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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

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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.
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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.
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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).

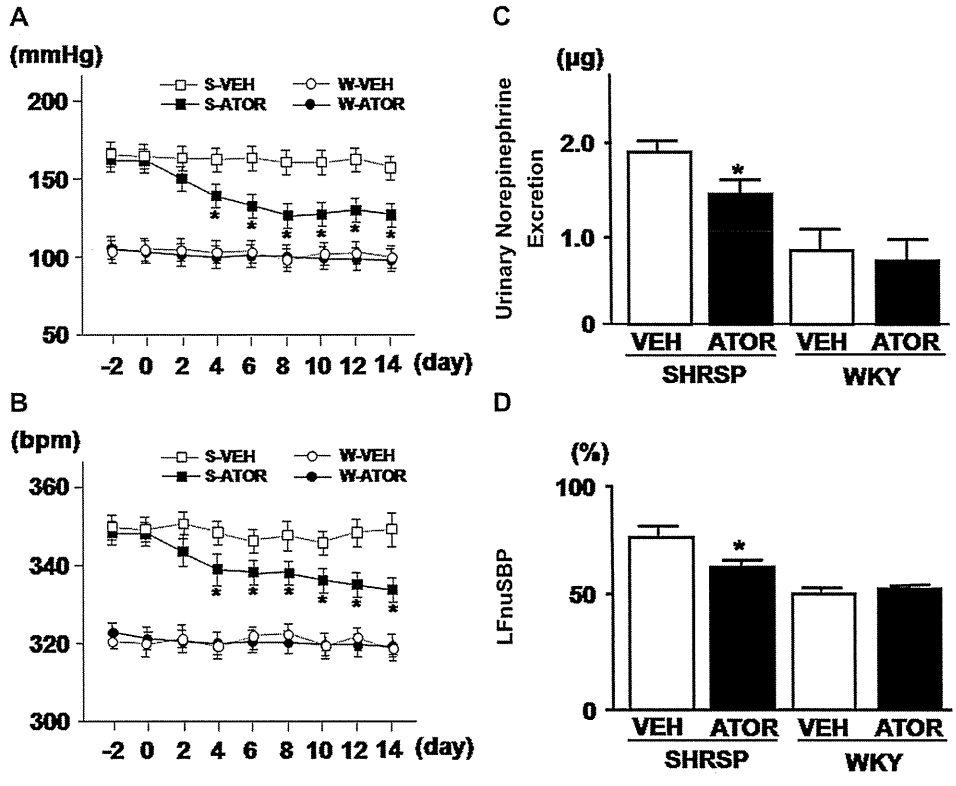

FIGURE 1. Time course of MBP (in mm Hg) (A) and HR (in beats per minute) (B) in S-ATOR (n = 5), S-VEH (n = 5), W-ATOR (n = 5), and W-VEH (n = 5). *$P < 0.05$ for ATOR versus VEH values in each strain. C, D, Urinary norepinephrine excretion for 24 hours (in micrograms) (C) and LFnuSBP (percentage) (D) 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.

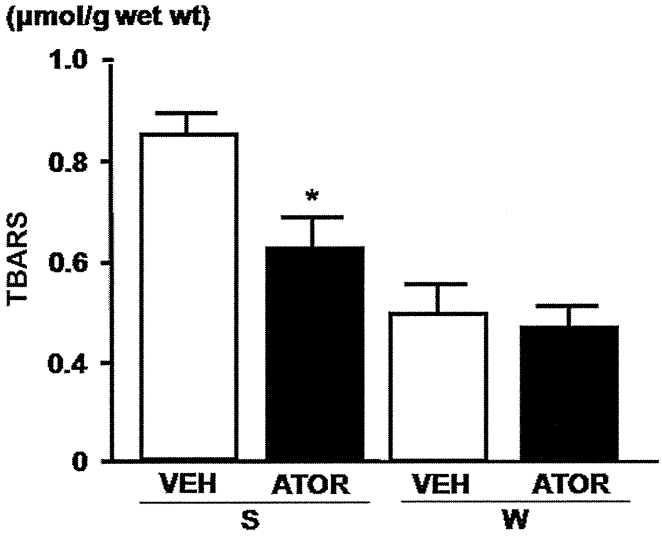

FIGURE 2. TBARS levels (in micromolars per gram wet weight) in the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). *$P < 0.05$ for ATOR versus VEH in each strain. †$P < 0.05$ compared with VEH-treated WKY. Data are shown as mean ± standard error of the mean.

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.

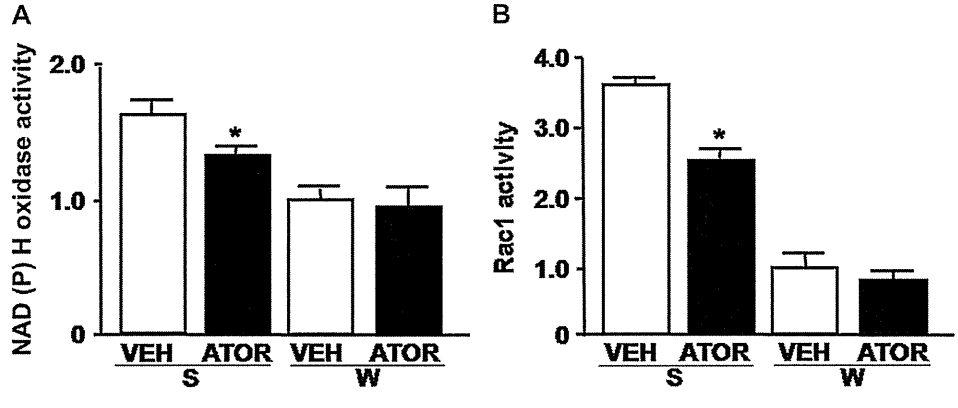

FIGURE 3. NAD(P)H oxidase activity (A) and Rac1 activity (B), in the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). *$P < 0.05$ for ATOR versus VEH in each strain. †$P < 0.05$ compared with VEH-treated WKY. NAD(P)H oxidase or Rac1 activity was expressed relative to that in W-VEH,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

