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Pharmacology

Chemerin fragments show different effects on systemic blood pressure dependent on carboxyl-terminal cleavage site

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ABSTRACT. Chemerin is an adipocytokine whose concentration in blood correlates positively with blood pressure (BP). We have recently revealed that acute intracerebroventricular (i.c.v.) injection of chemerin-9, an active fragment of human chemerin, increased systemic BP in normal Wistar rats, suggesting that chemerin is involved in the central nervous control of peripheral BP. After secreted as an inactive form as prochemerin, a mature form of active chemerin is produced through the cleavage of its carboxyl (C)-terminus by proteases. Although the activity of cleaved products of chemerin has been examined *in vitro*, *in vivo* effects remained to be elusive. In order to explore them, we performed acute i.c.v. injection of mouse chemerin-9 (mChemerin-9; 148F-156S), mouse chemerin-8 (mChemerin-8; 148F-155F), and mouse chemerin-7 (mChemerin-7; 148F-154A) into Wistar rats, and examined the effects on systemic BP. After chemerin fragment (1–30 nmol/head, i.c.v.) was cumulatively administered, systemic BP was measured by a cannulation method under an isoflurane anesthesia. mChemerin-9 but not mChemerin-8 and -7 induced a pressor response, which was concentration-dependent. In conclusion, we for the first time demonstrated that mChemerin-9 that corresponds to the C-terminal nine amino acids of active mouse chemerin156S increased systemic BP in rats, and also that chemerin fragments showed different effects on systemic BP dependent on how their C-terminus was cleaved.

KEYWORDS: blood pressure, carboxyl-terminus, chemerin, intracerebroventricular injection

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Chemerin is a secretory protein encoded by *retinoic acid receptor responder protein* (*RARRES*)*2/ tazarotene-induced gene* (*TIG*)*2* gene [12]. Chemerin is highly expressed in white adipose tissue, while it is also expressed in immune cells, liver, and lung [4, 5]. While chemerin was initially identified as a chemoattractant, it also mediates a differentiation of adipocytes [11, 15].

Chemokine-like receptor 1 (CMKLR1), chemeokine (C-C motif) receptor-like 2, and G protein-coupled receptor 1 have been identified as receptors for chemerin [12]. CMKLR1 mainly mediates the previously identified functions of chemerin, including chemotaxis and adipocyte differentiation [11, 15]. Additionally, we have previously revealed that mouse chemerin156S stimulated the proliferation and migration via CMKLR1 in vascular smooth muscle cells, which resulted in increased systemic blood pressure (BP) in mice [8].

After secreted as an inactive form as prochemerin, a mature form of active chemerin is produced through the cleavage of its carboxyl (C)-terminus by serine proteases or cysteine proteases [18]. It is demonstrated that the activity of chemerin was different dependent on the sites of cleavage [16]. In humans and rats, chemerin157S and 156F are active, while the activity of other cleaved products is low [1]. In addition, it was reported that the activity of mouse chemerin156S and chemerin155F, which are homologous to human chemerin157S (hChemerin157S) and human chemerin156F (hChemerin156F), respectively, was high [19]. Taken together, it is suggested that the activity of chemerin is dependent on the C-terminal sequence. Nonetheless, since the activity of cleaved products of chemerin including hChemerin157S, 156F, or 155A has been examined *in vitro* (e.g. the activity of hChemerin157S is the highest for elevating intracellular Ca^{2+} level), *in vivo* effects remained to be elusive so far [1]. Human chemerin-9 (hChemerin-9) is a frequently used C-terminal active fragment of hChemerin157S [3, 10]. It also remains to be determined whether the species differences in active chemerin fragment may affect the biologic activity.

The cardiovascular center and several crucial nuclei that control systemic BP exist in the brain [6]. Specifically, through integrating information from peripheral tissues, paraventricular nucleus participates in control of BP via regulating sympathetic nerve activity

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[7, 9]. We have recently revealed that acute intracerebroventricular (i.c.v.) injection of hChemerin-9 increased systemic BP in normal Wistar rats [17]. However, the effects of other C-terminal fragments on systemic BP as well as their species-dependent differences still remain to be determined. In order to explore them, we performed acute i.c.v. injection of mouse chemerin-9 (mChemerin-9; 148F-156S), mouse chemerin-8 (mChemerin-8; 148F-155F), and mouse chemerin-7 (mChemerin-7; 148F-154A) into Wistar rats, and examined the effects on systemic BP.

