Synthetic Salusins as Cardiac Depressors in Rat

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Abstract—Using bioinformatic analyses of full-length, enriched human cDNA libraries, we recently identified salusins, multifunctional related peptides ubiquitously expressed in major human tissues. Salusins cause transient and profound hypotension when injected intravenously to rats, the hypotensive effect of salusin-β being especially striking. However, the mechanisms of this hypotensive action remain elusive. To determine whether salusins modulate cardiac function in rats, we studied serial changes of systemic hemodynamics and functions of isolated perfused working and nonworking hearts before and after salusin administration. Intravenous salusin-β administration to intact anesthetized rats caused a temporary rapid, profound decrease in aortic blood flow concomitantly with hypotension and bradycardia without affecting systemic vascular resistance. Salusin-β–induced hypotension and bradycardia were completely blocked by pretreatment with atropine, a muscarinic receptor antagonist, but not by propranolol. In isolated perfused working rat hearts, salusin-β significantly decreased cardiac output, aortic flow, and stroke work. However, it did not affect coronary flow in isolated working and nonworking hearts. Our results indicate that salusins induce potent hypotension via negative inotropic and chronotropic actions. Salusin-β promotes its actions by facilitating vagal outflows to the heart, whereas the negative inotropism of salusin-β is also mediated via a direct myotropic effect. (Hypertension. 2005; 45:419-425.)

Key Words: cardiac output ■ heart rate ■ blood pressure ■ vascular resistance

The availability of a massive amount of information on human genome sequences has allowed for the faster elucidation of gene functions. Functional characterization of putative bioactive proteins is an indispensable process in elucidating the roles of secretory proteins. Very recently, we identified 2 novel multifunctional related peptides of 28- and 20-residues that we designated as salusin-α and salusin-β, respectively,1 after screening a number of secretory protein-encoding cDNAs using receptor ligand–facilitated gene transfer protocols.2,3 These salusins are considered to be generated simultaneously through proteolytic processing of prosalusin, a commonly occurring alternatively spliced product of the TOR2A gene, which has structural homologies to torsion dystonia genes (DYTI and DQ1).4,5 Alternative splicing responsible for biosynthesis of salusin peptides is probably not a rare event, and salusins are expressed ubiquitously throughout human tissues.1

Systemic administration of salusins to rats is associated with rapid and profound hypotension and bradycardia; the maximal hypotensive response to salusin-β in rats is almost equivalent to or even exceeds those of the hypotensive peptide hormones identified thus far. Further, other potent endogenous hypotensive peptide hormones do not induce hypotension and concomitant bradycardia with such a time course, implying an as yet undescribed mechanism for salusins. Although the expression of preprosalusin in heart is very limited, salusin-like immunoactivities are present in human plasma and urine. Thus, it is very likely that they act on the cardiovascular system as circulating peptides to regulate hemodynamic homeostasis. However, it remains undetermined whether salusins decrease cardiac performance in intact animals and whether their systemic hemodynamic effects are elicited by direct cardiotoxic effects. Therefore, we investigated whether salusins, especially salusin-β, which has more potent hypotensive activity, modulate cardiac function in intact rats and isolated perfused rat hearts. Our results indicate that the hypotensive activity of salusin-β is caused by depressing cardiac contractility.

Materials and Methods

Animals
Male Sprague-Dawley rats (Sankyo Lab Service; Tokyo, Japan) were placed on a diet of normal chow and water ad libitum until the time of the experiments. All aspects of animal care and experimentation were performed in accordance with the guidelines for animal experimentation; the protocol was approved by the animal care and use committee of Tokyo Medical and Dental University.

Peptides
Human salusin-α and salusin-β were synthesized by an automated peptide synthesizer using the F-moc solid-phase method and purified by reverse-phase high-performance liquid chromatography.
N-terminal 20 amino acid residues of putative rat salusin are 70% homologous with human salusin.