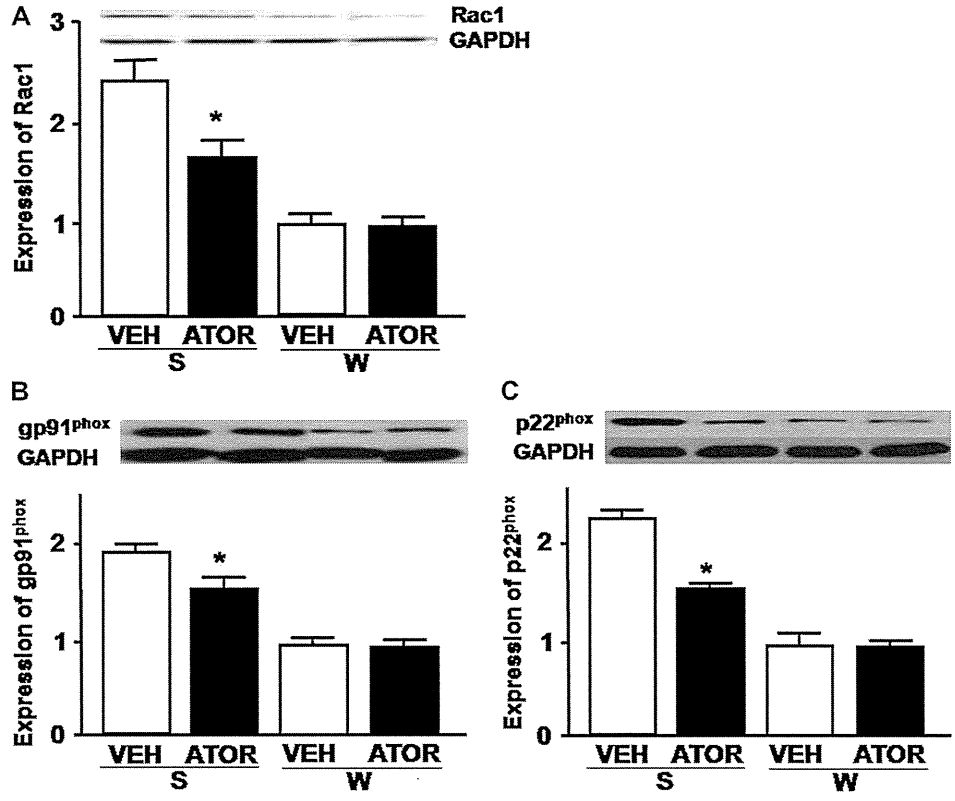

FIGURE 4. Western blot analysis showing the level of expression of Rac1 (A), gp91^{phox} (B), and p22^{phox} (C) in the membrane fraction of the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). *$P < 0.05$ for ATOR versus VEH in each strain. †$P < 0.05$ compared with VEH-treated 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

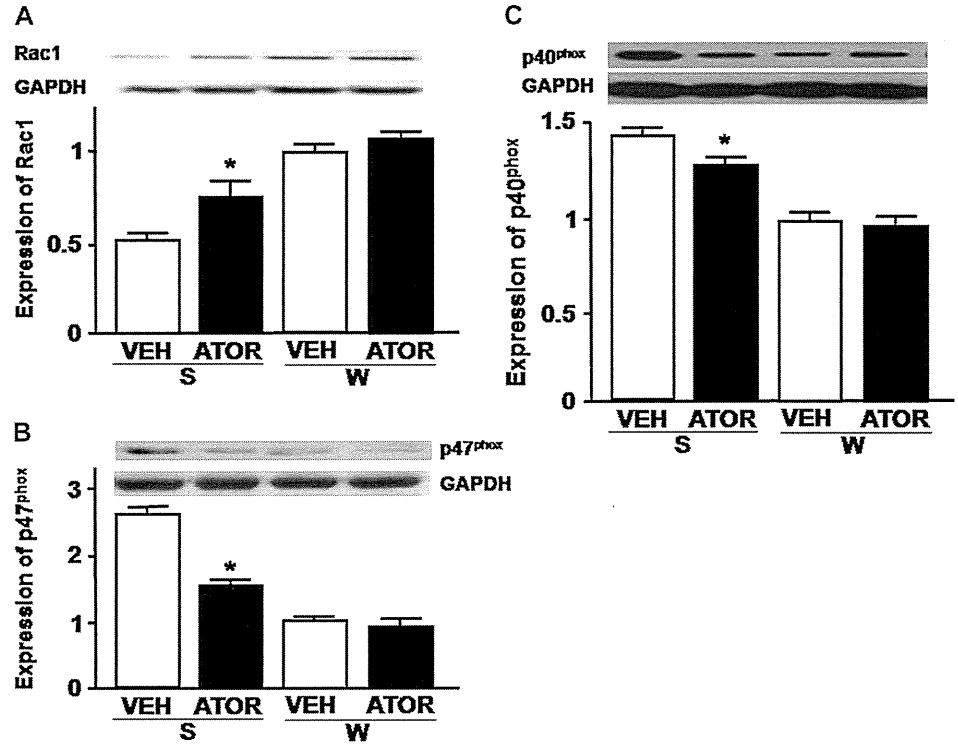

FIGURE 5. Western blot analysis showing the level of expression of Rac1 (A), $p47^{phox}$ (B), and $p40^{phox}$ (C) in the cytosolic fraction of the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 per group). $*P < 0.05$ for ATOR versus VEH in each strain. $\dagger P < 0.05$ compared with 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.

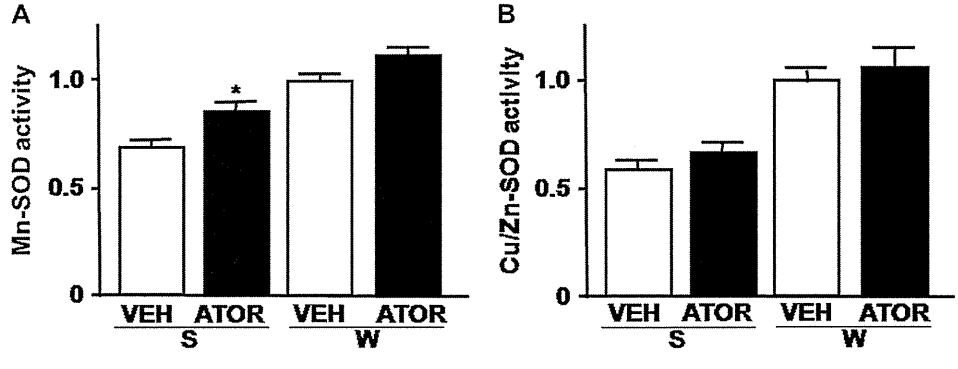

FIGURE 6. The activities of Mn-SOD (A) and Cu/Zn-SOD (B) in the RVLM at day 14 in ATOR- or VEH-treated SHRSP or WKY (n = 5 for each). $*P < 0.05$ for ATOR versus VEH in each strain. $\dagger P < 0.05$ compared with VEH-treated WKY. The activities of Mn-SOD and Cu/Zn-SOD were expressed relative to that 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.

