell multiple comparison test indicating $P < 0.001$ or $P < 0.05$, respectively. The number of patients included in each antihypertensive group is shown in **Table 2** ("Class of antihypertensives"). As indicated in that table, it includes patients treated with two or more drugs in combination as well as patients actually taking the specified drug alone. (**a**) Using the model shown in **Table 4**, ΔSBP2 was adjusted for age, gender, height, BMI, DBP, coadministered drugs (including Nitro = "0") other than the specified antihypertensive class, and the interactive term. (**b**) In addition to the adjustment in **Figure 3a**, ΔSBP2 values were adjusted for pulse rate. ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CCB, calcium channel blockers; Diur, diuretics; αBL, α-blockers; βBL, β-blockers.

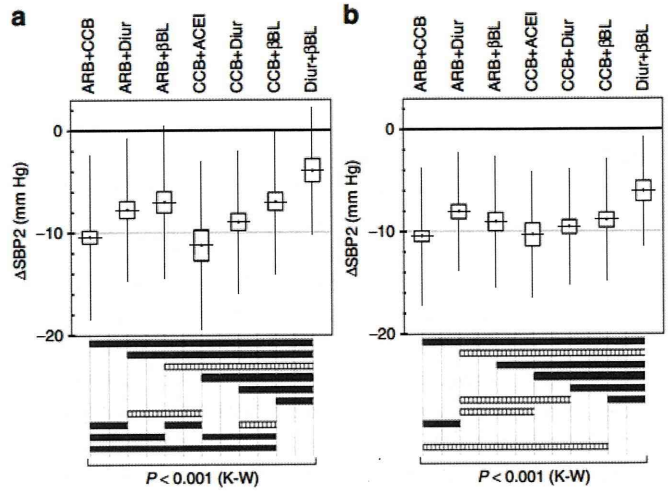

Figure 4 | Adjusted comparisons of ΔSBP2 among frequently used combinations of antihypertensive agents. The number of cases included in each treatment group is indicated in **Table 2** ("Drug classes used in combination"). As indicated in that table, it includes patients treated with three or more drugs as well as patients actually taking only two specified drugs in combination. The format of each graph is as in **Figure 3**. (**a**) Using the model shown in **Table 4**, data were adjusted for age, gender, height, BMI, DBP, and coadministered drugs (including Nitro = "0") other than the specified two-drug combination if applicable. The interactive term was set as "0" except for the CCB+Diur combination. (**b**) In addition to the adjustment in **Figure 4a**, data were also adjusted for pulse rate. ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CCB, calcium channel blockers; Diur, diuretics; βBL, β- blockers.

Central effects of major combinations of antihypertensive drugs. **Figure 4a** suggested some additive CBP-lowering effects of two different vasodilatory classes. In contrast, along with higher ΔSBP2 above the physiological level with non-VD (**Figure 2**), the findings with non-VD-including combinations suggested that the central effects of non-VD antihypertensives were CBP-raising rather than less potent CBP-lowering.

The negative *B* estimate of the significant interactive term, "CCB × Diur", suggested some synergistic CBP-lowering effect.

Comparison with other studies

The results of this study are consistent with reported studies, such as the CAFE study,[1] and other small-scale studies.[3–11] More recently, other small-scale treatment trials dealing with the effects on CBP of a newer class of antihypertensives, ARB, compared with βBL, have been reported.[27–29] The results of these studies can be summarized as the superiority of vasodilatory antihypertensives, including CCB, ARB, and ACEI, to nonvasodilatory agents, such as diuretics and βBL. Similar to the CAFE study, the higher CBP level with βBL-including treatments was evident in this study; however, these studies used only limited antihypertensive regimens. This cross-sectional observation, including a data-mining model-based estimation process, enabled tentative but simultaneous comparisons of all commonly used antihypertensive agents specifically in terms of central effects by individual class as well as by common combination regimens. Additionally, this study reported ΔSBP2 levels in HT compared with an adjusted physiological reference level.

Limitations

Issues relating to the observational study design. This study was designed as an exploratory study to generate rather than to test hypotheses; therefore, the results cannot confirm the causal effects of each drug class on CBP but provide hypotheses to be assessed. Because of the cross-sectional and observational design, in which the selection of antihypertensive drugs was left to the clinician, and might have been related to patients' clinical characteristics, an indication bias was inevitable. Although we examined available clinical variables in some participants (**Supplementary Tables S1a and S1b** online), they included only some of the population studied and all data could not be adjusted for clinical confounders. The influence of indication bias could therefore not be avoided and should be taken into consideration when interpreting the results of this study.

Issues relating to model-based adjustment. In addition, the results should be interpreted with caution for the following reasons. Data adjustments were based on linear regression models. The influences of adjusted variables are not necessarily linear. Also, the doses and duration of specified antihypertensive medications were not taken into consideration due

to the limited study design. Although the findings obtained from such analysis are not conclusive, we believe that they can provide information to develop hypotheses.

Interpretation of nitrates. As nitrates are not classified as antihypertensive agents, data were compared adjusting for the use of this type of drugs. Only a minority of subjects was given nitrates (**Table 2**), but significantly lower ΔSBP2 was observed. This may be attributable to, at least in part, cardiac dysfunction,[30,31] because nitrates are usually prescribed for cardiac patients. We could not adjust for cardiac function because of the absence of required information. It is well-known that nitrates markedly reduce aortic wave reflections or late systolic BP augmentation.[4,32,33] In this study, to compare each class of antihypertensives in terms of central effects, the DBP level was adjusted, indicating that the mean pressure-lowering effect was ignored, which was likely to exaggerate the effect of nitrates as a central antihypertensive. Although a small-scale uncontrolled trial using extended-release isosorbide mononitrate has already been reported,[7] randomized intervention trials are necessary to elucidate whether significant associations with lower ΔSBP2 are from pharmacological effects or cardiac dysfunction, as well as its clinical benefit for HT without cardiac dysfunction.

In summary, among all classes of antihypertensive drugs, any single VD antihypertensive agent (CCB, ARB, ACEI, or α-blockers) might lower CBP without interclass difference, whereas nonvasodilators (Diur and βBL) might raise CBP above the physiological level when peripheral BP is adjusted to the same level. The other novel findings obtained in this study are that (i) among assessable combinations, only CCB+Diur showed synergistic interaction; (ii) otherwise, coadministered VD antihypertensives did not affect the CBP-raising features of nonvasodilators; (iii) the CBP-raising effect of βBL is chiefly attributable to negative chronotropism; and (iv) total cholesterol level, serum creatinine, and hemoglobin showed modest but significant associations with ΔSBP2.

Finally, the hypothetical feature of each antihypertensive class in terms of CBP and its prognostic predictive value should be assessed by large-scale randomized intervention trials.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ajh

Acknowledgments: This study was supported by an unrestricted grant from Omron Healthcare. Some of the devices used to measure radial augmentation index and related parameters were rented from Omron Healthcare.

Disclosure: The authors declared no conflict of interest.

1. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, Hughes AD, Thurston H, O'Rourke M. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 2006; 113:1213–1225.
2. Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, Umans JG, Howard BV. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension* 2007; 50:197–203.
3. Guerin AP, Pannier BM, Marchais SJ, Metivier F, Safar M, London GM. Effects of antihypertensive agents on carotid pulse contour in humans. *J Hum Hypertens* 1992; 6 Suppl 2:S37–S40.
4. Takazawa K, Tanaka N, Takeda K, Kurosu F, Ibukiyama C. Underestimation of vasodilator effects of nitroglycerin by upper limb blood pressure. *Hypertension* 1995; 26:520–523.
5. Cholley BP, Shroff SG, Sandelski J, Korcarz C, Balasia BA, Jain S, Berger DS, Murphy MB, Marcus RH, Lang RM. Differential effects of chronic oral antihypertensive therapies on systemic arterial circulation and ventricular energetics in African-American patients. *Circulation* 1995; 91:1052–1062.
6. Chen CH, Ting CT, Lin SJ, Hsu TL, Yin FC, Siu CO, Chou P, Wang SP, Chang MS. Different effects of fosinopril and atenolol on wave reflections in hypertensive patients. *Hypertension* 1995; 25:1034–1041.
7. Stokes GS, Ryan M. Can Extended-Release Isosorbide Mononitrate be Used as Adjunctive Therapy for Systolic Hypertension? An Open Study Employing Pulse-Wave Analysis to Determine Effects of Antihypertensive Therapy. *Am J Geriatr Cardiol* 1997; 6:11–19.
8. Pannier BM, Guerin AP, Marchais SJ, London GM. Different aortic reflection wave responses following long-term angiotensin-converting enzyme inhibition and beta-blocker in essential hypertension. *Clin Exp Pharmacol Physiol* 2001; 28:1074–1077.
9. Asmar R, Gosse P, Topouchian J, N'tela G, Dudley A, Shepherd GL. Effects of telmisartan on arterial stiffness in Type 2 diabetes patients with essential hypertension. *J Renin Angiotensin Aldosterone Syst* 2002; 3:176–180.
10. de Luca N, Asmar RG, London GM, O'Rourke MF, Safar ME; REASON Project Investigators. Selective reduction of cardiac mass and central blood pressure on low-dose combination perindopril/indapamide in hypertensive subjects. *J Hypertens* 2004; 22:1623–1630.
11. Morgan T, Lauri J, Bertram D, Anderson A. Effect of different antihypertensive drug classes on central aortic pressure. *Am J Hypertens* 2004; 17:118–123.
12. O'Rourke M. Systolic blood pressure: arterial compliance and early wave reflection, and their modification by antihypertensive therapy. *J Hum Hypertens* 1989; 3 Suppl 1:47–52.
13. O'Rourke MF. Arterial mechanics and wave reflection with antihypertensive therapy. *J Hypertens Suppl* 1992; 10:S43–S49.
14. Ting CT, Chen CH, Chang MS, Yin FC. Short- and long-term effects of antihypertensive drugs on arterial reflections, compliance, and impedance. *Hypertension* 1995; 26:524–530.
15. Melenovsky V, Borlaug BA, Fetics B, Kessler K, Shively L, Kass DA. Estimation of central pressure augmentation using automated radial artery tonometry. *J Hypertens* 2007; 25:1403–1409.
16. Pauca AL, Wallenhaupt SL, Kon ND, Tucker WY. Does radial artery pressure accurately reflect aortic pressure? *Chest* 1992; 102:1193–1198.
17. Pauca AL, Kon ND, O'Rourke MF. The second peak of the radial artery pressure wave represents aortic systolic pressure in hypertensive and elderly patients. *Br J Anaesth* 2004; 92:651–657.
18. Takazawa K, Kobayashi H, Shindo N, Tanaka N, Yamashina A. Relationship between radial and central arterial pulse wave and evaluation of central aortic pressure using the radial arterial pulse wave. *Hypertens Res* 2007; 30:219–228.
19. Richardson CJ, Maki-Petaja KM, McDonnell BJ, Hickson SS, Wilkinson IB, McEniery CM. Comparison of estimates of central systolic blood pressure and peripheral augmentation index obtained from the Omron HEM-9000AI and SphygmoCor systems. *Artery Research* 2009; 3:24–31.
20. Hickson SS, Butlin M, Mir FA, Graggaber J, Cheriyan J, Khan F, Grace AA, Yasmin, Cockcroft JR, Wilkinson IB, McEniery CM; Anglo-Cardiff Collaboration Trial Investigators. The accuracy of central SBP determined from the second systolic peak of the peripheral pressure waveform. *J Hypertens* 2009; 27:1784–1788.
21. Kelly R, Hayward C, Avolio A, O'Rourke M. Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 1989; 80:1652–1659.
22. Gatzka CD, Kingwell BA, Cameron JD, Berry KL, Liang YL, Dewar EM, Reid CM, Jennings GL, Dart AM. Gender differences in the timing of arterial wave reflection beyond differences in body height. *J Hypertens* 2001; 19:2197–2203.
23. London GM, Guerin AP, Pannier BM, Marchais SJ, Metivier F. Body height as a determinant of carotid pulse contour in humans. *J Hypertens Suppl* 1992; 10:S93–S95.
24. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol (Lond)* 2000; 525 Pt 1:263–270.
25. Wilkinson IB, Mohammad NH, Tyrrell S, Hall IR, Webb DJ, Paul VE, Levy T, Cockcroft JR. Heart rate dependency of pulse pressure amplification and arterial stiffness. *Am J Hypertens* 2002; 15:24–30.
26. Nürnberger J, Dammer S, Opazo Saez A, Philipp T, Schäfers RF. Diastolic blood pressure is an important determinant of augmentation index and pulse wave velocity in young, healthy males. *J Hum Hypertens* 2003; 17:153–158.

27. Dhakam Z, McEniery CM, Yasmin, Cockcroft JR, Brown MJ, Wilkinson IB. Atenolol and eprosartan: differential effects on central blood pressure and aortic pulse wave velocity. *Am J Hypertens* 2006; 19:214–219.
28. Schneider MP, Delles C, Klingbeil AU, Ludwig M, Kolloch RE, Krekler M, Stumpe KO, Schmieder RE. Effect of angiotensin receptor blockade on central haemodynamics in essential hypertension: results of a randomised trial. *J Renin Angiotensin Aldosterone Syst* 2008; 9:49–56.
29. Vyssoulis GP, Karpanou EA, Kyvelou SM, Adamopoulos DN, Antonakoudis GC, Deligeorgis AD, Cokkinos DV, Stefanadis CI. Beneficial effect of angiotensin II type 1 receptor blocker antihypertensive treatment on arterial stiffness: the role of smoking. *J Clin Hypertens (Greenwich)* 2008; 10:201–207.
30. Westerhof N, O'Rourke MF. Haemodynamic basis for the development of left ventricular failure in systolic hypertension and for its logical therapy. *J Hypertens* 1995; 13:943–952.
31. Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries*, 5th edn. Hodders Arnold: London, 2005.
32. Yaginuma T, Avolio A, O'Rourke M, Nichols W, Morgan JJ, Roy P, Baron D, Branson J, Feneley M. Effect of glyceryl trinitrate on peripheral arteries alters left ventricular hydraulic load in man. *Cardiovasc Res* 1986; 20:153–160.
33. Fitchett DH, Simkus GJ, Beaudry JP, Marpole DG. Reflected pressure waves in the ascending aorta: effect of glyceryl trinitrate. *Cardiovasc Res* 1988; 22: 494–500.

厚生労働省科学研究補助金
医療技術実用化総合研究事業

平成２１年度～平成２３年度
総合研究報告書

バイオニック血圧制御システムの実用化開発
（Ｈ２１－トランス－一般－０１３）

主任研究者：砂川　賢二
（九州大学大学院医学研究院）

Vol. 2

平成２４（２０１２）年５月

厚生労働省科学研究補助金
医療技術実用化総合研究事業

平成２１年度～平成２３年度
総合研究報告書

バイオニック血圧制御システムの実用化開発
（Ｈ２１－トランス－一般－０１３）

主任研究者：砂川　賢二
（九州大学大学院医学研究院）

Vol. 2

平成２４（２０１２）年５月

目　　次
Vol. 2

研究成果の刊行に関する一覧表

【報告書 Vol.2 掲載分】

雑誌

50. Kishi T, Hirooka Y, Konno S, Sunagawa K. Sympathoinhibition induced by centrally administered atorvastatin is associated with alteration of NAD(P)H and Mn superoxide dismutase activity in rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats. J Cardiovasc Pharmacol. 2010 Feb;55(2):184-90.

51. Takemoto M, Nakashima A, Muneuchi J, Yamamura K, Shiokawa Y, Sunagawa K, Tominaga R. Para-Hisian pacing for a pediatric patient with a congenitally corrected transposition of the great arteries (SLL). Pacing Clin Electrophysiol. 2010 Jan;33(1):e4-7.

52. Kamiya A, Kawada T, Mizuno M, Shimizu S, Sugimachi M. Parallel resetting of arterial baroreflex control of renal and cardiac sympathetic nerve activities during upright tilt in rabbits. Am J Physiol Heart Circ Physiol. 298: H1966-H1975, 2010

53. Kawada T, Li M, Kamiya A, Shimizu S, Uemura K, Yamamoto H, Sugimachi M. Open-loop dynamic and static characteristics of the carotid sinus baroreflex in rats with chronic heart failure after myocardial infarction. J Physiol Sci. 60: 283-298, 2010

54. Kawada T, Akiyama T, Shimizu S, Kamiya A, Uemura K, Sata Y, Shirai M, Sugimachi M. Large conductance Ca2+-activated K+ channels inhibit vagal acetylcholine release at the rabbit sinoatrial node. Auton Neurosci. 156: 149-151, 2010

55. Mizuno M, Kawada T, Kamiya A, Miyamoto T, Shimizu S, Shishido T, Smith SA, Sugimachi M. Dynamic characteristics of heart rate control by the autonomic nervous system in rats. Exp Physiol. 95: 919-925, 2010

56. Shimizu S, Shishido T, Une D, Kamiya A, Kawada T, Sano S, Sugimachi M. Right ventricular stiffness constant as a predictor of postoperative hemodynamics in patients with hypoplastic right ventricle: a theoretical analysis. J Physiol Sci. 60: 205-212, 2010

57. Takahama H, Asanuma H, Sanada S, Fujita M, Sasaki H, Wakeno M, Kim J, Asakura M, Takashima S, Minamino T, Komamura K, Sugimachi M, Kitakaze M. A histamine H receptor blocker ameliorates development of heart failure in dogs independently of beta-adrenergic receptor blockade. Basic Res Cardiol. 105: 787-794, 2010

58. Uemura K, Zheng C, Li M, Kawada T, Sugimachi M. Early short-term vagal nerve stimulation attenuates cardiac remodeling after reperfused myocardial infarction. J Card Fail. 16: 689-699, 2010

59. Une D, Shimizu S, Kamiya A, Kawada T, Shishido T, Sugimachi M. Both skeletonized and pedicled internal thoracic arteries supply adequate graft flow after coronary artery bypass grafting even during intense sympathoexcitation. J Physiol Sci. 60: 407-413, 2010

60. Yokokawa M, Chugh A, Ulfarsson M, Takaki H, Han L, Yoshida K, Sugimachi M, Morady F, Oral H. Effect of linear ablation on spectral components of atrial fibrillation. Heart Rhythm. 7: 1732-1737, 2010

61. Sato K, Urbano R, Yu C, Yamasaki F, Sato T, Jordan J, Robertson D, Diedrich A. The effect of donepezil treatment on cardiovascular mortality. Clin Pharmacol Ther. 88: 335-338, 2010

62. Yamakoshi K Bioinstrumentation. IEEE Rev. Biomed. Eng 3: 3-6, 2010

63. Ogawa M, Nogawa M, Yamakoshi T, and Yamakoshi K. Evaluation of cardiovascular stress reaction using HPCD method on a beat-by-beat basis. Advances in Natural Science. 3: 128-132, 2010

64. Yamakoshi K. Current status of non-invasive bioinstrumentation for healthcare. Sensors and Materials. 20: 1-20, 2010

65. 佐藤隆幸 アルツハイマー病に用いられるドネペジルの抗心不全作用循環器内科 68: 496-498, 2010

66. Hirooka Y, Kishi T, Sakai K, Takeshita A, Sunagawa K. Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. Am J Physiol Regul Integr Comp Physiol. 300: R818-26, 2011.

