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INTEGRATIVE PHYSIOLOGY

Acute intracerebroventricular injection of chemerin-9 increases systemic blood pressure through activating sympathetic nerves via CMKLR1 in brain

Atsunori Yamamoto[1] · Kengo Matsumoto[1] · Kiko Hori[1] · Satoshi Kameshima[2] · Naoko Yamaguchi[3] · Shoshiro Okada[3] · Muneyoshi Okada[1] · Hideyuki Yamawaki[1]

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Abstract

Chemerin is an adipocytokine involved in inflammation and lipid metabolism via G protein-coupled receptor, chemokine-like receptor (CMKLR)1. Since the important nuclei regulating pressure (BP) exist in the brain, we examined the effects of acute intracerebroventricular (i.c.v.) injection of chemerin-9 on systemic BP and explored underlying mechanisms. We examined the effects of acute i.c.v. injection of chemerin-9 (10 nmol/head) on systemic BP by a carotid cannulation method in the control or CMKLR1 small interfering (si) RNA-treated Wistar rats (0.04 nmol, 3 days, i.c.v.). We examined protein expression of CMKLR1 around brain ventricles by Western blotting. We examined the effects of acute i.c.v. injection of chemerin-9 on serum adrenaline by a high performance liquid chromatography. In the control siRNA-treated rats, chemerin-9 significantly increased mean BP, which reached a peak at 2 to 4 min after injection. On the other hand, in the CMKLR1 siRNA-treated rats, chemerin-9 did not affect the mean BP. Protein expression of CMKLR1 specifically in subfornical organ (SFO) and paraventricular nucleus (PVN) from the CMKLR1 siRNA-treated rats decreased compared with the control siRNA-treated rats. In the control siRNA-treated rats, chemerin-9 increased serum adrenaline level. On the other hand, in the CMKLR1 siRNA-treated rats, chemerin-9 did not affect the serum adrenaline level. Further, pretreatment with prazosin, an α-adrenaline receptor blocker, significantly prevented the pressor responses induced by chemerin-9. In summary, we for the first time demonstrated that chemerin-9 stimulates the sympathetic nerves via CMKLR1 perhaps expressed in SFO and PVN, which leads to an increase in systemic BP.

Keywords Adipocytokine · Blood pressure · Brain · Intracerebroventricular injection · Sympathetic nerve

Introduction

The role of adipose tissue had long been recognized as a storage of extra energy [47]. However, it is recently recognized as an endocrine organ secreting various cytokines (adipocytokine) [47]. Chemerin, one of the adipocytokines, is a secretory protein encoded by retinoic acid receptor responder protein (*RARRE*) *2*/tazarotene-induced gene (*TIG*) *2* [39]. After secreted in an immature form as prochemerin (18 kD), its carboxyl-terminal is cleaved by serine proteases, and the mature form of active chemerin (16 kD) is formed [48]. Chemerin is highly expressed in the white adipose tissue (WAT) and is also expressed in the liver, lung, and skin [10, 15]. Initially, the function of chemerin was identified as a chemoattractant for dendritic cells and macrophages; however, it has been demonstrated that chemerin also regulates the adipocyte differentiation and lipid metabolism [38, 45]. Chemerin is useful as a biomarker for obesity because its

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✉ Hideyuki Yamawaki
yamawaki@vmas.kitasato-u.ac.jp

[1] Laboratory of Veterinary Pharmacology, School of Veterinary Medicine, Kitasato University, Higashi 23 Bancho 35-1, Towada, Aomori 034-8628, Japan

[2] Small Animal Internal Medicine, School of Veterinary Medicine, Kitasato University, Higashi 23 Bancho 35-1, Towada, Aomori 034-8628, Japan

[3] Department of Pharmacology, School of Medicine, Aichi Medical University, Yazakokarimata 1-1, Nagakute, Aichi 480-1195, Japan

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concentration in blood correlates positively with the body mass index (BMI) [1].

Chemerin binds to the receptors including chemokine-like receptor (CMKLR)1, chemokine (CC motif) receptor-like (CCRL)2, and G protein-coupled receptor (GPR)1 [39]. CMKLR1 is expressed in the antigen-presenting cells (e.g., dendritic cells and macrophages), WAT, lung, heart, placenta, vascular smooth muscle cells (VSMCs), and vascular endothelial cells [10, 15, 22, 26]. Moreover, CMKLR1 is expressed in the central nervous systems such as hippocampus, cerebellum, and ependymal cells [11, 14]. We have previously demonstrated that chemerin stimulated the proliferation and migration of rat VSMCs via CMKLR1 and that systolic blood pressure (BP) of mouse was increased by the long-term intraperitoneal administration with chemerin [28]. Further, it has been revealed that chemerin amplified contraction of rat isolated superior mesenteric artery via CMKLR1 expressed in the sympathetic nerves in perivascular adipose tissue [7]. Thus, it is suggested that chemerin/CMKLR1 in the peripheral tissues is involved in the pressor responses as well as in the etiology of systemic hypertension. On the other hand, CCRL2 is a decoy receptor that does not induce any signal transduction [16]. GPR1 is expressed in the skeletal muscle and WAT [40]. It is reported that GPR1 is related to an infection of human immunodeficiency virus and also to a glucose homeostasis [24].