Effects of the Intravenous Infusion of Salusins on the Hemodynamics of Intact Rats

Male Sprague-Dawley rats (weighing 400 to 525 g) were anesthetized with pentobarbital sodium (50 mg/kg body weight intraperitoneally). The femoral vein was cannulated for the administration of salusins, and a saline-filled PE50 polyethylene catheter placed in the femoral artery was connected to a pressure transducer for continuous recordings of blood pressure and heart rate (HR) as described. An electromagnetic flow probe was attached to the abdominal aorta to estimate arterial blood flow (ABF) with an electromagnetic flowmeter (MFV-1100; Nihon Koden). Data were acquired with a polygraph (RM-6000; Nihon Koden). Salusins were then infused intravenously at the indicated doses over a period of 15 seconds, and systolic pressure (SP), diastolic pressure (DP), mean aortic pressure (MAP), HR, and ABF recorded over the 30-minute period that followed. The effect of parasympathetic withdrawal was assessed by bolus injection of atropine sulfate (0.75 mg/kg) 10 minutes before intravenous administration of salusin-β (1.0 nmol/L per kg), whereas the effect of β-adrenoreceptor blockade was evaluated by intravenous injection of propranolol hydrochloride (1.0 mg/kg) before salusin-β (1.0 nmol/L per kg).

To measure blood pressure and HR in conscious rats, a catheter was advanced into the abdominal aorta via the femoral artery, exteriorized at the back of the neck, and filled with heparinized saline under pentobarbital anesthesia. Recordings for conscious rats were started after connecting the arterial catheter to a pressure transducer and stable tracings had been obtained.

Analysis of left ventricular performance was measured in anesthetized rats using a Millar Microtip catheter pressure transducer system (Millar Instruments). A microtip catheter transducer (SPC-320) was inserted into the carotid artery and advanced into the left ventricle. HR, left ventricular SP, left ventricular end-diastolic pressure (LVEDP), and the maximal rate of increment (dP/dtmax) and decrement (dP/dtmin) in left ventricular pressure were recorded before and after intravenous infusion of salusin-β. Each rat was tested for just 1 concentration of the drug.

Effects of Salusin-β on Hemodynamics in Isolated Rat Working and Nonworking Hearts

Sodium heparin (200 IU intravenously) was injected after anesthetizing rats with sodium pentobarbital. Hearts were excised, rapidly
cannulated, and perfused in nonworking heart mode at a pressure of 60 mm Hg using an IPH-W2 apparatus (Primetech Corporation) with modified Krebs–Henseleit buffer (KHB) gassed with 95% O2/5% CO2. After stabilization of hearts beating in a nonworking mode for 15 minutes at a pressure of 60 mm Hg at 37°C, perfusion was switched to a working mode with a preload of 15 cm H2O and an afterload of 60 mm Hg. After 15 minutes of control perfusion with drug-free KHB, perfusate was then switched to KHB containing the indicated doses of salusin-β ([1] 0 mol/L [n=5]; [2] 3×10−8 mol/L [n=5]; and [3] 10−7 mol/L [n=5]) for 15 minutes. HR, SP, DP, MAP, and dP/dt were recorded simultaneously using the RM-6000, whereas aortic flow (AF) was monitored using the MFV-1100. Coronary flow (CF) was measured by collecting coronary sinus effluent at the end of stabilization and at 5 and 10 minutes after the start of salusin-β treatment. Cardiac output (CO) was calculated by adding AF to CF. Stroke works (SWs) were taken as a measure of relative contractility and calculated by (AF+CF)×MAP/HR. Stroke volume (SV) and SW values were normalized per gram of heart weight.

To determine the effect of salusin-β on CF in nonworking hearts, Langendorf heart perfusions were also performed using IPH-W2. Spontaneously beating hearts were perfused via the aorta in the nonworking heart mode at a constant pressure of 60 mm Hg with KHB at 37°C, and HR and dP/dt were monitored. After 15 minutes of equilibration, perfusate was replaced with KHB containing salusin-β or vehicle. CF was measured by collecting coronary sinus effluent before and at 5 and 10 minutes after the start of salusin-β. Each heart was excised from an intact rat that had not been used for any other experiment and was tested for just 1 concentration of the drug.