67. Chen L, Nakano K, Kimura S, Matoba T, Iwata E, Miyagawa M, Tsujimoto H, Nagaoka K, Kishimoto J, Sunagawa K, Egashira K. Nanoparticle-mediated delivery of pitavastatin into lungs ameliorates the development and induces regression of monocrotaline-induced pulmonary artery hypertension. Hypertension. 57: 343-50, 2011.

68. Fujino T, Nishizaka M, Yufu T, Sunagawa K. A case of multiple focal nodular hyperplasia in the liver which developed after heart transplantation. Intern Med. 2011;50(1):43-6.

69. Kawada T, Shimizu S, Kamiya A, Sata Y, Uemura K, Sugimachi M. Dynamic characteristics of baroreflex neural and peripheral arcs are preserved in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol. 300: R155-165, 2011

70. Mizuno M, Kawada T, Kamiya A, Miyamoto T, Shimizu S, Shishido T, Smith SA, Sugimachi M. Exercise training augments the dynamic heart rate response to vagal but not sympathetic stimulation in rats. Am J Physiol Regul Integr Comp Physiol. 300: R969-977, 2011

71. Yamamoto H, Kawada T, Kamiya A, Miyazaki S, Sugimachi M. Involvement of the mechanoreceptors in the sensory mechanisms of manual and electrical acupuncture. Auton Neurosci 160: 27-31, 2011.

72. Sugimachi M, Uemura K, Kawada T, Shishido T, Sunagawa K. Reduction of myocardial oxygen demand by controlling heart rate and hemodynamics simultaneously by novel circulatory model. Conf Proc IEEE Eng Med Biol Soc. 2011:4297-300, 2011.

73. Sakamoto T, Murayama Y, Tanaka A, Sakamoto K, Tobushi T, Saku K, Hosokawa K, Onitsuka K, Fujino T, Sunagawa K. Impact of baroreflex on venous return surface. Conf Proc IEEE Eng Med Biol Soc. 2011: 4295-4296, 2011.

74. Kishi T, Sunagawa K. Experimental 'jet lag' causes sympathoexcitation via oxidative stress through AT1 receptor in the brainstem. Conf Proc IEEE Eng Med Biol Soc. 2011: 1969-1972, 2011.

75. Hosokawa K, Funakoshi K, Tanaka A, Sakamoto T, Onitsuka K, Sakamoto K, Tobushi T, Fujino T, Saku K, Murayama Y, Ide T, Sunagawa K. Artificial baroreflex system restores volume tolerance in the absence of native baroreflex. Conf Proc IEEE Eng Med Biol Soc. 2011: 697-699, 2011.

76. Kawada T, Shimizu S, Sata Y, Kamiya A, Sunagawa K, Sugimachi M. Consideration on step duration to assess open-loop static characteristics of the carotid sinus baroreflex in rats. Conf Proc IEEE Eng Med Biol Soc. 2011: 689-692, 2011.

77. Masuda S, Nakano K, Funakoshi K, Zhao G, Meng W, Kimura S, Matoba T, Miyagawa M, Iwata E, Sunagawa K, Egashira K. Imatinib mesylate-incorporated nanoparticle-eluting stent attenuates in-stent neointimal formation in porcine coronary arteries. J Atheroscler Thromb. 18: 1043-1053, 2011.

78. Matsuura H, Ichiki T, Ikeda J, Takeda K, Miyazaki R, Hashimoto T, Narabayashi E, Kitamoto S, Tokunou T, Sunagawa K. Inhibition of prolyl hydroxylase domain-containing protein downregulates vascular angiotensin II type 1 receptor. Hypertension. 58: 386-393, 2011.

79. Ogawa K, Hirooka Y, Kishi T, Sunagawa K. Brain AT1 receptor activates the sympathetic nervous system through toll-like receptor 4 in mice with heart failure. J Cardiovasc Pharmacol. 58: 543-549, 2011.

80. Matsukawa R, Hirooka Y, Nishihara M, Ito K, Sunagawa K. Neuregulin-1/ErbB signaling in rostral ventrolateral medulla is involved in blood pressure regulation as an antihypertensive system. J Hypertens. 29: 1735-1742, 2011.

81. Kishi T, Hirooka Y, Ogawa K, Konno S, Sunagawa K. Calorie restriction inhibits sympathetic nerve activity via anti-oxidant effect in the rostral ventrolateral medulla of obesity-induced hypertensive rats. Clin Exp Hypertens. 33: 240-245, 2011.

82. Nakagaki T, Hirooka Y, Ito K, Kishi T, Hoka S, Sunagawa K. Role of angiotensin-(1-7) in rostral ventrolateral medulla in blood pressure regulation via sympathetic nerve activity in Wistar-Kyoto and spontaneous hypertensive rats. Clin Exp Hypertens. 33: 223-230, 2011.

83. Hashimoto T, Ichiki T, Ikeda J, Narabayashi E, Matsuura H, Miyazaki R, Inanaga K, Takeda K, Sunagawa K. Inhibition of MDM2 attenuates neointimal hyperplasia via suppression of vascular proliferation and inflammation. Cardiovasc Res. 91: 711-719, 2011.

84. Uemura K, Kawada T, Sunagawa K, Sugimachi M. Peak systolic mitral annulus velocity reflects the status of ventricular-arterial coupling-theoretical and experimental analyses. J Am Soc Echocardiogr. 24: 582-591, 2011.

85. Shimizu S, Akiyama T, Kawada T, Sata Y, Mizuno M, Kamiya A, Shishido T, Inagaki M, Shirai M, Sano S, Sugimachi M. Medetomidine, an α(2)-Adrenergic Agonist, Activates Cardiac Vagal Nerve Through Modulation of Baroreflex Control. Circ J. 76: 152-159, 2011.

86. Abe C, Kawada T, Sugimachi M, Morita H. Interaction between vestibulo-cardiovascular reflex and arterial baroreflex during postural change in rats. Appl Physiol. 111: 1614-1621, 2011.

87. Kawada T, Shimizu S, Li M, Kamiya A, Uemura K, Sata Y, Yamamoto H, Sugimachi M. Contrasting effects of moderate vagal stimulation on heart rate and carotid sinus baroreflex-mediated sympathetic arterial pressure regulation in rats. Life Sci. 89: 498-503, 2011.

88. Shimizu S, Une D, Shishido T, Kamiya A, Kawada T, Sano S, Sugimachi M. Norwood procedure with non-valved right ventricle to pulmonary artery shunt improves ventricular energetics despite the presence of diastolic regurgitation: a theoretical analysis. J Physiol Sci. 61: 457-465, 2011.

89. Shimizu S, Akiyama T, Kawada T, Sonobe T, Kamiya A, Shishido T, Tokudome T, Hosoda H, Shirai M, Kangawa K, Sugimachi M. Centrally administered ghrelin activates cardiac vagal nerve in anesthetized rabbits. Auton Neurosci. 162: 60-65, 2011.

90. Komamura K, Tatsumi R, Tsujita-Kuroda Y, Onoe T, Matsumoto K, Nakamura T, Miyazaki J, Horio T, Sugimachi M. Cellular injury of cardiomyocytes during hepatocyte growth factor gene transfection with ultrasound-triggered bubble liposome destruction. J Drug Deliv. 2011: 453619, 2011.

91. Kamiya A, Kawada T, Shimizu S, Sugimachi M. Closed-loop spontaneous baroreflex transfer function is inappropriate for system identification of neural arc but partially appropriate for peripheral arc: predictability analysis. J Physiol. 589: 1769-1790, 2011.

92. Furuno T, Yamasaki F, Yokoyama T, Sato K, Sato T, Doi Y, Sugiura T. Effects of various doses of aspirin on platelet activity and endothelial function. Heart Vessels. 28: 267-273, 2011.

93. Yamauchi K, Nagafuji H, Nakamura T, Sato T, Kohno N. Feasibility of ICG fluorescence-guided sentinel node biopsy in animal models using the HyperEye Medical System. Ann Surg Oncol. 18: 2042-2047, 2011.

94. Arikawa M, Kakinuma Y, Handa T, Yamasaki F, Sato T. Donepezil, anti-Alzheimer's disease drug, prevents cardiac rupture during acute phase of myocardial infarction in mice. PLoS ONE 6: e20629, 2011.

95. Yamamoto M, Sasaguri S, Sato T. Assessing intraoperative blood flow in cardiovascular surgery. Surg Today 41: 1467-1474, 2011.

96. Morita T, Kakinuma Y, Kurabayashi A, Fujieda M, Sato T, Shuin T, Furihata M, Wakiguchi H. Conditional VHL gene deletion activates a local NO-VEGF axis in a balanced manner reinforcing resistance to endothelium-targeted glomerulonephropathy. Nephrol Dial Transplant. 26: 4023-4031, 2011.

97. Yamakoshi K Bioinstrumentation. IEEE Rev. Biomed. Eng. 4: 6-8, 2011.

98. 杉本健樹、花崎和弘、佐藤隆幸： HyperEye Medical System を用いた乳癌センチネルリンパ節生検手技 手術 65: 421-425, 2011.