The cardiovascular center that participates in the BP control by regulating a stroke volume, vascular contractility, and secretion of hormones including vasopressin exists in the brain [18, 23]. It locates in the rostral ventrolateral medulla (RVLM) in the medulla oblongata [18, 27]. Subfornical organ (SFO) and paraventricular nucleus (PVN) are other important nuclei that also regulate BP [27]. The nervous conductions from these nuclei are transmitted to RVLM, which affects the activity of peripheral organs, including heart, vasculature, and kidney, via intermediolateral nucleus (IML) in the spinal cord [18, 27]. On the other hand, a blood brain barrier (BBB) limits the exchange of blood and substances between the brain and peripheral organs. However, SFO, organum vasculosum lamina terminalis (OVLT), and area postrema are lacking BBB due to the fenestrated capillaries [19]. SFO regulates BP through monitoring blood hormone and Na^+ levels [27]. In the central nervous systems, the balance of inhibitory neurotransmitters (e.g., γ-aminobutyric acid (GABA) and glycine) and excitatory neurotransmitters (e.g., glutamic acid, angiotensin II, and acetylcholine) is important for regulating BP [17, 18, 20, 27]. It has been reported that the balance of these neurotransmitters is disrupted during the development of systemic hypertension [27]. Thus, it is recognized that the BP control mechanisms not only by the periphery organs but also by the central nervous systems are important for the pathogenesis of systemic hypertension.

Recently, some of the roles for chemerin in the central nervous systems are reported. For example, a microinjection of chemerin to hypothalamic arcuate nucleus increased the expression of anorexigenic and orexigenic genes [2]. In addition, chemerin protected the nerves from an inflammation induced by the germinal matrix hemorrhage [49]. Taken together, it is suggested that chemerin has multiple roles in the brain. Thus, we hypothesized that the intracerebroventricular (i.c.v.) injection of chemerin affects systemic BP. In the present study, in order to test this hypothesis, we examined the effects of the acute i.c.v. injection of chemerin-9 on systemic BP and explored underlying mechanisms.

Materials and methods

Materials

Reagent sources were as follows; recombinant chemerin-9 (RP20248, Genscript, Piscataway, NJ, USA), [Arg^8]-Vasopressin (4085-v, PEPTIDE INSTITUTE, Osaka, Japan), and prazosin (P0938, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan).

Antibody sources were as follows; anti-ChemR23 (CMKLR1; sc-398769, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-glyceraldehude-3-phosphate dehydrogenase (GAPDH; 016–25,523, Wako, Osaka, Japan), and anti-mouse IgG horseradish peroxidase (HRP)-linked whole antibody (Cell Signaling Technology, Beverly, MA, USA).

Animal

Male Wistar rats (9–11 weeks old) were obtained from CLEA (Tokyo, Japan). Animal study was approved by the ethical committee of School of Veterinary Medicine, the Kitasato University, and performed in conformity with the institutional guideline of the Kitasato University.

i.c.v. injection

Wistar rats under an isoflurane anesthesia (induction 5%, maintenance 2–3%; Wako) and a buprenorphine analgesia (50 μg/kg, s.c.; Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan) were placed in a stereotaxic apparatus (NARISHIGE, Tokyo, Japan), and the skull was exposed. The coordinates of bregma and lambda were checked, and the head was adjusted to be horizon. The skull was drilled for i.c.v. injection at the position of 0.8 mm posterior and 1.5 mm right side from the bregma. An injection cannula (outer diameter of 0.3 mm) connected to a micromanipulator was took down by 4.5 mm from the surface of skull [43]. Chemerin-9 (10 nmol/2 μl/head), artificial cerebro-spinal fluid (aCSF; 2 μl/head), a vehicle, or vasopressin (0.2 nmol/2 μl/head), a positive control was

injected (rate; 1 μl/min) every 20 min by a microsyringe (ITO CORPORATION, Shizuoka, Japan). At the end of experiment, an Evans blue solution (2 μl/head) was injected in order to check if the i.c.v. injection was succeeded.

Measurement of BP

Systemic BP of Wistar rats was measured [25] under an isoflurane anesthesia (induction 5%, maintenance 2–3%) and a buprenorphine analgesia (50 μg/kg, s.c). The catheters filled with a 1% heparin (AY PHARMACEUTICSLS, Tokyo, Japan)-saline solution were inserted into carotid artery and femoral vein. The catheter inserted into carotid artery was connected to an MLT0670BP transducer (AD Instruments, Colorado Springs, CO, USA), ML117BP Amp (AD Instruments), and ML825 PowerLab 2/25 (AD Instruments), and the BP was invasively measured and recoded in a computer.