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Baroreflex Sensitivity Might Predict Responders to Milrinone in Patients With Heart Failure

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SUMMARY

The phosphodiesterase III inhibitor milrinone (MIL) is considered to be effective for "wet and cold" heart failure. In some cases, however, the inotropic effects of milrinone are insufficient. A previous study suggested that baroreflex sensitivity (BRS) predicts the cases in which MIL increases left ventricular *dp/dt*. The aim of this study was to determine whether BRS measured using the spontaneous sequence method predicts the MIL responders. Twenty-four patients with "wet and cold" heart failure whose systolic blood pressure > 100 mmHg were enrolled. At 2 hours MIL improved dyspnea, general fatigue, urine volume, and tricuspid regurgitant pressure gradient in 13 patients (responders; R group), whereas it failed to improve in 11 patients (nonresponders; NR group). BRS in the R group was significantly higher than that in the NR group prior to the MIL infusion. At 2 hours after the MIL infusion, BRS was further increased in the R group, but did not increase in the NR group. The sensitivity and specificity of BRS at a cut-off level of 5 ms/mmHg for the prediction of R group were 0.94 and 0.93, respectively. BRS might be useful for identifying potential responders to milrinone in patients with blood pressure-preserved "wet and cold" heart failure. (Int Heart J 2010; 51: 411-415)

Key words: Heart failure, Baroreflex sensitivity, Milrinone

Milrinone, a phosphodiesterase-III inhibitor (PDEIII-I), has an inotropic and vasodilator effect for "wet and cold" heart failure,[1,2] which is determined as heart failure with congestion and hypoperfusion.[3] In some cases, however, the inotropic effects of milrinone are insufficient and combined treatment with dobutamine is necessary.[1] A previous study suggested that baroreflex sensitivity (BRS) can predict the cases in which milrinone increases left ventricular *dp/dt*.[4] However, whether arterial baroreflex function is related to the inotropic responsiveness to milrinone has not been clarified in human heart failure.

Baroreflex control is one of the key mechanisms responsible for the short-term control of blood pressure.[5-7] Impairment of this reflex has been found in a number of conditions, such as aging,[8] post myocardial infarction,[9,10] hypertension,[7] and heart failure.[11] Baroreflex sensitivity was originally assessed by intra-arterial measurement of the change in pulse interval following a pharmacologically induced change in blood pressure. However, for some time now, noninvasive monitoring of blood pressure using finger plethysmography has been available, and further methods for measuring baroreflex sensitivity have been developed, which assess spontaneous changes in blood pressure and pulse interval, and do not require pharmacological manipulation of blood pressure-spectral analysis.[12-16]

The aim of this study was to determine whether the baroreflex sensitivity measured using the spontaneous sequence method can identify potential milrinone responders or not in patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure.

METHODS

The present study was approved by the Ethics Committee for Human Research of Kyushu University Graduate School of Medical Sciences. Data collected retrospectively were fully de-identified.

Patient populations: We retrospectively studied patients with symptomatic acute heart failure admitted to Kyushu University Hospital from January 2006 to December 2007 who were treated with intravenous infusion of milrinone. The criteria for enrollment in the study were clinical evidence of acute heart failure diagnosed by Framingham criteria[17] and low cardiac output, which is called "wet and cold" heart failure.[3] We defined low cardiac output from the clinical state of "cold and wet". In those patients, the New York Heart Association (NYHA) functional classification on admission ranged between III and IV. We excluded patients whose systolic blood pressure was < 100 mmHg or who had atrial fibrillation, chronic obstructive pulmonary disease, dehydration, right ventricular myocardial infarction, or right heart failure. Prior medication by intravenous injection of diuretics, nitrates, and morphine was permitted. The dose of milrinone was adjusted according to the condition of each individual patient, and if symptoms of heart failure were not adequately improved by milrinone, concomitant use of or replacement with other agents indicated for the treatment of acute heart failure was

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Received for publication June 17, 2010.

Revised and accepted September 9, 2010.

permitted. Finally, we enrolled 24 patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure. We did not use the intravenous infusion of digitalis, and none of the patients in the present study were taking oral digitalis. Before the initiation of treatment, all patients underwent blood sampling, and an electrocardiogram, echocardiogram, and chest x-rays.

Measurement of blood pressure and heart rate: Blood pressure monitoring was performed using the TaskForce Monitor 3040i (CNSystems, Austria). The cuff was attached to a finger on the left hand and supported at heart level. Electrocardiogram electrodes were attached to the chest. Once a reading of blood pressure and heart rate had stabilized, 3 consecutive 5-minute recordings were made of the blood pressure and electrocardiogram tracing. Noninvasive brachial blood pressure readings were taken with an appropriate sized cuff.

Measurement of BRS by spontaneous sequence method: Sequence analysis detected 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). The sequences involving premature ventricular contraction were excluded. BRS was estimated as the mean slope of the up sequences (Up BRS), the down sequences (Down BRS), and also the mean slope of all sequences (Sequence BRS).[14,15] Previous reports showed that this protocol measures BRS accurately in animals compared with standard pharmacological techniques.[14-16]

MIL responders and nonresponders: Blood sampling was performed in all patients, and the severity of tricuspid regurgitation (TR) was evaluated by color-flow Doppler (graded as trivial, mild, moderate, or severe). TR pressure gradient (TRPG) was measured by echocardiography and BRS was measured by the spontaneous sequence method before and 2 hours after the initiation of intravenous infusion of milrinone (0.25 μg/kg/minute). We did not do an initial bolus infusion. The effects of MIL are reported to be stable and plateau at 2 hours after the initiation of MIL.[18] At 2 hours after administration, milrinone improved dyspnea, urine volume (> 100 mL/hour), and the severity of tricuspid regurgitation in 13 patients (responders; R), whereas no improvement was observed in 11 patients (nonresponders; NR) (Figure 1). The degree of dyspnea was assessed by the modified Borg scale.[19] An improvement in dyspnea was defined as a reduction of 2 or more in the modified Borg scale score.[20]

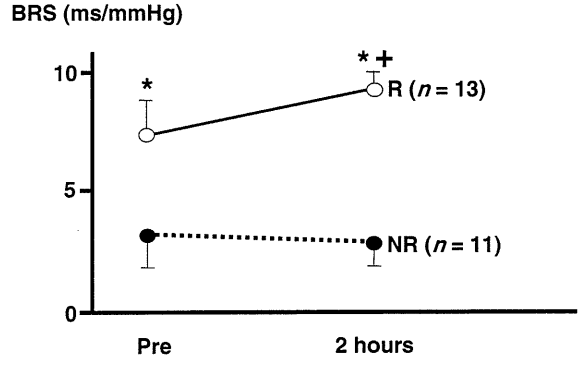

Figure 1. Baseline BRS was significantly lower in the NR group than in the R group. At 2 hours after milrinone treatment, BRS was also significantly lower in the NR than in the R group. Furthermore, in the R group, BRS at 2 hours was significantly higher than that at baseline. *$P < 0.05$ versus NR group. +$P < 0.05$ versus pre.

Statistical analysis: Normally distributed variables are expressed as the mean ± SD. The unpaired t test or Mann-Whitney U test was used to compare the differences in normally distributed variables, respectively, between the R group and NR group. All statistical tests were carried out against the baseline characteristics. Differences were considered significant at a P value of < 0.05. A receiver operating characteristic (ROC) curve was used to determine the discriminatory ability of BRS on the occurrence of response to MIL. An ROC-curve (plot of sensitivity versus 1-specificity) analysis is a powerful tool for assessing a test's ability to discriminate between R and NR groups of subjects.