99. Inoue E, Ichiki T, Takeda K, Matsuura H, Hashimoto T, Ikeda J, Kamiharaguchi A, Sunagawa K. Beraprost sodium, a stable prostacyclin analogue, improves insulin resistance in high-fat diet-induced obese mice. J Endocrinol. 213: 285-291, 2012

100. Miyazaki R, Ichiki T, Hashimoto T, Ikeda J, Kamiharaguchi A, Narabayashi E, Matsuura H, Takeda K, Sunagawa K. Acetylcholinesterase inhibitors attenuate angiogenesis. Clin Sci (Lond). 123: 241-249, 2012.

101. Takemoto M, Mukai Y, Inoue S, Matoba T, Nishizaka M, Ide T, Chishaki A, Sunagawa K. Usefulness of non-contact mapping for radiofrequency catheter ablation of inappropriate sinus tachycardia: new procedural strategy and long-term clinical outcome. Intern Med. 51: 357-362, 2012.

102. Nakagaki T, Hirooka Y, Matsukawa R, Nishihara M, Nakano M, Ito K, Hoka S, Sunagawa K. Activation of mineralocorticoid receptors in the rostral ventrolateral medulla is involved in hypertensive mechanisms in stroke-prone spontaneously hypertensive rats. Hypertens Res. 35: 470-476, 2012.

103. Nishihara M, Hirooka Y, Matsukawa R, Kishi T, Sunagawa K. Oxidative stress in the rostral ventrolateral medulla modulates excitatory and inhibitory inputs in spontaneously hypertensive rats. J Hypertens. 30: 97-106, 2012.

104. Ito K, Hirooka Y, Matsukawa R, Nakano M, Sunagawa K. Decreased brain sigma-1 receptor contributes to the relationship between heart failure and depression. Cardiovasc Res. 93: 33-40, 2012.

105. Miyamoto T, Inagaki M, Takaki H, Kawada T, Shishido T, Kamiya A, Sugimachi M. Adaptation of the respiratory controller contributes to the attenuation of exercise hyperpnea in endurance-trained athletes. Eur J Appl Physiol 112: 237-251, 2012

106. Kishi T, Hirooka Y, Katsuki M, Ogawa K, Shinohara K, Isegawa K, Sunagawa K. Exercise Training Causes Sympathoinhibition through Antioxidant Effect in the Rostral Ventrolateral Medulla of Hypertensive Rats. Clin Exp Hypertens. In press, 2012

107. Kishi T, Hirooka Y, Sunagawa K. Sympathoinhibition caused by orally administered telmisartan through inhibition of the AT(1) receptor in the rostral ventrolateral medulla of hypertensive rats. Hypertens Res. In press, 2012

108. Ichiki T, Miyazaki R, Kamiharaguchi A, Hashimoto T, Matsuura H, Kitamoto S, Tokunou T, Sunagawa K. Resveratrol attenuates angiotensin II-induced senescence of vascular smooth muscle cells. Regul Pept. In press, 2012

109. Shinohara K, Hirooka Y, Ogawa K, Kishi T, Yasukawa K, Utsumi H, Sunagawa K. Combination Therapy of Olmesartan and Azelnidipine Inhibits Sympathetic Activity Associated with Reducing Oxidative Stress in the Brain of Hypertensive Rats. Clin Exp Hypertens. In press, 2012

110. Hara M, Tabata K, Suzuki T, Do MK, Mizunoya W, Nakamura M, Nishimura S, Tabata S, Ikeuchi Y, Sunagawa K, Anderson JE, Allen RE, Tatsumi R. Calcium influx through a possible coupling of cation channels impacts skeletal muscle satellite cell activation in response to mechanical stretch. Am J Physiol Cell Physiol. In press, 2012

Sympathoinhibition Induced by Centrally Administered Atorvastatin Is Associated With Alteration of NAD(P)H and Mn Superoxide Dismutase Activity in Rostral Ventrolateral Medulla of Stroke-Prone Spontaneously Hypertensive Rats

Takuya Kishi, MD, PhD, Yoshitaka Hirooka, MD, PhD, Satomi Konno, MD, and Kenji Sunagawa, MD, PhD

Abstract: Oxidative stress in the rostral ventrolateral medulla (RVLM) increases sympathetic nervous system activity (SNA). Oral treatment with atorvastatin decreases SNA through antioxidant effects in the RVLM of stroke-prone spontaneously hypertensive rats (SHRSP). We aimed to examine whether centrally administered atorvastain reduces SNA in SHRSP and, if so, to determine whether it is associated with the reduction of oxidative stress induced by alteration of activities of nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase and superoxide dismutase (SOD) in the RVLM of SHRSP. SHRSP received atorvastatin (S-ATOR) or vehicle (S-VEH) by continuous intracerebroventricular infusion for 14 days. Mean blood pressure, heart rate, and SNA were significantly lower in S-ATOR than in S-VEH. Oxidative stress, Rac1 activity, NAD(P)H oxidase activity, Rac1, gp91^{phox} and p22^{phox} expression in the membrane fraction, and p47^{phox} and p40^{phox} expression in the cytosolic fraction in the RVLM were significantly lower in S-ATOR than in S-VEH. Rac1 expression in the cytosolic fraction and Mn-SOD activity, however, were significantly higher in S-ATOR than in S-VEH. Our findings suggest that centrally administered atorvastatin decreases SNA and is associated with decreasing NAD(P)H oxidase activity and upregulation of Mn-SOD activity in the RVLM of SHRSP, leading to suppressing oxidative stress.

Key Words: hypertension, sympathetic nerve activity, atorvastatin, oxidative stress, brain

(*J Cardiovasc Pharmacol*™ 2010;55:184–190)

INTRODUCTION

In the brainstem, the rostral ventrolateral medulla (RVLM) is known as one of the vasomotor centers that regulates sympathetic nervous system activity (SNA).[1,2] Previously, we reported that the levels of reactive oxygen species (ROS) in the RVLM are increased in stroke-prone spontaneously hypertensive rats (SHRSP), which is a hypertensive rat model exhibiting increased SNA. We also demonstrated that the increase in SNA was due to ROS activation,[3] consistent with the findings of other studies.[4–6] Furthermore, oral administration of atorvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, suppresses SNA probably through the inhibition of ROS in the RVLM of SHRSP.[7] Other studies suggest that central infusion of simvastatin suppresses SNA in heart failure models.[8–10] Our previous study was based on the oral administration of atorvastatin, however, and it is not known whether atorvastatin directly and chronically administered into the brain reduces the central sympathetic outflow via its effects on oxidative stress in the brain, particularly in the RVLM of hypertensive models.

In the brain, ROS are produced mainly through the activation of nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase by the small G protein Rac1.[11,12] NAD(P)H oxidase is a multicomponent enzyme complex that comprises a membrane-bound heterodimer of gp91^{phox} (phagocytic oxidase) and p22^{phox}, and the cytosolic regulatory subunits p40^{phox}, p47^{phox}, p67^{phox}, and Rac1.[13–15] Transfection of dominant-negative Rac1 in the nucleus tractus solitarius decreases ROS and SNA.[12] Atorvastatin is also suggested to inhibit NAD(P)H oxidase activity in the vasculature,[16] the quadriceps muscle of diabetic rats,[17] and cardiomyocytes.[18] Furthermore, atorvastatin inhibits membrane translocation of Rac1, which is required for the activation of NAD(P)H oxidase in the vasculature.[16] In the kidney, rosuvastatin attenuates NAD(P)H oxidase activity through the inhibition of Rac1 and p22^{phox}.[18,19] In the brain, however, the contribution of atorvastatin to reducing ROS and its involvement in the inhibition of the membrane translocation of Rac1 and NAD(P)H oxidase activity is unknown. We previously demonstrated that Mn superoxide dismutase (SOD) activity is decreased in the RVLM of SHRSP, and the decrease contributes to the increase

Received for publication September 7, 2009; accepted November 10, 2009.
From the Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan.
This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (B19390231).
The authors report no conflicts of interest.
Reprints: Yoshitaka Hirooka, MD, PhD, FAHA, Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan (e-mail: hyoshi@cardiol.med.kyushu-u.ac.jp).

in ROS.[3] A number of reports suggest that statins upregulate SOD in the vasculature.[20–23] Furthermore, the upregulation of Rac1 and NAD(P)H oxidase and the inhibition of SOD in the RVLM and nucleus tractus solitarius have major roles in increasing SNA and blood pressure (BP).[3,24] However, the mechanisms involved by which atorvastatin reduces ROS in the RVLM of SHRSP are not evaluated. The aim of the present study was thus to determine whether the sympathoinhibitory effect of atorvastatin due to the reduction of ROS in the RVLM is caused by the inhibition of Rac1-NAD(P)H oxidase activity and upregulation of Mn-SOD and Cu/Zn-SOD in the RVLM of SHRSP. Therefore, the aim of the present study was to examine the effects of atorvastatin administered into the brain and evaluate the changes in BP and SNA in SHRSP and to evaluate the oxidative stress and the NAD(P)H oxidase activity in the RVLM as the ROS generation. For this purpose, we determined the expression of Rac1, gp91^{phox}, and p22^{phox} in the membrane fraction and the expression of Rac1 and p40^{phox} in the cytosolic fraction of the RVLM. In addition, the activity of Cu/Zn-SOD, and Mn-SOD as scavenging enzymes of ROS was measured in the RVLM of intracerebroventricular (ICV) atorvastatin–treated and vehicle-infused SHRSP and Wistar Kyoto (WKY) rats.