Data Analyses
The effects of treatment were compared with repeated measures of ANOVA where applicable, and differences before and after treatment were compared with post hoc Fisher protected least significant difference (PLSD). All data were presented as mean±SEM.

Results
Effects of Salusin-β on Intact Rats
The mean values of baseline cardiac functions of intact anesthetized rats measured before treatment were 97.8±4.0 mm Hg of MAP, 355±5 bpm of HR, and 29.0±1.3 mL/min of ABF. To determine whether hypotension and bradycardia induced by salusins are accompanied by changes of ABF, we simultaneously continuously monitored SP, DP, MAP, and ABF before and during intravenous infusion of salusins. Salusin-β dose-dependently (0.1 to 1.0 nmol/L per kg) caused a rapid temporary but marked decrease in MAP and HR (Figure 1; repeated measures of ANOVA; P<0.0001 for the entire observation period for Figure 1A through 1C and 1E through 1G experiments). These changes were accompanied by a concomitant and profound dose-dependent decrease in ABF (0.1 to 1.0 nmol/L per kg; Figure 2; repeated measures of ANOVA; P<0.0001 for Figure 2A through 2C). Larger doses of salusin-α were required to cause less hypotensive responses. Intravenous administration of 10 nmol/L per kg salusin-α decreased MAP 17.5±2.5% below pretreatment values, and this hypotensive response was accompanied by a 13.3±2.8% decrease in HR and a 13.5±3.9% decrease in ABF (repeated measures of ANOVA; P<0.0001 for MAP, HR, and ABF). In conscious rats, baseline MAP and HR were 120.1±9.7 mm Hg and 469±7 bpm (n=5), respectively. Salusin-β (1.0 nmol/L per kg) rapidly decreased MAP and HR, reaching nadir in 1.5 minutes (MAP −33.1±6.9% of baseline; HR −37.5±10.4% of baseline; repeated measures of ANOVA; P<0.0001 for MAP and HR), and returned to baseline levels after ~15 minutes. These results indicated that both salusins have negative inotropic and chronotropic effects in intact rats.

To assess the possible influence of the parasympathetic nervous system on cardiac function, the effects of atropine, a muscarinic receptor antagonist, on blood pressure and HR were investigated in anesthetized rats. Pretreatment with
atropine abrogated the hypotensive and bradycardiac responses to salusin-β (1.0 nmol/L per kg; Figure 3A and 3B). In contrast, pretreatment with propranolol (1.0 mg/kg), a β-adrenergic receptor antagonist, did not antagonize the effect of salusin-β (1.0 nmol/L per kg). Propranolol significantly decreased MAP and HR, showing further marked declines after salusin-β administration (data not shown).

The profound salusin-β–induced hypotension and bradycardia were not associated by any significant changes in systemic vascular resistance (SVR; Figure 3C; repeated measures of ANOVA; \(P = 0.960\)). Therefore, we measured LVEDP, dP/dt\(_{\text{max}}\), and dP/dt\(_{\text{min}}\) using a Millar catheter-based method to determine whether the salusin-β–induced decrease in ABF is associated with decreased ventricular performance. In 1 minute after intravenous infusion of 1.0 nmol/L per kg salusin-β, LVEDP increased by 37% from the baseline levels of 8.55±0.78 mm Hg (Figure 3D; repeated measures of ANOVA; \(P < 0.05\)), dP/dt\(_{\text{max}}\) decreased by 47% from the baseline levels of 4857±228 mm Hg per second (Figure 3E; repeated measures of ANOVA; \(P < 0.005\)), and dP/dt\(_{\text{min}}\) increased by 45% from the baseline value of -3699±282 mm Hg per second (Figure 3F; repeated measures of ANOVA; \(P < 0.005\)). Infusion of vehicle did not cause any changes in SP, DP, MAP, ABF, SVR, dP/dt\(_{\text{max}}\), or dP/dt\(_{\text{min}}\). These results indicated that salusin-β markedly decreases systolic and diastolic functions in rats.