Small interfering (si) RNA injection

Wistar rats under an isoflurane anesthesia (induction 5%, maintenance 2–3%) and a buprenorphine analgesia (50 μg/kg, s.c.) were placed in a stereotaxic apparatus. CMKLR1 siRNA (CCAUCGUCUUCAAGUUGCA-dTdT) or control-nonsilencing (cont) siRNA (0.04 nmol; Nippon Gene Material, Toyama, Japan) mixed with an in vivo jet-PEI reagent (Polyplus transfection, Illkirch-Graffenstanden, France) at a nitrogen/phosphorus ratio of ten was diluted to 4.3 μl with 10% glucose [21]. The i.c.v. injection of siRNA was achieved by the methods as described above.

Isolation of brain tissues

Wistar rats were euthanized by a deep isoflurane anesthesia (5%). The whole brain tissues were isolated. In order to separate SFO, PVN, and lateral ventricle, they were cut into two slices on ice. The slices were isolated at the position of approximately 0.5–1.5 mm posterior and 1.5–2.5 mm posterior from bregma (thickness: approximately 1 mm). Then, SFO at the former slice and PVN at the latter slice were isolated. Lateral ventricle was isolated from both slices. The remaining regions neighboring the third ventricle of brain tissues were isolated as the other regions (Fig. 1) [36].

Western blotting

Western blotting was performed as described previously [35]. Brain tissues were rapidly frozen by a liquid nitrogen and mashed. Protein lysates were obtained from the mashed brain tissues with a lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophoshate, 1 mM Na_3Vo_4, 1 μg/ml leupeptin (Cell Signaling Technology)) containing a 0.1% protease inhibitor mixture (Nacalai tesque, Kyoto, Japan). Protein concentration was determined by a bicinchoninic acid method (Pierce, Rockford, IL, USA). Equal amount of protein (10 μg) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%, 80–120 V, 1.5–2 h) and transferred to a nitrocellulose membrane (400 mV, 1.5 h; Pall Corporation, Ann, Arbor, MI, USA). After being blocked with 0.5% skim milk, the membranes were incubated with a primary antibody (1:500 dilution in Tris-buffered saline with Tween 20; TBS-T) at 4 °C overnight. The membranes were incubated with HRP-conjugated secondary antibody (1:10,000 dilution in TBS-T, 45 min) and treated with chemiluminescent reagent (EZ-ECL system; Biological Industries Kibbutz, Beit-Haemek, Israel) for 3 min, and the bands on membrane were visualized by a Light-capture apparatus (AE-6971/2 c/FC; ATTO, Tokyo, Japan). For confirming equal loading of protein, the expression of GAPDH was examined. The visualized bands were analyzed using a CS analyzer 3.0 software (ATTO).

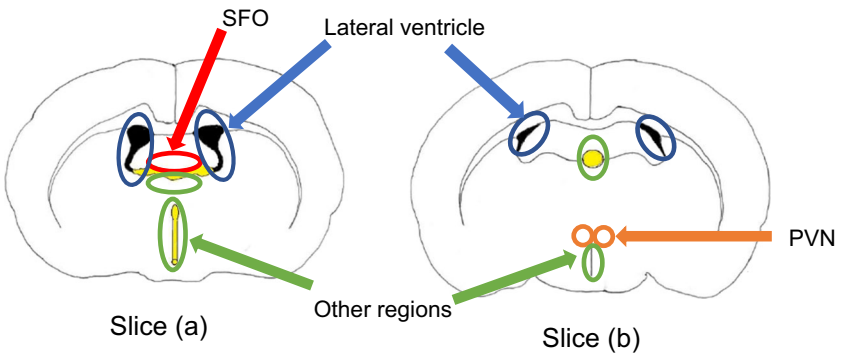

Fig. 1 The slices of isolated brain tissues at the position of approximately (a) 0.5–1.5 mm posterior and (b) 1.5–2.5 mm posterior from bregma were isolated (thickness: approximately 1 mm). Subfornical organ (SFO, red circle) at slice (a) and paraventricular nucleus (PVN, orange circle) at slice (b) were isolated. Lateral ventricle (blue circle) was isolated from both slice (a) and (b). The remaining regions neighboring the third ventricle of brain tissues were isolated as the other regions (green circle). Yellow zone: the third ventricle