MATERIALS AND METHODS

Animals and General Procedures

Male SHRSP/Izm rats and age-matched WKY rats (14–16 weeks old) were obtained from SLC Japan, Hamamatsu, Japan. Rats were fed a standard diet, and each strain was divided into 4 groups (SHRSP treated with atorvastatin, S-ATOR; SHRSP treated with vehicle, S-VEH; WKY treated with atorvastatin, W-ATOR; and WKY treated with vehicle, W-VEH; n = 5 per group). Atorvastatin (Pfizer, Inc, New York, NY) was dissolved in dimethyl sulfoxide and further diluted in artificial cerebrospinal fluid for a final concentration of 40 μg/mL. Atorvastatin or dimethyl sulfoxide in artificial cerebrospinal fluid was infused at 1 μL/h for 14 days with an osmotic minipump (Alzet 1003D; Alza Scientific Products, Palo Alto, CA) into the left lateral ventricle of the brain (from bregma: anteroposterior, −0.8 mm; lateral, 1.5 mm; and depth, 3.5 mm). The flow rate of agents in ICV methods was determined to have the significant effect in brainstem.[25] In a preliminary experiment, this dose of atorvastatin did not affect BP and heart rate (HR) when administered intravenously. Food and tap water were available ad libitum throughout the study. BP and HR were measured using the UA-10 radio-telemetry system (Data Science International, Dallas, TX) as described previously.[3,26–28] Urinary norepinephrine excretion (uNE) for 24 hours was calculated as an indicator of SNA, as described previously.[3,25–27] In addition, spectral analysis was performed using an adaptive autoregressive model to provide power spectra for systolic BP (SBP). Low frequency power of SBP was computed by integrating the spectra between 0.04 and 0.15 Hz, and SNA is presented as the normalized unit of the low frequency component of SBP (LFnuSBP).[29–31] Baroreflex sensitivity (BRS) was measured using the spontaneous sequence method as a parameter of autonomic control. Sequence analysis was performed to detect sequences of 3 or more beats in which there was either an increase in SBP and pulse interval (up sequence) or a decrease in SBP and pulse interval (down sequence). BRS was estimated as the mean slope of the up and down sequences.[32–34] The RVLM was defined according to a rat brain atlas as described previously.[3,26–28] The study protocol was reviewed and approved by the Committee on the Ethics of Animal Experiments at the Kyushu University Graduate School of Medical Sciences and conducted according to the Guidelines for Animal Experiments of Kyushu University.

Measurement of TBARS

The RVLM tissues were homogenized, and thiobarbituric acid (0.3%) was added to the homogenate. The mixture was extracted with a mixture of distilled water and *n*-butanolpyridine (15:1) and centrifuged at 1600*g* for 10 minutes. The amount of thiobarbituric acid reactive substances (TBARS) was determined by absorbance measured at 532 nm, as described previously.[3,7]

Expression of Rac1, gp91^{phox}, and p22^{phox} in the Membrane Fraction and Rac1, p47^{phox} and p40^{phox} in the Cytosolic Fraction

Western blot analysis was used to determine the expression of Rac1 (Upstate Biotechnology, Lake Placid, NY),[12] gp91^{phox}, and p22^{phox} in the membrane fraction (Santa Cruz Biotechnology, Santa Cruz, CA), and the expression of Rac1, p47^{phox}, and p40^{phox} in the cytosolic fraction (Santa Cruz Biotechnology, Santa Cruz, CA) of the RVLM.

Activity of Rac1 in the RVLM

Rac1 activity can be monitored by its interaction with p21-activated kinase, which only occurs when Rac1 is active. We used a Rac1 Activation kit (Upstate Biotechnology, Lake Placid, NY) to evaluate Rac1 activity in the RVLM, as previously described.[12]

NAD(P)H Oxidase Activity

NAD(P)H-dependent superoxide production in the RVLM was measured using a lucigenin luminescence assay as described previously.[35,36] Quantification of NAD(P)H oxidase activity was expressed relative to that in WKY rats, which was assigned a value of 1.

Cu/Zn-SOD and Mn-SOD Activity in the RVLM

Cu/Zn-SOD or Mn-SOD activity was assayed by monitoring the inhibition of the rate of xanthine-mediated/xanthine oxidase–mediated reduction of cytochrome c (pH 7.4). To discriminate between Cu/Zn-SOD and Mn-SOD activities, the assay was also performed after incubation in the presence of KCN, which selectively inhibits the Cu/Zn-SOD isoform.[37] Cu/Zn- and Mn-SOD activities were expressed relative to those in vehicle-treated WKY rats, which were assigned a value of 1.

Microinjection of Apocynin Into the Bilateral RVLM

In other S-ATOR and S-VEH, (n = 5 for each) on day 14, the NAD(P)H oxidase inhibitor apocynin (1 nmol) was microinjected bilaterally into the RVLM, as described previously.[3]

Statistical Analysis

Normally distributed variables were expressed as mean ± SD. An unpaired t test was used to compare the differences between groups of normally distributed variables, and the Mann–Whitney U test was used to compare differences between groups of non–normally distributed variables. A 2-factor repeated-measures analysis of variance was used to compare differences between groups. Differences were considered to be statistically significant with a P value of less than 0.05.

RESULTS

BP, HR, SNA, and BRS

Mean BP (MBP) and HR were significantly decreased on day 4 after the administration of atorvastatin in S-ATOR. On day 14, MBP, HR, 24-hour uNE, and LFnuSBP were significantly higher in S-VEH than in W-VEH and lower in S-ATOR than in S-VEH (Fig. 1A–D). BRS was significantly lower in S-VEH than in W-VEH (12.8 ± 2.3 vs. 19.7 ± 1.8 ms/mm Hg, n = 5 for each; $P < 0.05$) and significantly higher in S-ATOR than in S-VEH (16.4 ± 1.6 vs. 12.8 ± 2.3 ms/mm Hg, n = 5 for each; $P < 0.05$). Mean BP, HR, 24-hour uNE, LFnuSBP, and BRS values did not significantly differ between W-ATOR and W-VEH (Fig. 1A–D).

Oxidative Stress Measured by TBARS Methods in the RVLM

Oxidative stress in the RVLM measured by the TBARS method was significantly lower in S-ATOR than in S-VEH (Fig. 2). Oxidative stress did not differ significantly between W-ATOR and W-VEH (Fig. 2).

Activity of NAD(P)H Oxidase and Rac1 in the RVLM

The activity of NAD(P)H oxidase was significantly lower in S-ATOR than in S-VEH (Fig. 3A). The activity of Rac1 was also significantly lower in S-ATOR than in S-VEH (Fig. 3B). NAD(P)H oxidase activity and Rac1 activity did not significantly differ between W-ATOR and W-VEH (Fig. 3A, B).

Expression of Rac1, gp91^{phox}, and p22^{phox} in the Membrane Fraction and Rac1, p47^{phox}, and p40^{phox} in the Cytosolic Fraction

The expression of Rac1, gp91^{phox}, and p22^{phox} in the membrane fraction was significantly lower in S-ATOR than in S-VEH (Fig. 4A–C). The expression of p47^{phox} and p40^{phox} in the cytosolic fraction was also significantly lower in S-ATOR than in S-VEH (Fig. 5B, C). The expression of Rac1 in the cytosolic fraction was significantly higher, however, in S-ATOR than in S-VEH (Fig. 5A). The expression of Rac1, gp91^{phox}, and p22^{phox} in the membrane fraction and the expression of Rac1, p47^{phox}, and p40^{phox} in cytosolic fraction did not differ significantly between W-ATOR and W-VEH (Figs. 4A–C, 5A–C).

Cu/Zn- and Mn-SOD Activity in the RVLM

Mn-SOD activity in the RVLM was significantly higher in S-ATOR than in S-VEH, but Cu/Zn-SOD activity did not significantly differ between S-ATOR and S-VEH (Fig. 6A, B).

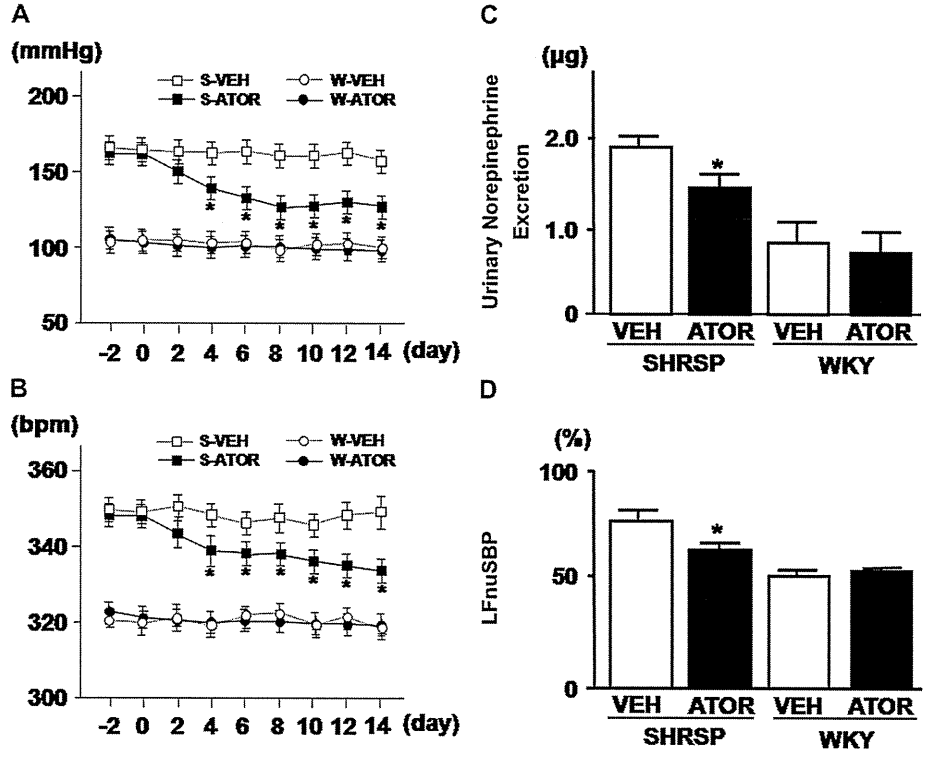

FIGURE 1. Time course of MBP (in mm Hg) (A) and HR (in beats per minute) (B) in S-ATOR (n = 5), S-VEH (n = 5), W-ATOR (n = 5), and W-VEH (n = 5). *$P < 0.05$ for ATOR versus VEH values in each strain. C, D, Urinary norepinephrine excretion for 24 hours (in micrograms) (C) and LFnuSBP (percentage) (D) at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). *$P < 0.05$ for ATOR versus VEH values in each strain. †$P < 0.05$ compared with VEH-treated WKY. Data are shown as mean ± standard error of the mean.