Results

Patient characteristics at baseline: The patient profiles at enrollment are summarized in Tables I and II. As can be seen in Table I, there were no significant differences in age, gender, or the prevalence of dilated cardiomyopathy, hypertensive heart disease, or valvular heart disease between the R and NR group just before the milrinone therapy. Prior medications were not different either.

As shown in Table II, hemoglobin, brain natriuretic peptide (BNP) measured at discharge, and the estimated glomerular filtration rate (eGFR) did not differ between the two groups. Left ventricular ejection fraction (LVEF), left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), and TRPG assessed by echocardiography

Table I. Baseline Characteristics

	R	NR	P
n	13	11	
Male	8	7	NS
Age	58 ± 7	59 ± 5	NS
BMI	22 ± 4	23 ± 3	NS
Body weight (kg)	56 ± 7	53 ± 9	NS
Current smoker	4 (31%)	3 (27%)	NS
Causes of heart failure			
Coronary artery disease	4 (31%)	4 (36%)	NS
Dilated cardiomyopathy	4 (31%)	3 (27%)	NS
Hypertensive heart disease	3 (23%)	2 (18%)	NS
Valvular heart disease	2 (15%)	2 (18%)	NS
NYHA functional classification			
III	9 (69%)	8 (73%)	NS
IV	4 (31%)	3 (27%)	NS
Modified Borg scale	6.1 ± 1.8	6.4 ± 2.2	NS
Systolic blood pressure (mmHg)	118 ± 11	121 ± 14	NS
Diastolic blood pressure (mmHg)	78 ± 14	73 ± 9	NS
Heart rate (bpm)	108 ± 9	111 ± 13	NS
Medications			
Diuretics	7 (54%)	6 (55%)	NS
β-Blockers	9 (69%)	8 (73%)	NS
ACE inhibitors	10 (77%)	8 (73%)	NS
Angiotensin receptor blocker	3 (23%)	3 (27%)	NS

Data are presented as number (%) or mean ± SD. BMI indicates body mass index; NYHA, New York Heart Association; and NS, not significant.

Table II. Baseline Characteristics (2)

	R (n = 13)	NR (n = 11)	P
Total cholesterol (mg/dL)	187 ± 33	172 ± 44	NS
LDL cholesterol (mg/dL)	94 ± 31	102 ± 43	NS
HDL cholesterol (mg/dL)	42 ± 8	45 ± 7	NS
Triglycerides (mg/dL)	119 ± 27	120 ± 31	NS
Hemoglobin (g/dL)	9.8 ± 1.4	10.3 ± 2.1	NS
Hematocrit (%)	35 ± 3	34 ± 3	NS
Total bilirubin (mg/dL)	1.3 ± 0.2	1.4 ± 0.3	NS
AST/ALT (U/L)	47 ± 4 / 36 ± 8	57 ± 4 / 45 ± 6	< 0.05 / < 0.05
Serum sodium (mmol/L)	131 ± 3	132 ± 2	NS
FBS (mg/dL)	82 ± 17	99 ± 15	NS
HbA1c (%)	5.2 ± 0.7	5.3 ± 0.4	NS
BNP (pg/mL)	188 ± 27	194 ± 36	NS
eGFR (mL/minute/1.73m^2)	61.4 ± 7.9	62.8 ± 4.9	NS
LVEF (%)	37 ± 7	34 ± 5	NS
LVEDD (mm)	58 ± 6	59 ± 7	NS
LVESD (mm)	42 ± 4	43 ± 7	NS
IVC (mm)	18 ± 4	24 ± 3	< 0.05
TRPG (mmHg)	47 ± 8	51 ± 9	NS
BRS (ms/mmHg)	7.3 ± 1.2	3.2 ± 1.6	< 0.05

Data are presented as number (%) or mean ± SD. LDL indicates low-density lioprotein; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, creatinine-based estimate of glomerular filtration rate; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; TRPG, tricuspid regurgitant pressure gradient; BRS, baroreflex sensitivity; and NS, not significant.

Table III. Effects of Milrinone at 2 Hours

	R (n = 13)	NR (n = 11)	P
Modified Borg scale	3.3 ± 1.6	6.2 ± 1.9	< 0.05
TRPG (mmHg)	29 ± 4	49 ± 7	< 0.05
Urine volume (mL/hour)	128 ± 18	42 ± 23	< 0.05
SBP (mmHg)	106 ± 15	114 ± 18	NS
Serum sodium level (mmol/L)	135 ± 2	129 ± 3	< 0.05
LVEF (%)	42 ± 3	36 ± 2	< 0.05
HR (bpm)	82 ± 15	106 ± 12	< 0.05

Data are presented as number (%) or mean ± SD. TRPG indicates tricuspid regurgitant pressure gradient; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction; and HR, heart rate.

were not different between the two groups. AST and inferior vena cava diameter were significantly higher in the NR group than in the R group. Median BRS was significantly higher in the R group than in NR prior to the milrinone infusion (7.3 ± 1.2 versus 3.211.6 ms/mmHg, $P < 0.05$).

Effects of MIL at 2 hours: In the R group, the modified Borg scale score and TRPG at 2 hours were significantly lower than in the NR group (Table III). Urine volume per hour at 2 hours was over 100 mL/hour in all patients in the R group and significantly higher than in the NR group (Table III). LVEF and the serum sodium level were significantly higher in the R group than in the NR group (Table III). Systolic blood pressure at 2 hours was not changed. However, heart rate at 2 hours was significantly lower in the R group than in the NR group (Table III).

BRS at 2 hours: At 2 hours after the milrinone infusion, BRS was further increased in the R group, whereas it failed to increase in the NR group (Figure 1). The sensitivity and specificity for BRS at a cut-off level of 5 ms/mmHg were 0.94 and 0.93, respectively. The negative and positive predictive values were 0.98 and 0.71 (Figure 2).

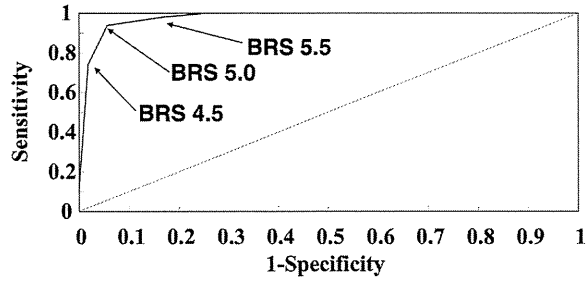

Figure 2. Receiver operator curve of BRS for prediction of responders to milrinone.

Discussion

In the present study, we demonstrated that baroreflex sensitivity measured by a spontaneous sequence method was significantly lower in the nonresponder group to milrinone than in the responder group to milrinone in patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure. Furthermore, the sensitivity and specificity for BRS at a cut-off level of 5 ms/mmHg was 0.94 and 0.93. These results suggested that BRS might be clinically useful for the prediction of responders to milrinone among patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure.