Measurement of serum adrenaline

We used rats treated with CMKLR1 siRNA or cont siRNA (3 days, i.c.v.). The i.c.v. injection of siRNA was achieved by the methods as described above. The catheters filled with a 1% heparin-saline solution were inserted into carotid artery for measurement BP, femoral artery for blood collection, and femoral vein for transfusion under an isoflurane anesthesia (induction 5%, maintenance 2–3%) and a buprenorphine analgesia (50 μg/kg, s.c.). Systemic BP was monitored by the method described above. The transfusion for a maintenance of a circulating blood volume was achieved by a MINIPULS 3 Peristaltic Pump (GILSON, Middleton, WI, USA). Then, the i.c.v. injection was performed by the method described above. Chemerin-9 (10 nmol/2 μl/head), aCSF (2 μl/head), or vasopressin (0.2 nmol/2 μl/head) was injected. Arterial blood samples (450 μl) were collected from femoral artery before and 2.5 min after i.c.v. injection. To obtain serum samples, the arterial blood samples were centrifuged (3000 rpm, 10 min, 4 °C). The serum samples, an activated alumina, 3,4-dehydroxybenzylamine (DHBA; an internal standard), and 0.5 M Tris Buffer (pH 8.6) were mixed, shaken for 10 min, and aspirated off the supernatant. The alumina was washed three times with ice-cold double deionized water. After eluting adrenaline adsorbed onto the alumina with 4% acetic acid solution, the samples were used for high performance liquid chromatography (HPLC) method. Mobile phase (85% 0.1 M NaH_2PO_4-Na_2HPO_4 buffer (pH 6.0), 15% methanol, 750 mg/l 1-octane sulfate sodium, and 50 mg/l EDTA dihydrate) was flowed at a rate of 0.18 ml/min and the signal was detected by an electrochemical detector (450 mV; ECD-300; Eicom, Kyoto, Japan). The concentration of serum adrenaline was determined using the peak height ratio relative to that of DHBA. The retention times were as follows; adrenaline (7.8 min) and DHBA (14.2 min). The HPLC column used was Eicompak CA-50SD (Eicom) [34].

Statistics

Data were shown as mean ± standard error of the mean (SEM). Statistical evaluations were done with Student's *t* test (Fig. 3), one-way ANOVA followed by Bonferroni's post hoc test (Fig. 4), or two-way ANOVA followed by Bonferroni's post hoc test (Fig. 2 c, d, Fig. 5 b). Results were considered significant when *P* value was less than 0.05.

Results

Effects of CMKLR1 siRNA on pressor responses induced by acute i.c.v. injection of chemerin-9

We first examined the effects of CMKLR1 siRNA on base line systolic BP. Systolic BP was not different between cont

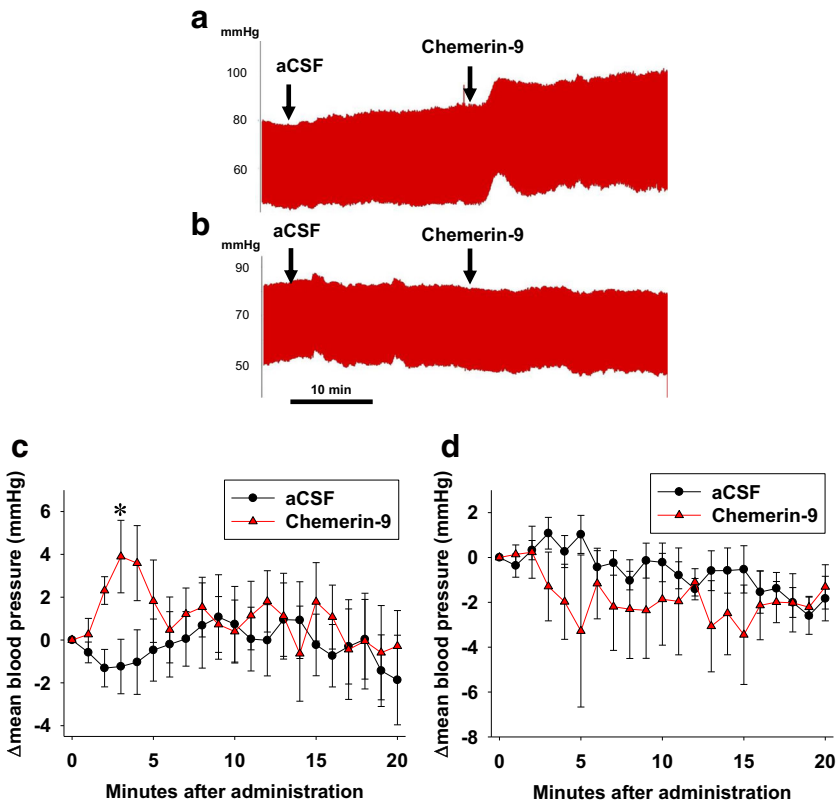

Fig. 2 Effects of chemokine-like receptor (CMKLR)1 small interfering (si) RNA on pressor responses induced by acute intracerebroventricular (i.c.v.) injection of chemerin-9. Control-nonsilencing (cont) siRNA (0.04 nmol/head, i.c.v.) (**a**, **c**) or CMKLR1 siRNA (0.04 nmol/head, i.c.v.) (**b**, **d**) was administered to male Wistar rats (9–11 weeks old). Three days later, blood pressure (BP) was measured under an isoflurane anesthesia by a carotid cannulation method. Artificial cerebro-spinal fluid (aCSF; 2 μl/head, i.c.v.), a vehicle or chemerin-9 (10 nmol/2 μl/head, i.c.v.) was administered ($n = 5$). Representative recordings (**a**, **b**) were shown. Quantitative results (**c**, **d**) were shown as mean ± standard error of the mean (SEM) ($n = 5$). *$P < 0.05$ vs. aCSF