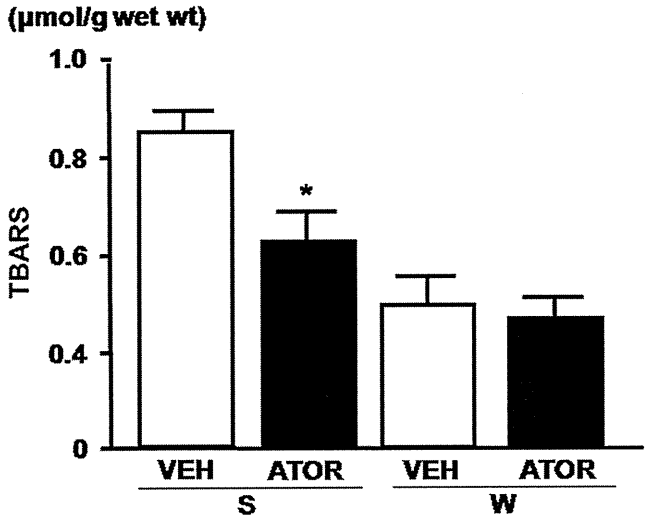

FIGURE 2. TBARS levels (in micromolars per gram wet weight) in the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). *$P < 0.05$ for ATOR versus VEH in each strain. †$P < 0.05$ compared with VEH-treated WKY. Data are shown as mean ± standard error of the mean.

Cu/Zn- and Mn-SOD activity did not significantly differ between W-ATOR and W-VEH (Fig. 6A, B).

Microinjection of Apocynin Into the RVLM

The degree of the change in MBP induced by the microinjection of apocynin into the bilateral RVLM was significantly smaller in S-ATOR than in S-VEH (−9.4 ± 1.9 vs. −26.4 ± 3.7 mm Hg; n = 5; $P < 0.05$).

DISCUSSION

The novel finding of the present study was that atorvastatin administered chronically into the brain in SHRSP reduced BP and SNA in SHRSP and that it was associated with reduced oxidative stress, probably due to the inhibition of NAD(P)H oxidase and the activation of Mn-SOD in the RVLM of SHRSP. This is supported by the following findings: (1) ICV injection of atorvastatin for 14 days decreased MBP, HR, SNA, and TBARS in the RVLM of SHRSP; (2) ICV injection of atorvastatin decreased NAD(P)H oxidase activity through the inhibition of Rac1 membrane translocation in the RVLM of SHRSP; (3) ICV injection of atorvastatin activated Mn-SOD in the RVLM of SHRSP; and (4) changes in MBP induced by microinjection of NAD(P)H oxidase inhibitor into the RVLM were significantly smaller in SHRSP treated with atorvastatin than in SHRSP treated with vehicle. Thus, atorvastatin inhibits Rac1 membrane translocation and Rac1 activity in the RVLM of SHRSP.

Atorvastatin decreased the expression of NAD(P)H membrane-bound subunits $gp91^{phox}$ and $p22^{phox}$ and the cytosolic regulatory subunit $p47^{phox}$ and $p40^{phox}$ and inhibited NAD(P)H oxidase activity in the RVLM of SHRSP. Oral administration of atorvastatin decreases ROS in the RVLM of SHRSP.[3] In the brain, ROS is produced mainly by NAD(P)H oxidase, which is activated through Rac1 membrane translocation.[11] In another area of the brainstem, the nucleus tractus solitarius, the inhibition of Rac1 decreases NAD(P)H oxidase activity and ROS formation.[12] Previous reports suggest that atorvastatin inhibits Rac1 membrane translocation and NAD(P)H oxidase activity in the vasculature of hypertensive rats.[13] We found that the depressor response elicited by apocynin into the RVLM was attenuated in SHRSP treated with ICV atorvastatin in the present study. Based on these findings, we suggest that the atorvastatin-induced reduction of ROS in the RVLM of SHRSP is caused by a decrease in NAD(P)H oxidase activity linked to the inhibition of Rac1 membrane translocation.

Atorvastatin activated Mn-SOD activity in the RVLM of SHRSP but not Cu/Zn-SOD. In the RVLM of SHRSP, Mn-SOD activity is decreased, and overexpression of Mn-SOD in the RVLM of SHRSP decreases ROS.[3] A number of reports suggest that statins activate total SOD[20–23] and Cu/Zn-SOD in the vasculature.[26,27] In the present study, however, atorvastatin did not activate Cu/Zn-SOD in the RVLM of SHRSP. In the nucleus tractus solitarius, Cu/Zn-SOD expression is decreased in SHRSP.[26] It is not clear why atorvastatin did not activate Cu/Zn-SOD in the present study. Recently, we reported that angiotensin II increases the intracellular Ca^{2+} concentration and that the increase in mitochondrial Ca^{2+} uptake leads to mitochondrial ROS production in the RVLM.[24] Therefore, it is possible that atorvastatin-induced activation of Mn-SOD in the RVLM of SHRSP contributes to inhibit ROS to an even greater extent than Cu/Zn-SOD.

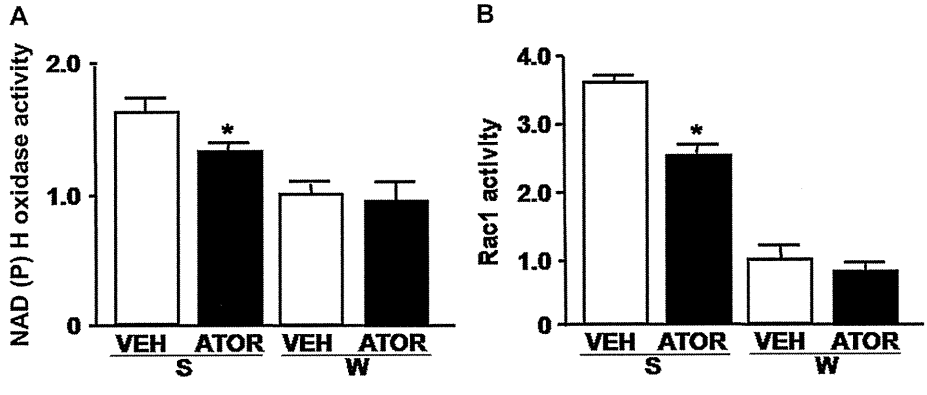

FIGURE 3. NAD(P)H oxidase activity (A) and Rac1 activity (B), in the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). *$P < 0.05$ for ATOR versus VEH in each strain. †$P < 0.05$ compared with VEH-treated WKY. NAD(P)H oxidase or Rac1 activity was expressed relative to that in W-VEH, which was assigned a value of 1. Data are shown as mean ± standard error of the mean.

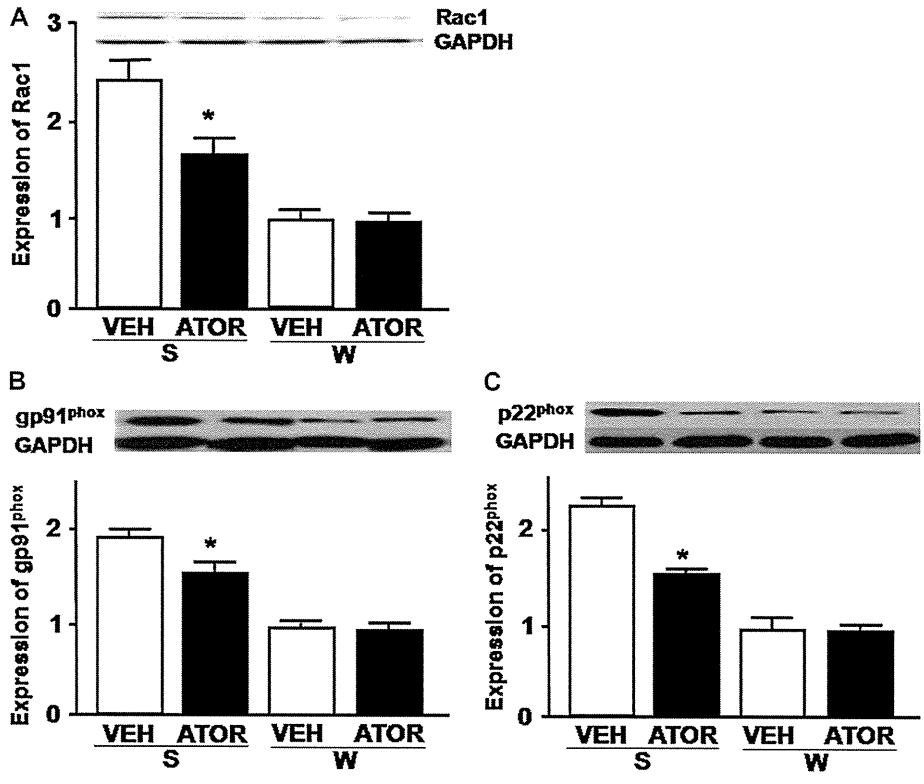

FIGURE 4. Western blot analysis showing the level of expression of Rac1 (A), gp91^{phox} (B), and p22^{phox} (C) in the membrane fraction of the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). *$P < 0.05$ for ATOR versus VEH in each strain. †$P < 0.05$ compared with VEH-treated WKY. The expression level of Rac1, gp91^{phox}, and p22^{phox} was expressed relative to that in W-VEH, which was assigned a value of 1. Data are shown as mean ± standard error of the mean.