Milrinone is recommended for patients with "wet and cold" heart failure,[2] which is determined as heart failure with congestion and hypoperfusion.[3] In cases in which the systolic blood pressure is under 100 mmHg, the combination of milrinone and dobutamine is necessary.[1] However, among patients with "wet and cold" heart failure whose systolic blood pressure is over 100 mmHg, milrinone monotherapy is often insufficient. In the present study, 11 of 24 patients with "wet and cold" heart failure whose systolic blood pressure was over 100 mmHg were nonresponders to milrinone. Based on these results, we believe that systolic blood pressure is not clinically useful for predicting the response to milrinone. This is the first study to demonstrate the cut-off levels of parameters for predicting the response to milrinone, and BRS assessed by a spontaneous sequence method might be a new and novel therapeutic parameter.

A sustained baroreflex-mediated increase in sympathetic activity may contribute to increased end-organ damage and to progression of the underlying disease, and a blunted baroreflex gain is predictive of increased cardiovascular risk in postmyocardial infarction and heart failure patients.[7] Recently, the prognostic value of BRS obtained noninvasively by the modified transfer function method has been assessed in a cohort of 317 mild-to-moderate clinically stable heart failure patients. In 55 of the 228 subjects with a measurable index, a depressed BRS (≤ 3.1 ms/mmHg) was significantly associated with a higher risk of cardiac death. The results of the present study are compatible with a previous study.[16] We believe that BRS might be clinically useful as a parameter with which to determine the severity of heart failure.

The spontaneous sequence method is a noninvasive and easy method for measuring BRS in patients with acute heart failure. The advantages of this method are twofold: (i) computations are automatic and standardized, which virtually eliminates intra- and intersubject measurement variability, and (ii) distinct measurements are obtained for increasing and decreasing arterial pressure values, thus allowing one to take into account the well-known asymmetry of the baroreceptor response.[7] Further studies are necessary to determine whether other noninvasive methods to measure BRS are clinically useful or not.

The mechanisms in which BRS predicts the responses to milrinone were not fully determined in the present study. Previous studies have suggested that arterial baroreflex control of HR is diminished in heart failure,[11,21] and that the contribution of baroreflex control in myocardial contractility is markedly impaired in animals with a lower baseline inotropic state.[22] Furthermore, in heart failure, baroreflex changes in cardiac output are less related to changes in HR and more related to changes in stroke volume.[23] Milrinone is considered to exert a positive inotropic action and to decrease left ventricular end-systolic pressure reflecting the decrease in left ventricular afterload.[24] However, milrinone-induced reductions in effective arterial elastance reflecting the fall in total peripheral resistance and changes in stroke volume are not significant.[24] These previous reports suggested that baroreflex control is important to obtain the inotropic effect of milrinone. Another previous study suggested that BRS predicts the cases in which milrinone increases left ventricular *dp/dt*.[4] The increase in left ventricular *dp/dt* indicates milrinone has an inotropic effect. In the present study, in the R group, the treatment with milrinone for 2 hours was considered to increase cardiac output because LVEF and serum sodium levels were significantly increased. Moreover, AST and inferior vena cava diameter were significantly higher in the NR group than in the R group. These results suggest that right ventricular function was impaired in the NR group to a greater extent than in the R group. Nonresponders to milrinone can be considered to be a higher severity group of heart failure than responders to milrinone. However, we did not have the direct data of cardiac output, chest x-rays, and electrocardiogram in the present study. Further studies monitoring hemodynamic parameters (involving cardiac output) of the patients with acute heart failure treated with milrinone are necessary.

There are several limitations to the present study. First, it is a small and retrospective study. It is necessary to examine the predictive value of BRS in a large-scale population in which the distribution of BRS is normal. Second, we excluded patients with atrial fibrillation. In patients with atrial fibrillation, the spontaneous sequence method is not feasible. However, among patients with acute heart failure, there are many patients with atrial fibrillation. We believe that BRS measured by the spontaneous sequence method can only be used in patients with sinus rhythm. Third, we examined the effects of milrinone for only 2 hours. Furthermore, the present study is retrospective. Fourth, we did not perform the hemodynamic assessment using a Swan-Ganz catheter, and we defined low cardiac output from the clinical state of "cold and wet". There were no variables to show the fact by echocardiography or Swan-Ganz catheter. Randomized study with the assessment of hemodynamic data obtained by Swan-Ganz catheter should be performed.

Conclusions: The results of the present study suggested that baroreflex sensitivity to milrinone measured by the spontaneous sequence method was significantly lower in the nonresponder group than in the responder group in patients with "wet and cold" heart failure and that the cut-off level of BRS is 5 ms/mmHg. We believe that BRS might be clinically useful for the prediction of responders to milrinone in patients with "wet and cold" heart failure.

ACKNOWLEDGMENTS

We are grateful to the staff of the Department of Cardiovascular Medicine of Kyushu University Graduate School of Medical Sciences. Furthermore, we wish to thank Dr. Kazuhisa Kodama, the chair of the Nakanoshima Heart Failure Conference.

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32nd Annual International Conference of the IEEE EMBS
Buenos Aires, Argentina, August 31 - September 4, 2010

Development of artificial bionic baroreflex system

Kenji Sunagawa, *Senior Member, IEEE* and Masaru Sugimachi, *Member, IEEE*

Abstract—**The baroreflex system is the fastest mechanism in the body to regulate arterial pressure. Because the neural system (i.e., autonomic nervous system) mediates the baroreflex and the system operates under the closed-loop condition, the quantitative dynamic characteristics of the baroreflex system remained unknown until recently despite the fact that a countless number of observational and qualitative studies had been conducted. In order to develop the artificial baroreflex system, i.e., the bionic baroreflex system, we first anatomically isolated the carotid sinuses to open the baroreflex loop and identified the open-loop transfer function of the baroreflex system using white noise pressure perturbations. We found that the baroreflex system is basically a lowpass filter and remarkably linear. As an actuator to implement the bionic baroreflex system, we then stimulated the sympathetic efferent nerves at various parts of the baroreflex loop and identified the transfer functions from the stimulation sites to systemic arterial pressure. We found that the actuator responses can be described remarkably well with linear transfer functions. Since transfer functions of the native baroreflex and of the actuator were identified, the controller that is required to reproduce the native baroreflex transfer function can be easily derived from those transfer functions. To examine the performance of bionic baroreflex system, we implemented it animal models of baroreflex failure. The bionic baroreflex system restored normal arterial pressure regulation against orthostatic stresses that is indistinguishable from the native baroreflex system.**

I. Introduction

Baroreflex is known to be the fastest mechanism in the body to stabilize arterial pressure. The reflex makes use of negative feedback mechanism. The baroreceptors sitting in the arterial wall sense arterial pressure and send the pressure signal to the brainstem through the afferent nerve fibers. The brainstem receives the pressure signal and judges the level of arterial pressure. If the level is low, the brainstem activates the sympathetic system innervating the heart and vascular system to increase arterial pressure. If the level of arterial pressure is high, the brainstem withdraws the sympathetic activation.