**Effects of Salusin-β on Isolated Perfused Rat Hearts**

Rats used for isolated perfused heart preparation weighed 481.9±17.6 g and their excised hearts 1.95±0.04 g. Baseline functions of isolated working hearts measured after stabilization were 53.4±2.2 mL/min of AF, 13.5±0.5 mL/min of CF, 66.9±2.4 mL/min of CO, 92.6±7.3 mm Hg of SP, 54.0±5.2 mm Hg of DP, 66.8±5.3 mm Hg of MAP, and 2398±239 mm Hg per second of dP/dt\(_{\text{max}}\). Perfusion with salusin-β significantly decreased AF within 8 minutes (Figure 4A through 4C; repeated measures of ANOVA; \(P < 0.0001\) for A; \(P < 0.05\) for B). However, in contrast to the in vivo results, salusin-β did not cause any significant decrease in HR (Figure 4D through 4F). Salusin-β decreased MAP (Figure 5A through 5C; repeated measures of ANOVA; \(P < 0.0001\) for A; \(P < 0.05\) for B), and dP/dt\(_{\text{max}}\) in a dose-dependent manner (Figure 5D through 5F; repeated measures of ANOVA; \(P < 0.0001\) for D and E). Perfusion with salusin-β significantly decreased CO and AF at 5 and 10 minutes; this effect was more potent with 1.0 nmol/L than with 0.3 nmol/L (Figure 6A and 6B). Salusin-β did not significantly affect CF in isolated working
To confirm the negative constrictive effects of salusin-β on coronary vessels, we used a nonworking Langendorf setting to measure the effects of salusin-β on CF. Salusin-β did not affect CF (Figure 6C). Salusin-β (1.0 nmol/L) significantly reduced SV from a baseline value of 149.4 ± 5.8 μL/g heart weight to 126.8 ± 3.0 and 121.0 ± 3.0 μL/g heart weight at 5 and 10 minutes, respectively (P < 0.01 versus baseline). Perfusion with 1.0 nmol/L salusin-β also decreased SW by 22.3% at 10 minutes (Figure 7). These results, demonstrating negative inotropism and negative coronary vasoconstriction in isolated perfused rat hearts, indicate the direct myotropic effects of salusin-β.

Discussion
This study provides the first evidence that salusins, newly identified bioactive peptides, are cardiotropic factors that potently suppress cardiac performance. Many potent endogenous hypotensive factors, such as adrenomedullin and NO, reduce blood pressure via their vasorelaxant activity. Adrenomedullin, after exerting vasorelaxation, increases CO and HR in intact animals and, independently of its vasodilatory effect, exerts a positive inotropic effect in isolated perfused rat hearts. On the other hand, the involvement of NO or urotensin II in cardiac physiology is more complex and multifaceted. Natriuretic peptides induce diuresis and reduce myocardial contractility via the cGMP-dependent pathway. In contrast to these factors, the rapid and marked hypotension caused by salusins was always accompanied by profound bradycardia but not by any significant changes in SVR. Pretreatment of NO inhibitor to anesthetized rats did not abrogate salusin-induced hypotension and bradycardia. Furthermore, salusins did not show any vasodilatory effects on isolated thoracic aorta strips. Our present results using intact rats present further evidence that salusin-β also decreases ABF in a dose-dependent fashion concomitantly with the development of hypotension and bradycardia. Furthermore, salusins did not show any vasodilatory effects on isolated thoracic aorta strips. Pretreatment of NO inhibitor to anesthetized rats did not abrogate salusin-induced hypotension and bradycardia. Furthermore, salusins did not show any vasodilatory effects on isolated thoracic aorta strips.
intact rats, whereas washing out salusin-β for 15 minutes did not reverse its negative inotropic influence (data not shown). As demonstrated in the present study, the effect of salusin-β to reduce CO, SV, and SW in rats appears to be greater than other known factors, such as atrial natriuretic peptides or NO, whereas the magnitude of the decrease in SW after 1.0 nmol/L salusin-β reached 22.3% of baseline. Thus, our data lend strong credence to the notion that the hypotensive