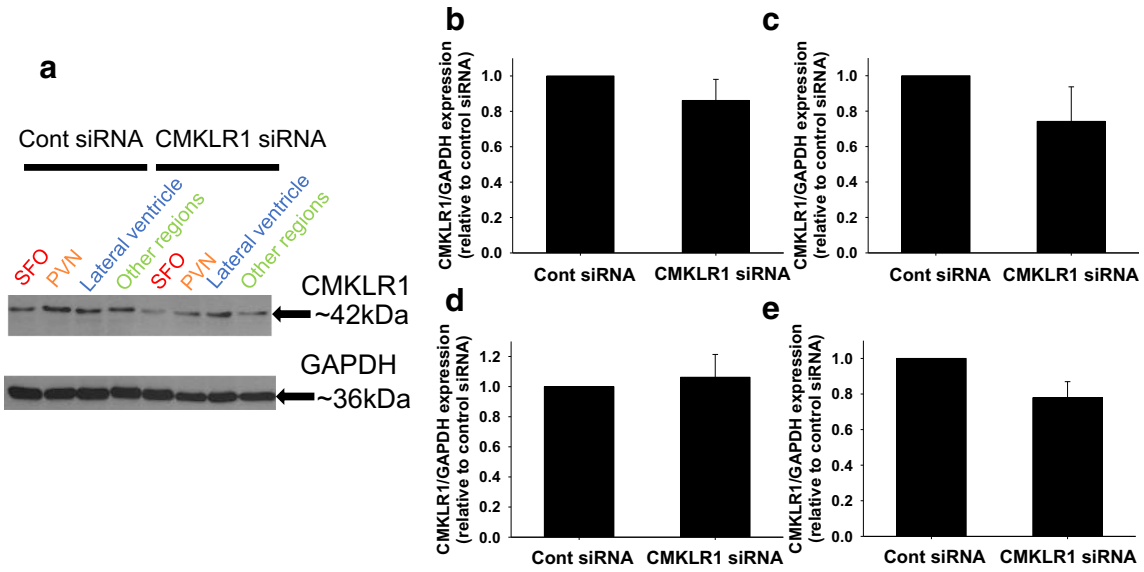

Fig. 3 Effects of CMKLR1 siRNA on expression of CMKLR1 protein around brain ventricles. Cont siRNA (0.04 nmol/head, i.c.v.) or CMKLR1 siRNA (0.04 nmol/head, i.c.v.) was administered to male Wistar rats (9–11 weeks old). Three days later, expression of CMKLR1 protein in SFO, PVN, lateral ventricle, and other regions around brain ventricles in isolated brain tissues was determined by Western blotting. Representative blots for CMKLR1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were shown (**a**). The normalized CMKLR1 expression relative to GAPDH expression in SFO (**b**), PVN (**c**), lateral ventricle (**d**), and other regions (**e**) was shown as relative to cont siRNA. Data were shown as mean ± SEM ($n = 4$)

siRNA- and CMKLR1 siRNA-treated rats (systolic BP: pre; 118.0 ± 1.9 mmHg, 1 day; 113.0 ± 2.0 mmHg, 2 day; 114.7 ± 2.9 mmHg, 3 day; 116.3 ± 1.7 mmHg in Cont siRNA, pre; 122.8 ± 2.1 mmHg, 1 day; 115.1 ± 1.1 mmHg, 2 day; 111.4 ± 1.3 mmHg, 3 day; 111.8 ± 2.4 mmHg in CMKLR1 siRNA, $n = 4$, Supplementary Fig. 1). We next examined the effects of acute i.c.v. injection of chemerin-9 (10 nmol/2 μl/head) on systemic BP, and then examined the effects of CMKLR1 siRNA on the responses. In the rats treated with cont siRNA (0.04 nmol, 3 days, i.c.v.), acute i.c.v. injection of chemerin-9 significantly increased mean BP, which reached a peak at 2 to 4 min after injection ($n = 5$, $P < 0.05$ at 3 min, Fig. 2a, c). This pressor response returned to base line within 10 min after injection. On the other hand, in the rats treated with CMKLR1 siRNA (0.04 nmol, 3 days, i.c.v.), acute i.c.v. injection of chemerin-9 did not affect the mean BP ($n = 5$, Fig. 2b, d).

Effects of CMKLR1 siRNA on expression of CMKLR1 protein in brain tissues

We next examined the effects of CMKLR1 siRNA (0.04 nmol, 3 days, i.c.v.) on expression of CMKLR1 protein around brain ventricles by Western blotting. Expression of CMKLR1 protein in the SFO, PVN, and other regions around brain ventricles from CMKLR1 siRNA-treated rats was decreased compared with cont siRNA (0.04 nmol, 3 days, i.c.v.)-treated rats ($n = 4$, Fig. 3b, c, e). On the other hand, expression of CMKLR1 protein in the lateral ventricle was not different between CMKLR siRNA- and cont siRNA-treated rats ($n = 4$, Fig. 3d). It is speculated that this was due to the relatively