The baroreflex system is critically important in animal, particularly in human. This is because, unlike animals with four legs, the position dependent gravitational effect on circulation is most prominent in human. It is well known that once we lose the normal function of baroreflex, we no longer keep sitting and/or standing positions because of position induced profound hypotension and hypoperfusion of the brain. Baroreflex failure destroys normal life and is a devastating pathological state in human. However, since the baroreflex failure is a disease of the neural system, no effective treatment has ever developed to save those patients.

Baroreflex failure could happen under various conditions. In some patients, they lost baroreflex function because they have problems in the baroreceptors, the brainstem and/or the spinal cord. In those patients, if we can develop a mechanism to activate their sympathetic efferent system in response to changes in arterial pressure just like the native brainstem does, in theory, normal baroreflex function can be restored.

The purpose of this investigation is to develop an artificial baroreflex system, so called the bionic baroreflex system, to restore normal baroreflex function to overcome such a serious pathological condition.

II. Bionic Baroreflex System

Shown in Fig. 1 are how we identify the transfer function of the controller of bionic baroreflex system. First we identify the transfer function of the baroreflex open loop (H_{NATIVE}) from baroreceptor pressure to arterial pressure responses. We then electrically stimulate a particular site in the baroreflex loop and identify the transfer function of the actuator from the stimulation to arterial pressure responses ($H_{STM \rightarrow AOP}$). Since the controller will be in series with the actuator, the transfer function of bionic baroreflex system becomes identical to the native baroreflex system when the transfer function of controller (H_{BIONIC}) satisfies the following equation:

$$H_{NATIVE} = H_{BIONIC} \times H_{STM \rightarrow AOP}$$

In theory both H_{NATIVE} and $H_{STM \rightarrow AOP}$ can be experimentally determined. Therefore, H_{BIONIC} can be determined. However whether such a simple approach works or not highly depends on the simplicity of the native baroreflex system including the system linearity. We therefore examined the dynamic characteristics of baroreflex system.

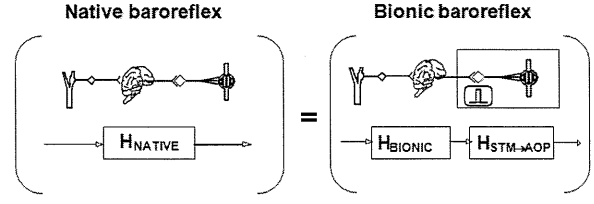

Fig. 1 Native vs. Bionic baroreflex

We vascularily isolated the baroreceptors (carotid sinuses) in rats (n=10) to open the baroreflex feedback loop and connected the carotid sinuses to a servo-controlled piston pump. This preparation allowed us to manipulate the carotid sinus pressure (CSP) independent of arterial pressure. We then perturbed CSP with random binary pressure sequences and identified the transfer function from CSP to arterial pressure. Shown in the left panels of Fig. 2 are the time series of CSP and aortic pressure. As can be seen, aortic pressure changes slowly toward the opposite direction in response to changes in CSP. This becomes even more evident in the transfer function (the right panel). The transfer function has low-pass filter characteristics. The phase response becomes nearly out-of-phase in the low frequency range suggesting the negative feedback nature of baroreflex system. Note that the magnitude squared coherence function is about 0.8 over the frequency range of interest. This is to say that most dominant characteristics of the total baroreflex open loop are captured by the linear transfer function.

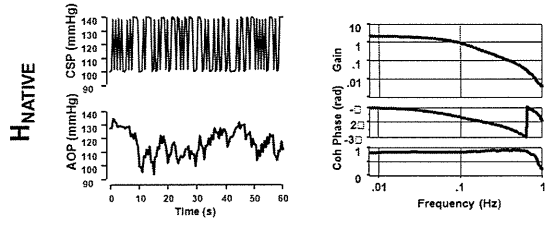

Fig. 2 Dynamic characteristics of native baroreflex system

In order to identify the actuator transfer function, we electrically stimulated the celiac ganglia with random binary pressure perturbations. Illustrated in the left panels of Fig. 3 are the time series of stimulation of celiac ganglia and aortic pressure responses. As can be seen, aortic pressure changes slowly toward the same direction in response to changes in stimulation. As anticipated the transfer function (the right panel) has low-pass filter characteristics. Unlike the total baroreflex loop, however, the phase response becomes nearly in-phase in the low frequency range. The magnitude squared coherence function is about 0.8 over the frequency range of interest. Again, it is reasonable to assume that most dominant characteristics of the actuator are captured by the linear transfer function.

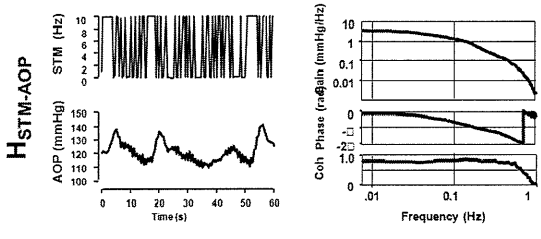

Fig. 3 Dynamic characteristics of sympathetic stimulation

We identified the transfer function (H_{BIONIC}) required for the controller by taking the ratio of H_{NATIVE} to $H_{STM \rightarrow AOP}$. Since both dynamic characteristics of the total baroreflex loop and actuator are well represented by the linear transfer functions, the resultant H_{BIONIC} should reproduce the native characteristics of the baroreflex system when the feedback loop is closed. Shown in Fig. 4 are the changes in arterial pressure in response to orthostatic stresses under the open-loop baroreflex condition (baroreflex failure), the closed-loop baroreflex condition (native baroreflex) and the bionic baroreflex condition. Orthostatic stresses profoundly lowered arterial pressure in the absence of the native baroreflex. Closing the native baroreflex loop markedly attenuated the hypotensive responses. The activation of bionic baroreflex system also attenuated the hypotensive response as much as the native baroreflex system did. Statistical analysis indicated that the pressure regulation achieved by the bionic baroreflex system was indistinguishable from that achieved by the native baroreflex system.

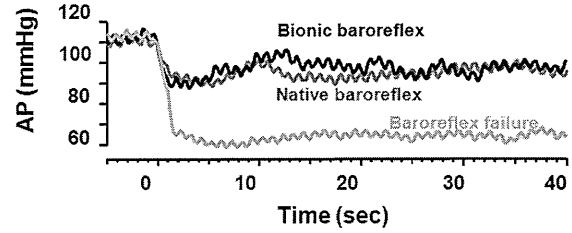

Fig. 4 Native baroreflex system vs. bionic baroreflex system

III. Discussion

We have shown that the bionic baroreflex system was as good as the native baroreflex system in regulating arterial pressure. The dynamic pressure responses to orthostatic stresses were indistinguishable between the native baroreflex system and the bionic baroreflex system. Although the baroreflex system is known to be nonlinear over the wide pressure range, we found that it is remarkably linear in the physiological pressure range. Because of this, the linear transfer function could represent the dominant characteristics of the baroreflex loop, and thereby allowed us to develop the bionic baroreflex system.