In the present study, we measured SNA by spectral analysis. Low frequency power of SBP was computed by integrating the spectra between 0.04 and 0.15 Hz, and SNA is presented as LFnuSBP, as described in previous reports.[29–31] On day 14, the LFnuSBP values were comparable to those of uNE. Therefore, this method seems to be useful for measuring SNA in awake animals. Furthermore, atorvastatin improved the impaired baroreflex control in the SHRSP in the present study. Whereas we did not measure cardiac output in the present study and the reduction of BP and HR due to atorvastatin might cause a potential fall in cardiac output, the effects of atorvastatin are due to the decrease in sympathetic nerve activity. It is generally accepted that SNA is enhanced in SHRSP,[3,5,26–28,40] and atorvastatin attenuates the enhanced central sympathetic outflow to various organs including heart, kidney, and vasculature. At least, atorvastatin did not induce heart failure due to low cardiac output. We consider that the decrease in central sympathetic outflow reduced the peripheral vascular resistance by which cardiac output keep constant instead of the reduction of sympathetic outflow to the heart.

Another intriguing finding of the present study is that the BP-lowering and sympathoinhibitory effects are comparable between oral administration (50 mg/kg^{-1}/day^{-1})[7] and ICV injection (2 μg/kg^{-1}/day^{-1}) of atorvastatin. We confirmed the direct effects of atorvastatin administered into the brain on BP, SNA, and baroreflex function in SHRSP as one of the hypertensive models in the present study. The changes in TBARS levels are also similar between oral administration and ICV injection of atorvastatin. In SHRSP, the blood–brain barrier might be disrupted[38] and oral administration of atorvastatin is considered to affect the brain directly.[39] The present findings suggest that orally administered atorvastatin crosses the blood–brain barrier and affects the brain of SHRSP. The abnormal activation of sympathetic nervous system causes hypertension, heart failure, and ischemic heart diseases, and we consider that oral administration of atorvastatin has a potential to treat cardiovascular diseases due to the sympathoinhibition through the antioxidant effect in the RVLM.

We previously demonstrated that oral administration of atorvastatin increases the expression of endothelial nitric oxide synthase (eNOS) in the brainstem.[40] Overexpression of eNOS in the RVLM decreases SNA in WKY and SHRSP.[26–28] In the present study, we did not investigate whether an increase in NO production in the RVLM is involved in the reduction of BP and oxidative stress. It is possible, however, that ICV injection of atorvastatin increases eNOS in the RVLM of SHRSP and that an increase in eNOS contributes to the sympathoinhibitory effect. Further study is needed to clarify this issue.

In WKY rats, atorvastatin does not alter SNA and oxidative stress in the RVLM; these results are compatible with our previous report.[7] Moreover, atorvastatin also does not alter Rac1-induced NAD(P)H oxidase activity and Mn-SOD activity in the RVLM of WKY rats. In the present study, the mechanisms by which atorvastatin affected Rac1-induced NAD(P)H oxidase activity and Mn-SOD activity in SHRSP, but not in WKY, were not determined. It may be that there are thresholds for the induction of Rac1-induced NAD(P)H oxidase activity and Mn-SOD activity in the RVLM, which

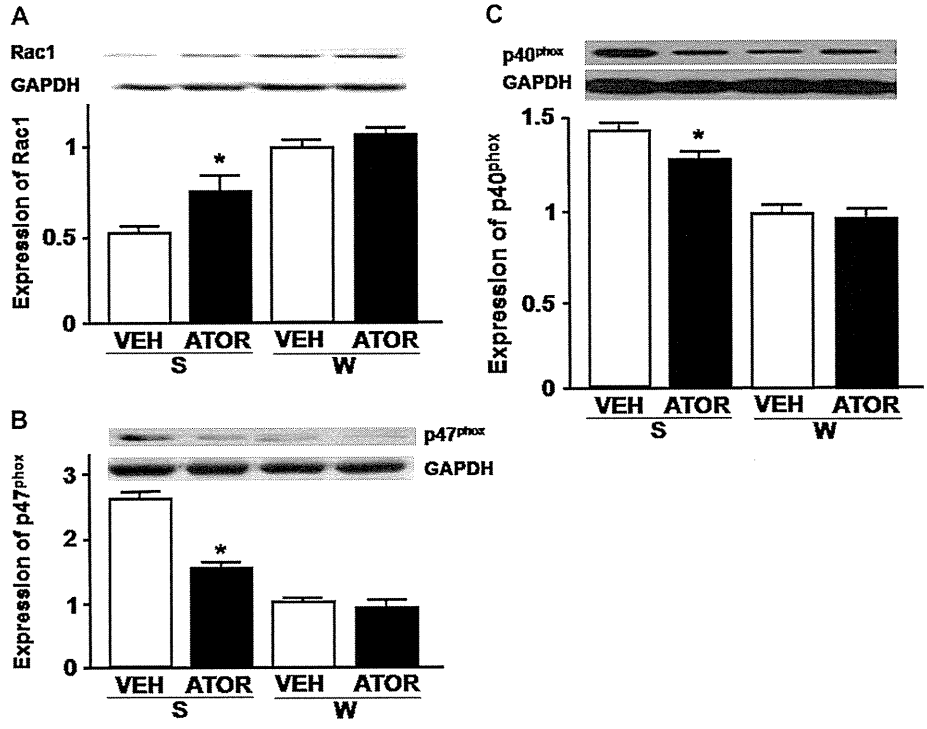

FIGURE 5. Western blot analysis showing the level of expression of Rac1 (A), $p47^{phox}$ (B), and $p40^{phox}$ (C) in the cytosolic fraction of the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 per group). $*P < 0.05$ for ATOR versus VEH in each strain. $\dagger P < 0.05$ compared with VEH-treated WKY. The expression level of Rac1, $p47^{phox}$, and $p40^{phox}$ was expressed relative to that in W-VEH, which was assigned a value of 1. Data are shown as mean ± standard error of the mean.

are differently affected by atorvastatin between SHRSP and WKY rats.

STUDY LIMITATIONS

The present study has several limitations. First, we examined the effects of atorvastatin specifically in only the RVLM, and its effects in other brain areas cannot be excluded at this time. Nevertheless, neural activity in the RVLM has a direct influence on SNA,[1,2] and the present results identified an antioxidant effect of atorvastatin and its mechanisms in the RVLM. Angiotensin II type 1 receptors (AT_1R) are abundantly distributed in the RVLM, and there is a close link between AT_1R stimulation and NAD(P)H oxidase activation.[41] Therefore, in the present study, we focused on the RVLM, although other brain regions related to central autonomic control also contain AT_1R and NAD(P)H oxidase. Second, among all statins, we only studied the effect of atorvastatin, which is a lipophilic statin.[42] Our previous studies suggested that oral atorvastatin also reduces oxidative stress in the RVLM.[7] Further study is needed to clarify whether our results in the present study are broad class effects or are specific for atorvastatin. Finally, a recent study suggests that statins reduce BP in patients with hypertension.[43] It will be important to determine whether atorvastatin has this beneficial effect caused by the mechanism related to our suggestion in the present study, although we understand that this is difficult to examine in humans.

CONCLUSIONS

In conclusion, atorvastatin administered directly into the brain of SHRSP decreases BP, SNA, and baroreflex function. The findings of the present study suggest that these effects are associated with inhibition of oxidative stress in the RVLM, probably resulting from a decrease in NAD(P)H oxidase activity and the upregulation of Mn-SOD activity in the RVLM.

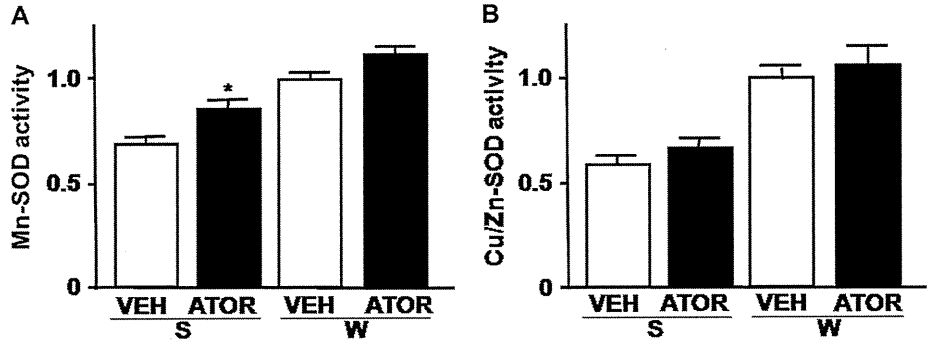

FIGURE 6. The activities of Mn-SOD (A) and Cu/Zn-SOD (B) in the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). $*P < 0.05$ for ATOR versus VEH in each strain. $\dagger P < 0.05$ compared with VEH-treated WKY. The activities of Mn-SOD and Cu/Zn-SOD were expressed relative to that in W-VEH, which was assigned a value of 1. Data are shown as mean ± standard error of the mean.