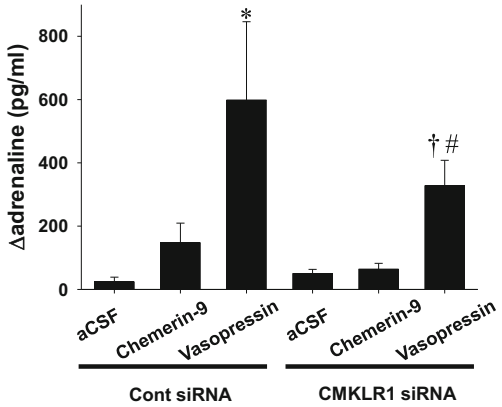

Fig. 4 Effects of acute i.c.v. injection of chemerin-9 on concentration of serum adrenaline. Cont siRNA or CMKLR1 siRNA (0.04 nmol/head, i.c.v.) was administered to male Wistar rats (9–11-week-old). Three days later, chemerin-9 (10 nmol/2 μl/head, i.c.v.), aCSF (2 μl/head, i.c.v.), or vasopressin (0.2 nmol/2 μl/head, i.c.v.), a positive control was administered to the rats under an isoflurane anesthesia. Arterial blood samples were collected from femoral artery before and 2.5 min after administration. Serum adrenaline was extracted by an activated alumina. Concentration of serum adrenaline was measured by a high performance liquid chromatography method. The change (Δ) of serum adrenaline was shown as mean ± SEM (Cont siRNA: $n = 4$, CMKLR1 siRNA: $n = 5$). *P < 0.05 vs. aCSF (in cont siRNA), †$P < 0.05$ vs. aCSF (in CMKLR1siRNA), #$P < 0.05$ vs. chemerin-9 (in CMKLR1 siRNA)

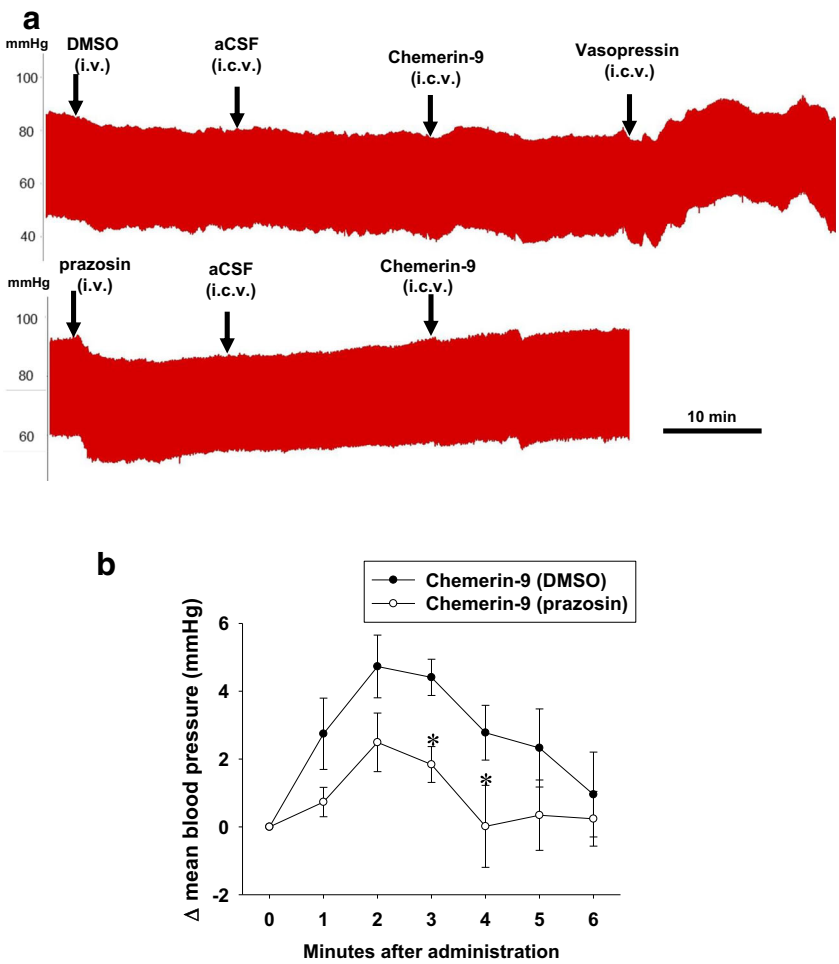

Fig. 5 Effects of prazosin on the pressor responses induced by acute i.c.v. injection of chemerin-9. BP of male Wistar rats (9–11 weeks old) under an isoflurane anesthesia was measured by a carotid cannulation method. After administration of dimethyl sulfoxide (DMSO, vehicle) or prazosin (α_1 blocker, 5 μg/kg) through the catheter inserted into femoral vein, aCSF (2 μl/head, i.c.v.) or chemerin-9 (10 nmol/2 μl/head, i.c.v.) was applied. Vasopressin (0.2 nmol/2 μl/head, i.c.v.) was applied as a positive control for pressor responses. Representative recordings (**a**) were shown. Quantitative results (**b**) were shown as mean ± SEM ($n = 5$). Chemerin-9 (DMSO): chemerin-9 application after DMSO, chemerin-9 (prazosin): chemerin-9 application after prazosin. *$P < 0.05$ vs. chemerin-9 (DMSO)

lower expression of CMKLR1 in this region (Supplementary Fig. 2).