Figure 6. Effects of perfusion with salusin-β on CO, aortic perfusate flow, and CF in isolated and perfused unpaced working rat hearts (A and B) and on CF in the isolated perfused nonworking hearts (C). Excised hearts receiving vehicle (saline) only did not show any significant change (data not shown). Data represent the mean±SEM of quintuplicate measurements relative to baseline levels. *P<0.05 vs baseline; **P<0.001 versus baseline.

Figure 5. Time course of the change in MAP (A and B) and aortic dP/dt max (D and E) after addition of salusin-β to left ventricular perfusate in isolated and perfused unpaced working rat hearts. Excised hearts receiving vehicle (saline) only did not show any significant change (C and F). Data represent the mean±SEM of quintuplicate measurements relative to mean baseline levels. Boxes indicate the time during which the indicated concentration of salusin-β or vehicle was perfused. ANOVA; P<0.0001 (A); P<0.01 (B); P=0.266 (C); P<0.0001 (D); P<0.0001 (E); and P=0.806 (F) for the entire observation period; post hoc Fisher’s PLSD; *P<0.05 vs baseline.
activities of salusin peptides are mediated in part by their direct myotropic effect as well. Together, our results indicate that rat heart is a major target for the hemodynamic functions of salusins.

We explored the reasons for the discrepancy between in vivo and in vitro effects of salusin-β. The present results show that the marked hypotensive and bradycardiac responses to salusin-β were completely blocked by parasympathetic withdrawal induced by atropine pretreatment. In contrast, infusion of propranolol, a β-adrenoreceptor antagonist, did not antagonize the effects. These results demonstrate an involvement of muscarinic receptors in salusin-β-induced hemodynamic actions. Stimulation of atrial M₂ receptors, a dominant form of muscarinic receptors in mammalian heart, is known to cause direct negative chronotropic and inotropic effects, whereas the stimulation of M₁ receptors in ventricles causes only indirect negative inotropic effects. Parasympathetic stimulation is also known to reduce systolic and diastolic functions of the heart. Thus, hemodynamic changes observed to be associated with salusin-β infusion are similar to a parasympathomimetic action. Further, we previously demonstrated a potential role for salusin-β as a neuropeptide.

Considering the evidence, we conclude that salusin-β may promote negative inotropism and chronotropism by, at least, facilitating vagal outflows to the heart. Lines of evidence show that the salusin-β–caused decrease in cardiac contractility was not attributable to coronary vasoconstriction. First, rapid hypotension and bradycardia induced by salusin-β were not associated with any significant myocardial ischemic changes on electrocardiograms. Second, salusin-β did not decrease CF in either isolated perfused working or nonworking hearts. Third, the onset of profound hypotension and bradycardia after intravenous salusin-β is too rapid to be ascribed to the result of coronary vasoconstriction. So far, we have been unable to find any vasoconstrictive effects of salusins in the isolated rat vascular strips we have tested. These data exclude the possibility that the salusin-β–induced negative inotropism is mediated by a drop in CF.

In summary, the newly identified salusin peptides act as cardiac depressors with negative inotropic and chronotropic actions in intact rats. The profound hypotensive and bradycardiac responses to salusin-β are abrogated by the muscarinic receptor antagonist atropine. In isolated perfused rat hearts, salusin-β decreases cardiac contractility without affecting HR. Thus, salusin-β not only suppresses cardiac performance via a direct myotropic effect, but also by a cholinergic mechanism.

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References

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