We can think of many anatomical sites where we can manipulate the activity of sympathetic system. In 1992, we stimulated the carotid sinus nerve to control the sympathetic system [1]. In 2004, we stimulated the spinal cord to stimulate the sympathetic efferent fibers [2]. The bionic mechanism worked beautifully regardless of the site of stimulation. It equally worked well in rats [3], rabbits [2], and dogs [1]. Although our experience of baroreflex failure in patients is limited, judging from its robustness, the bionic baroreflex system would work in patients as well [4]. If the bionic baroreflex system works in patients, it has a major impact as the treatment of baroreflex failure [5] that has been considered to be an incurable devastating disease.

IV. Conclusion

The bionic baroreflex system restores normal baroreflex function in an animal model of baroreflex failure.

ACKNOWLEDGMENT

This study was supported in part by Health and Labour Sciences Research Grant for Research on Medical Devices for Improving Impaired QOL from the Ministry of Health Labour and Welfare of Japan, Health and Labour Sciences Research Grant for Clinical Research from the Ministry of Health Labour and Welfare of Japan, and Grant-in-Aid for Scientific Research(S) (18100006) from the Japan Society for the Promotion of Science.

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32nd Annual International Conference of the IEEE EMBS
Buenos Aires, Argentina, August 31 - September 4, 2010

The pressure-volume relationship of the heart: Past, Present and Future

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Abstract—**The pressure-volume relationship of the heart was first reported more than a century ago. It was not widely accepted, however, until the mid-1970s. The pressure-volume diagram became a central theme of cardiac mechanics once it was shown to be a good representation of ventricular mechanics. Early in 1980s, the introduction of the ventricular interaction with afterload using effective arterial elastance made it possible to translate ventricular mechanical properties represented by the pressure-volume relationship to the pumping ability of the heart. Furthermore incorporating the framework of ventricular arterial interaction into the classic Guyton's circulatory equilibrium early in 2000s enabled us to express quantitatively how mechanical properties of the ventricles and vascular systems determine the circulatory equilibrium. Successful quantitative descriptions of circulatory equilibrium using the pressure-volume concept would promote basic cardiovascular physiology and accelerate its clinical applications.**

I. PAST (~1980s)

In 1899 Otto Frank published a theoretical paper [1] entitled "Die Grundform des arteriellen Pulses," in which he characterized contractions of the frog ventricle in a pressure-volume (P-V) diagram. It is a schematic expression based on experimental data published in 1895. This schematic diagram is perhaps the first complete P-V diagram ever published on the heart. For various reasons, however, the approach was not well accepted. The marked loading history dependence of the end-systolic P-V relation (ESPVR) that Frank and his followers observed in the frog heart had left physiologists and cardiologists with a strong negative feeling about the use of the P-V diagram for understanding cardiac mechanics.

Our experience with isolated, blood-perfused canine hearts differed markedly from those earlier observations. In the physiological range, the ESPVR appeared to be linear and insensitive to changes in loading conditions. Encouraged by our own simple findings and by similar findings of other investigators, we proposed early in 1970s to scrutinize this relationship as a potential candidate for an index of ventricular contractility [2].

The discovery of the simple characteristics of the ESPVR in the canine heart was not the only attraction of the P-V analysis. We knew this approach would yield a great deal of information about cardiac pump function that is not explicit in the time function of the ventricular pressure and volume. The systolic P-V area [3] as a measure of the total mechanical energy released per ventricular contraction is an entirely new concept that could emerge only from the P-V diagram. Another essential production of this approach is the notion of effective arterial elastance [4] that functionally represents the mechanical properties of the afterload system in terms of P-V relationship. The concept of effective arterial elastance made it possible to couple the ventricle with the afterload on the P-V plane to analyze ventricular-arterial interaction. The qualitative expression of ventricular arterial interaction using the P-V diagram sharpened as well as deepened our understanding of the mechanism how the mechanical properties of the ventricle and arterial system determine stroke volume, thus cardiac output [5].

II. PRESENT (1990-2010)

Although the ventricular arterial interaction was quantitatively expressed by the P-V relationship, it remained unknown how the ventricle determines cardiac output by interacting with the total vascular system. Guyton established that cardiac output is determined as the equilibrium between the venous return curve and cardiac output curve [6]. In 2004 we developed and experimentally validated an algebraic expression of cardiac output curve by extensively using the ventricular arterial P-V relationship. Incorporating the derived cardiac output curves into a simultaneous expression of systemic and pulmonary venous return (the venous return surface) resulted in a new framework of circulatory equilibrium, i.e., the extended Guyton's model [7]. Since the new framework has an analytical solution of circulatory equilibrium for a given set of ventricular and vascular mechanical properties, it provides us an extremely powerful tool in understanding the mechanisms how cardiac output is determined under a variety of pathological as well as physiological conditions.

III. FUTURE (2010~)

The extended Guyton's model enables us to qualitatively predict a circulatory equilibrium once the mechanical properties of the ventricular and vascular system are known. This helps understand how complex physiological regulatory systems such as baroreflex systems regulate the cardiovascular system. In a

preliminary study reported at this conference, we indicated that the extended Guyton's model is capable of quantitatively synthesizing dynamic baroreflex arterial pressure regulation on the basis of baroreflexly modulated ventricular and vascular mechanical properties.

The fact that we can quantitatively predict circulatory equilibrium for a given set of ventricular and vascular mechanical properties opens up vast clinical applications. If we can develop a feedback mechanism to manipulate mechanical properties of ventricle and vascular system, we can in turn feedback regulate the circulatory equilibrium, and thereby hemodynamics. Recently we developed a prototype of fully automated closed-loop treatment system that stabilizes hemodynamics of decompensated left heart failure. The system outperformed as good as well trained cardiologists [8].

The P-V relationship will remain to be a central theme that bridges basic cardiac physiology to extensive clinical applications.

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32nd Annual International Conference of the IEEE EMBS
Buenos Aires, Argentina, August 31 - September 4, 2010

Physiological Significance of Pressure-Volume Relationship: a Load-Independent Index and a Determinant of Pump Function

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Kazunori Uemura, and Toshiaki Shishido

Abstract—**Pressure-volume relationship permits conceptual integration with time-varying elastance, stress-strain relationship, and pressure-volume area. It has also superior usefulness to other indexes, both as a load-independent index of ventricular contractility and as a determinant of ventricular pump function.**

PRESSURE-VOLUME relationship has become a standard framework [1] for discussing the mechanical properties of the ventricles and sometimes atria. It has gained popularity because of its conceptual integration and its superior usefulness, both as a load-independent index and as a determinant of pump function. The concept of pressure-volume relationship agrees with to that of time-varying elastance, that of (time-varying) material properties of myocardium (i.e., stress-strain relationship), and that of pressure-volume area as the major determinant of myocardial oxygen consumption [2].