Effects of acute i.c.v. injection of chemerin-9 on concentration of serum adrenaline

In order to explore the effects of acute i.c.v. injection of chemerin-9 on sympathetic nervous activity, we measured concentration of serum adrenaline before and 2.5 min after i.c.v. injection of chemerin-9 (10 nmol/2 μl/head) by an HPLC method. In the rats treated with cont siRNA (0.04 nmol, 3 days, i.c.v.), acute i.c.v. injection of chemerin-9 increased concentration of serum adrenaline (Δadrenaline 147.7 ± 61.8 pg/ml, chemerin-9 vs. 24.1 ± 14.6 pg/ml, aCSF, $n = 4$, Fig. 4). On the other hand, in the rats treated with CMKLR1 siRNA (0.04 nmol, 3 days, i.c.v.), chemerin-9 did not affect the concentration of serum adrenaline (Δadrenaline: 64.2 ± 18.1 pg/ml, chemerin-9 vs. 50.3 ± 13.2 pg/ml, aCSF, $n = 5$, Fig. 4). We confirmed that vasopressin significantly increased the concentration of serum adrenaline (Δadrenaline 598.5 ± 247.6 pg/ml in cont siRNA, $n = 4$, $P < 0.05$; 327.8 ± 80.3 pg/ml in CMKLR1 siRNA, $n = 5$, $P < 0.05$, Fig. 4).

Effects of prazosin on the pressor responses induced by acute i.c.v. injection of chemerin-9

In order to check if the pressor responses induced by acute i.c.v. injection of chemerin-9 was evoked by activating sympathetic nerves, we examined the effects of pretreatment with an α_1 blocker, prazosin (5 μg/kg). After pretreatment with DMSO (vehicle), acute i.c.v. injection of chemerin-9 (10 nmol/2 μl/head) increased mean BP (Fig. 5a, b). Pretreatment with prazosin significantly prevented it ($n = 5$, $P < 0.05$ at 3 and 4 min, Fig. 5a, b).

Discussion

In the present study, we examined the effects of acute i.c.v. injection of chemerin-9 on systemic BP in rats, and the major findings are as follows: (1) In the cont siRNA-treated rats, acute i.c.v. injection of chemerin-9 significantly increased mean BP, which reached a peak at 2 to 4 min after injection (Fig. 2a, c). On the other hand, in the CMKLR1 siRNA-treated rats, acute i.c.v. injection of chemerin-9 did not affect the mean BP (Fig. 2b, d). (2) Expression of CMKLR1 protein

in the SFO, PVN, and other regions from the CMKLR1 siRNA-treated rats decreased compared with the cont siRNA-treated rats (Fig. 3b, c, e). (3) In the cont siRNA-treated rats, acute i.c.v. injection of chemerin-9 increased serum adrenaline level. On the other hand, in the CMKLR1 siRNA-treated rats, chemerin-9 did not affect it (Fig. 4). (4) Pretreatment with prazosin significantly prevented the pressor responses induced by acute i.c.v. injection of chemerin-9 (Fig. 5). Collectively, we for the first time demonstrated that chemerin-9 stimulates the sympathetic nerves via CMKLR1 expressed in the brain including SFO and PVN, which leads to an increase in systemic BP.

In the present study, we for the first time demonstrated that acute i.c.v. injection of chemerin-9 increased rat systemic BP, which was abolished in the rats treated with CMKLR1 siRNA (Fig. 2). The nuclei that sense various stimulations and regulate BP exist in the brain [18, 27]. In the present study, however, we cannot identify the particular nuclei and/or cells that chemerin-9 affects in the brain. The SFO and glial cells (e.g., astrocytes and ependymal cells) around OVLT sense Na^+ concentration in the blood or cerebrospinal fluid [19, 32]. Voltage-gated sodium channel is expressed in the glial cells, which mediates a Na^+ influx. When H^+ is released from the cells due to the increase of Na^+ concentration in the glial cells, the OVLT neurons projecting to PVN are activated via acid-sensing ion channel, which leads to an increase in systemic BP [32]. Thus, it is proposed that the pressor response by acute i.c.v. injection of chemerin-9 was mediated via CMKLR1 expressed in the SFO or ependymal cells [14] around OVLT. On the other hand, chemerin activates L-type calcium channel and induces a calcium influx trough CMKLR1 in the VSMCs via Gi protein-dependent manner [9]. Then it might be possible that chemerin also mediates an increase in intracellular calcium though CMKLR1 expressed in neurons. An elevation of intracellular calcium concentration in the neurons induces a current via a-amino-3-hydroxy-5-methyl-4-isoxazoke propionic acid (AMPA) receptor through the activation of calcium and calmodulin-dependent kinase (CaMKII) [31]. The current occupies the majority of the excitatory postsynaptic current in the glutamatergic neuron [5]. Thus, it is suggested that chemerin/CMKLR1 affects neurons and induces the pressor response via the elevation of AMPA receptor current through the activation of CaMKII.