ACKNOWLEDGMENTS

We are grateful to Pfizer, Inc for supplying atorvastatin.

REFERENCES

1. Dampney RAL. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev.* 1994;74:323–364.
2. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci.* 2006;7:335–346.
3. Kishi T, Hirooka Y, Kimura Y, et al. Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. *Circulation.* 2004;109:2357–2362.
4. Peterson JR, Sharma RV, Davisson RL. Reactive oxygen species in the neuropathogenesis of hypertension. *Curr Hypertens Rep.* 2006;8:232–241.
5. Hirooka Y. Role of reactive oxygen species in brainstem in neural mechanisms of hypertension. *Auton Neurosci.* 2008;142:20–24.
6. Sheh YL, Hsu C, Chan SHH, et al. NADPH oxidase- and mitochondrion-derived superoxide at rostral ventrolateral medulla in endotoxin-induced cardiovascular depression. *Free Radic Biol Med.* 2007;42:1610–1623.
7. Kishi T, Hirooka Y, Shimokawa H, et al. Atorvastatin reduces oxidative stress in the rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats. *Clin Exp Hypertens.* 2008;30:3–11.
8. Pliquett RU, Cornish KG, Peuler JD, et al. Simvastatin normalizes autonomic neural control in experimental heart failure. *Circulation.* 2003; 107:2493–2498.
9. Gao L, Wang W, Li YL, et al. Simvastatin therapy normalizes sympathetic neural control in experimental heart failure: roles of angiotensin II type 1 receptors and NAD(P)H oxidase. *Circulation.* 2005;112:1763–1770.
10. Gao L, Wang W, Zucker IH. Simvastatin inhibits central sympathetic outflow in heart failure by a nitric-oxide synthase mechanism. *J Pharmacol Exp Ther.* 2008;326:278–285.
11. Zimmerman MC, Dunlay RP, Lazartigues E, et al. Requirement for Rac1-dependent NADPH oxidase in the cardiovascular and dipsogenic actions of angiotensin II in the brain. *Circ Res.* 2004;95:532–539.
12. Nozoe M, Hirooka Y, Koga Y, et al. Inhibition of Rac1-derived reactive oxygen species in NTS decreases blood pressure and heart rate in stroke-prone SHR. *Hypertension.* 2007;50:62–68.
13. Byrne JA, Grieve DJ, Bendall JK, et al. Contrasting roles of NADPH oxidase isoforms in pressure-overload versus angiotensin II-induced cardiac hypertrophy. *Circ Res.* 2003;93:802–805.
14. Privratsky JR, Wold LE, Sowers JR, et al. AT1 blockade prevents glucose-induced cardiac dysfunction in ventricular myocytes: role of the AT1 receptor and NADPH oxidase. *Hypertension.* 2003;42:206–212.
15. Maach C, Kartes T, Killer H. Oxygen free radical release in human failing myocardium is associated with increased activity of Rac1-GTPase and represents a target for statin treatment. *Circulation.* 2003;108:1567–1574.
16. Wassmann S, Laufs U, Muller K, et al. Cellular antioxidant effects of atorvastatin in vitro and in vivo. *Arterioscler Thromb Vasc Biol.* 2002;22: 300–305.
17. Riad A, Du J, Stiehl S, et al. Low-dose treatment with atorvastatin leads to anti-oxidative and anti-inflammatory effects in diabetes mellitus. *Eur J Pharmacol.* 2007;569:204–211.
18. Habibi J, Whaley-Connell A, Qazi MA, et al. Rosuvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, decreases cardiac oxidative stress and remodeling in Ren2 transgenic rats. *Endocrinology.* 2007;148:2181–2188.
19. Whaley-Connell A, Habibi J, Nistala R, et al. Attenuation of NADPH oxidase activation and glomerular filtration barrier remodeling with statin treatment. *Hypertension.* 2008;51:474–480.
20. Chen X, Touyz RM, Park JB, et al. Antioxidant effects of vitamin C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension.* 2001;38: 606–611.
21. Carneado J, Alvarez de Sotomayor M, Perez-Guerrero C, et al. Simvastatin improves endothelial function in spontaneously hypertensive rats through a superoxide dismutase mediated antioxidant effect. *J Hypertens.* 2002;20:429–437.
22. Yilmaz MI, Baykal Y, Kilic M, et al. Effects of statins on oxidative stress. *Biol Trace Elem Res.* 2004;98:119–127.
23. Umeji K, Umemoto S, Itoh S, et al. Comparative effects of pitavastatin and probucol on oxidative stress, Cu/Zn superoxide dismutase, PPAR-gamma, and aortic stiffness in hypercholesterolemia. *Am J Physiol.* 2006;291: H2522–H2532.
24. Nozoe M, Hirooka Y, Koga Y, et al. Mitochondria-derived reactive oxygen species mediate sympathoexcitation induced by angiotensin II in the rostral ventrolateral medulla. *J Hypertens.* 2008;26:2176–2184.
25. Nishimura M, Takahashi H, Yoshimura M. Upregulation of the brain renin-angiotensin system in rats with chronic renal failure. *Acta Physiol (Oxf).* 2007;189:369–377.
26. Kishi T, Hirooka Y, Sakai K, et al. Overexpression of eNOS in the RVLM causes hypotension and bradycardia via GABA release. *Hypertension.* 2001;38:896–901.
27. Kishi T, Hirooka Y, Ito K, et al. Cardiovascular effects of overexpression of endothelial nitric oxide synthase in the rostral ventrolateral medulla in stroke-prone spontaneously hypertensive rats. *Hypertension.* 2002;39: 264–268.
28. Kishi T, Hirooka Y, Kimura Y, et al. Overexpression of eNOS in RVLM improves impaired baroreflex control of heart rate in SHRSP. *Hypertension.* 2003;41:255–260.
29. Castiglioni P, Di Rienzo M, Veicsteinas A, et al. Mechanisms of blood pressure and heart rate variability: an insight from low-level paraplegia. *Am J Physiol.* 2007;292:R1502–R1509.
30. Cerutti C, Gustin MP, Paultre CZ. Autonomic nervous system and cardiovascular variability in rats: a spectral analysis approach. *Am J Physiol.* 1991;261:H1292–H1299.
31. Pagani M, Montano N, Porta A, et al. Relationship between spectral components of cardiovascular variabilities, and direct measures of muscle sympathetic nerve activity in humans. *Circulation.* 1997;95:1441–1448.
32. Waki H, Kasparov S, Wong LF, et al. Chronic inhibition of eNOS activity in nucleus tractus solitarii enhances baroreceptor reflex in conscious rats. *J Physiol.* 2003;546:233–242.
33. Waki H, Katahira K, Polson JW, et al. Automation of analysis of cardiovascular autonomic function from chronic measurements of arterial pressure in conscious rats. *Exp Physiol.* 2006;91:201–213.
34. Braga VA, Burmeister MA, Sharma RV, et al. Cardiovascular responses to peripheral chemoreflex activation and comparison of different methods to evaluate baroreflex gain in conscious mice using telemetry. *Am J Physiol.* 2008;295:R1168–R1174.
35. Tai MH, Wang LL, Wu KL, et al. Increased superoxide anion in rostral ventrolateral medulla contributes to hypertension in spontaneously hypertensive rats via interactions with nitric oxide. *Free Radic Biol Med.* 2005;38:450–462.
36. Tanaka M, Umemoto S, Kawahara S, et al. Angiotensin II type 1 receptor antagonist and angiotensin-converting enzyme inhibitor altered the activation of Cu/Zn-containing superoxide dismutase in the heart of stroke-prone spontaneously hypertensive rats. *Hypertens Res.* 2005;28: 67–77.
37. Romero RM, Canuelo A, Lara EM, et al. Aging affects but does not eliminate the enzymatic antioxidative response to hypoxia/reoxygenation in cerebral cortex. *Exp Gerontol.* 2006;41:25–31.
38. Iwanaga Y, Ueno M, Ueki M, et al. The expression of osteopontin is increased in vessels with blood-brain barrier impairment. *Neuropathol Appl Neurobiol.* 2008;34:145–154.
39. Cibickova L, Radomir H, Stanislav M, et al. The influence of simvastatin, atorvastatin and high-cholesterol diet on acetylcholinesterase activity, amyloid beta and cholesterol synthesis in rat brain. *Steroids.* 2009;74: 13–19.
40. Kishi T, Hirooka Y, Mukai Y, et al. Atorvastatin causes depressor and sympatho-inhibitory effects with upregulation of nitric oxide synthases in stroke-prone spontaneously hypertensive rats. *J Hypertens.* 2003;21: 379–386.
41. Hu L, Zhu DN, Yu Z, et al. Expression of angiotensin II type 1 (AT1) receptor in the rostral ventrolateral medulla in rats. *J Appl Physiol.* 2002; 92:2153–2161.
42. Cibickova L, Hyspler R, Ticha A, et al. Cholesterol synthesis in central nervous system of rat is affected by simvastatin as well as by atorvastatin. *Pharmazie.* 2008;63:819–822.
43. Golomb BA, Dimsdale JE, White HL, et al. Reduction in blood pressure with statins: results from the USCD Statin Study, a randomized trial. *Arch Intern Med.* 2008;168:721–727.