A. A Load-Independent Index

Pressure-volume relationship (PVR), especially the end-systolic pressure-volume relationship (ESPVR), has been repeatedly shown as one of the least load-sensitive index of ventricular contractility. Although preload-recruitable stroke work (PRSW) has been a rival, it is obvious that PRSW would no longer be load insensitive in extreme cases such as isovolumic beats.

Although detailed examination of ESPVR revealed its load-dependence (such as deactivation and activation associated with ejection) and curvilinearity [3], ESPVR is still the least load-dependent index of ventricular contractility. The apparent linearity of ESPVR seems to be observed just by chance, taking into consideration that ESPVR can be reconstructed from nonlinear (exponential) end-systolic stress-strain relationship of myocardium.

The most important advance what the concept of PVR has provided are the decoupling of heart from vasculature (preload and afterload), and the fact that actively contracting tissue would change its mechanical properties in cardiac cycles. Decoupling the heart enabled us to separately discuss the changes in the heart and the vasculature, rather than mix them and discuss only the measured hemodynamic variables. The uncovered complex load-dependence and curvilinearity would have not sacrificed the value of decoupling. The concept of changeable material property has simplified the explanation of complex time course of pressure development and ejection.

B. A Determinant of Pump Function

ESPVR has provided a method to precisely predict the stroke volume for given end-diastolic volume, heart rate and afterload resistance. This was accomplished by recoupling ESPVR with effective arterial elastance (mainly determined by heart rate and resistance). This is a major advantage over PRSW. What is more, even the pressure and flow waveform can be reconstructed by recoupling time-varying PVR (for the entire cardiac cycle) and arterial high-resolution impedance [4].

Manuscript received April 7, 2009. This work was supported in part by Grant-in-Aid for Scientific Research (B 20300164) from the Ministry of Education, Culture, Sports, Science and Technology, by Health and Labour Sciences Research Grants (H20-katsudo-shitei-007) from the Ministry of Health Labour and Welfare of Japan.

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32nd Annual International Conference of the IEEE EMBS
Buenos Aires, Argentina, August 31 - September 4, 2010

Automated drug delivery system for the management of hemodynamics and cardiac energetic in acute heart failure

Kazunori Uemura, Masaru Sugimachi, *Member, IEEE*,
Toru Kawada, and Kenji Sunagawa, *Member, IEEE*

Abstract— **We have developed a novel automated drug delivery system for simultaneous control of systemic arterial pressure (AP), cardiac output (CO), and left atrial pressure (P_{LA}) in acute heart failure. The circulatory equilibrium framework we established previously discloses that AP, CO, and P_{LA} are determined by equilibrium of the mechanical properties of the circulation, i.e. pumping ability of the left heart, stressed blood volume and systemic arterial resistance. Our system directly controls the three mechanical properties with cardiovascular drugs including inotropes and vasodilators, thereby controlling AP, CO, and P_{LA}. Furthermore, by precisely controlling bradycardia and LV inotropy, our system enables to improve cardiac energetic efficiency while preserving AP, CO, and P_{LA} within acceptable ranges. In conclusion, by directly controlling the mechanical properties of the heart and vessel, our automated system realizes comprehensive management of hemodynamics in acute heart failure.**

I. Introduction

In the management of patients with acute heart failure after myocardial infarction or following cardiac surgery, cardiovascular agents such as inotropes and/or vasodilators are commonly used to control systemic arterial pressure (AP), cardiac output (CO) and left atrial pressure (P_{LA}). Since responses to these agents vary between patients and within patient over time, strict monitoring of patient condition and frequent adjustments of drug infusion rates are usually required. This is a difficult and time-consuming process, especially in hemodynamically unstable patients.

Although several closed-loop systems [1, 2] to automate drug infusion have been developed to facilitate this process, no closed-loop system so far developed is capable of controlling the overall hemodynamics; i.e., controlling AP, CO and P_{LA} simultaneously. This is because all previous systems attempted to directly control AP and CO by estimating response of the variable to drug infusion [1, 2]. This approach is inapplicable because of the difficulties to estimate simultaneous AP, CO and P_{LA} responses to the infusion of multiple drugs.

In this study, we developed a new automated drug delivery system to control AP, CO and P_{LA} [3]. To overcome the difficulty of the previous systems, our system adopted a strikingly original approach. We previously developed a circulatory equilibrium framework by extending the Guyton's classic framework [4]. As shown in Fig. 1, the extended framework consists of an integrated cardiac output curve characterizing the pumping ability of the left and the right heart, and a venous return surface characterizing the venous return property of the systemic and pulmonary circulation [5-7]. The intersection point of the integrated CO curve and the venous return surface predicts the equilibrium point of CO, P_{LA} and right atrial pressure (P_{RA}) (Fig. 1). Once CO, P_{LA} and P_{RA} are predicted from the intersection point, systemic arterial resistance determines AP. Based on this framework, instead of directly controlling AP, CO, and P_{LA}, our system controls the integrated CO curve with dobutamine (DOB), the venous return surface with 10% dextran 40 (DEX) and furosemide (FUR), and systemic arterial resistance with sodium nitroprusside (SNP), thereby controlling AP, CO and P_{LA}. The purpose of this study was, therefore, to develop and validate the automated drug delivery system.

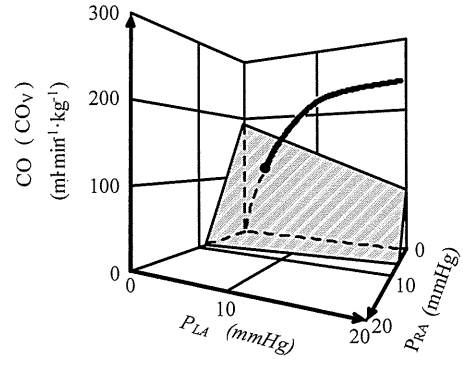

Fig. 1. Diagram of circulatory equilibrium for CO, venous return (CO_V), P_{LA}, and P_{RA}. The equilibrium CO, P_{LA} and P_{RA} are obtained as the intersection point of the venous return surface and integrated cardiac output curve.

Manuscript received April 23, 2010. This work was supported in part by Grant-in-Aid for Scientific Research (B 20300164, C 20500404) from the Ministry of Education, Culture, Sports, Science and Technology, by a research grant from Nakatani Foundation of Electronic Measuring Technology Advancement, by Health and Labour Sciences Research Grants (H20-katsudo-shitei-007) from the Ministry of Health Labour and Welfare of Japan.

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