PVN is constituted by the two regions, namely parvocellular and magnocellular parts [30]. Magnocellular parts project to pituitary gland [30]. On the other hand, parvocellular parts innervate IML and RVLM [30]. PVN regulates blood pressure via controlling sympathetic nerve activity through these nuclei [23]. It was reported that approximately 40% of PVN neurons (esp. in parvocellular parts) projecting to brainstem express vasopressin mRNA and V1a receptor, a vasopressin receptor [12, 30]. Thus, it is suggested that these neurons are activated by autocrine/paracrine effects of vasopressin. Additionally, it was reported that i.c.v. injection of vasopressin increased systemic blood pressure, sympathetic nerve activity, and plasma adrenaline and noradrenaline [8, 13, 33]. In the present study, we also demonstrated that acute i.c.v. injection of chemerin-9 increased systemic blood pressure and serum adrenaline. Thus, it is presumed that i.c.v. injection of chemerin-9 activates sympathetic nerves and induces pressor responses via the pathway similar to vasopressin presumably through IML and RVLM.

Chemerin is a hormone (adipocytokine) produced mainly in the adipose tissue and affects the functions of systemic organs [3]. So far, it is not revealed whether circulating chemerin is transferred to central nerves through BBB. However, it might be possible that chemerin produced in the periphery organs (for example, adipose tissue) induces the pressor response through the nuclei projecting to RVLM (cardiovascular center) including SFO and OVLT which lack BBB. In addition, since the expression of chemerin mRNA is confirmed in the brain [14], it is suggested that chemerin produced in the brain may also mediate the pressor response through CMKLR1.

Human chemerin is activated through the cleavage of carboxyl-terminal of prochemerin (hChem163) by various serine proteases [10]. The biological activity of active chemerin is different dependent on its length. For example, hChem157 (137 amino acid residues) has the highest activity and hChem156 (136 amino acid residues) also has a higher activity [3]. On the other hand, hChem158 (138 residues) and hChem155 (135 residues) have a lower activity [3]. In the present study, we used chemerin-9, a synthetic peptide constituted by the active sites of hChem157 (amino acids 149–157). The EC_{50} of hChem157 through CMKLR1 binding is approximately 4.5 nM, while that of chemerin-9 is approximately 26.4 nM, suggesting that the activity of chemerin-9 is approximately 40-fold lower than full length hChem157 [42, 46]. Considering that the amount of cerebrospinal fluid is approximately 280 μl per rat (12-week-old) [6], the concentration of injected chemerin-9 (10 nmol) is estimated to be 36 nmol/ml in the present study. Then, the concentration of chemerin-9 is 1700-fold higher than that in the blood, because the concentration of full length chemerin (hChem157) in the blood is reported to be approximately 350 ng/ml (0.22 nmol/ml) [41]. However, since the activity of chemerin-9 is lower than that of full length chemerin (40-fold lower), it is suggested that the concentration of chemerin-9 used in this study is not so high in examining BP control mechanisms in central nervous systems (particularly in the local pathological conditions).

The concentration of chemerin in the blood of healthy subjects is approximately 190 ng/ml, while it increases to approximately 350 ng/ml in the subjects with obesity [41]. It is reported that the concentration of chemerin in the blood and the expression of CMKLR1 in the blood vessels were increased in the obese rats, which lead to an increase in contractility of the

isolated blood vessels compared with normal rats [44]. Accordingly, it is suggested that the increased chemerin/CMKLR1 in the obesity is related to the development of systemic hypertension [44]. In the present study, we have revealed that chemerin/CMKLR1 induces the pressor response through the action on the central nervous systems. Thus, it is assumed that, in essential hypertension, chemerin-induced pressor response was mediated not only through the actions on the periphery organs (e.g. blood vessels) but also on the central nervous systems. Leptin and adiponectin are adipocytokines secreted from WAT [4, 29]. It is reported that these adipocytokines are related to a regulation of appetite and body weight through the central nervous system [37]. It is then presumed that chemerin produced in periphery organs (e.g. WAT) also affects the central nervous system. Thus, although the pressor responses induced by acute i.c.v. injection of chemerin-9 was transient in the present study, it is assumed that the high concentration of blood chemerin secreted from WAT continuously affects central nervous system and may contribute to the increase of BP in obesity subjects.

In conclusion, we for the first time revealed that chemerin activates the periphery sympathetic nerves perhaps acting on the CMKLR1 in the brain including SFO and PVN projecting to cardiovascular center (RVLM), which leads to the pressor response in the circulation. In the future, we have to identify the nuclei and/or cells that chemerin-9 affects and also to explore detailed mechanisms. Consequentially, it is expected to contribute to drug discovery targeting chemerin/CMKLR1 in the central nervous systems in essential hypertension.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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