MATERIALS AND METHODS

Materials

mChemerin-9 (FLPGQFAFS), -8 (FLPGQFAF), -7(FLPGQFA), and rat 88 chemerin-9 (FFPGQFAFS) synthesized in Biotech company (GenScript, Piscataway, NJ, USA) were purchased.

Animals

Male Wistar rats (9–11-week-old) (CLEA, Tokyo, Japan) were used. Animal study was approved by the ethical committee of School of Veterinary Medicine, the Kitasato University (approval no. 19-227), and performed in conformity with an institutional guideline of the Kitasato University.

Acute i.c.v. injection

Acute i.c.v. injection was performed as described previously [17]. A head of Wistar rats, under an isoflurane anesthesia (induction: 5%, maintenance: 2–3%; Wako, Osaka, Japan) and a buprenorphine analgesia (50 μg/kg, s.c.; Otsuka Pharmaceutical, Tokyo, Japan), was fixed on a stereotaxic apparatus (NARISHIGE, Tokyo, Japan), and the skull was exposed. 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. Injection cannula (outer diameter; 0.3 mm) connected to a micromanipulator was put down by 4.5 mm from the surface of skull. Vehicle (artificial cerebro-spinal fluid: aCSF; 8.66 g/L NaCl, 0.224 g/L KCl, 0.155 g/L $CaCl_2$, 0.163 g/L $MgCl_2$-$6H_2O$, 0.285 g/L Na_2HPO_4-$12H_2O$, 0.0234 g/L $NaHPO_4$, pH7.4) and each reagent was cumulatively administrated by a rate of 1 μL/min every 20 min by a microsyringe (ITO Corp., Fuji, Japan).

Measurement of BP

Measurement of systemic BP was performed as described previously [17]. Systemic BP of Wistar rats was measured under an isoflurane anesthesia (induction: 5%, maintenance: 2–3%) and a buprenorphine analgesia (50 μg/kg, s.c.). Systemic BP was measured by inserting a catheter filled with a 1% heparin (AY PHARMACEUTICSLS, Tokyo, Japan)-saline solution into femoral artery. The catheter was connected to an MLT0670BP transducer (AD Instruments, Colorado Springs, CO, USA), ML117BP Amp (AD Instruments), and ML825 PowerLab 2/25 (AD Instruments).

Statistics

Data were shown as mean ± standard error of the mean. Statistical evaluations were done with two-way ANOVA followed by Bonferroni's *post-hoc* test (Figs. 1–4). Results were considered significant when P value was less than 0.05.

RESULTS

Effects of i.c.v. injection of mChemerin-9 on BP in Wistar rats

Injection of mChemerin-9 (1–10 nmol/head, i.c.v.) did not significantly affect the mean BP, systolic BP, and diastolic BP in rats (Fig. 1A–C, Supplementary Fig. 1A–C, 1E–G, n=4). On the other hand, injection of mChemerin-9 (30 nmol/head, i.c.v.) significantly increased mean BP and systolic BP but not diastolic BP (Supplementary Fig. 1H, n=4), which reached a peak at 2–4 min (Fig. 1D, Supplementary Fig. 1D, n=4, P<0.05 vs. aCSF at 3–4 min).

Effects of i.c.v. injection of mChemerin-8 on BP in Wistar rats

We next examined the effects of injection of mChemein-8 on systemic BP in rats. Injection of mChemerin-8 (1–30 nmol/head, i.c.v.) did not affect the mean BP, systolic BP, and diastolic BP compared with vehicle injection (Fig. 2A–D, Supplementary Fig. 2A–H, n=4).

Effects of i.c.v. injection of mChemerin-7 on BP in Wistar rats

We further examined the effects of injection of mChemein-7 on systemic BP in rats. Injection of mChemerin-7 (1, 10, 30 nmol/head, i.c.v.) did not affect mean BP (Fig. 3A, 3C, 3D, n=6). On the other hand, mChemerin-7 (3 nmol/head, i.c.v.) induced a slight but significant pressor response (Δmean BP; 1.7 ± 1.6 mmHg at 2 min, P<0.05 vs. aCSF, Δmean BP; 2.2 ± 1.5 mmHg at 3 min, P<0.01 vs. aCSF, Fig. 3B, n=6). We anticipate that this was due to the decrease of BP following vehicle injection. Similar results were obtained following mChemerin-7 injection compared with vehicle injection in systolic BP and diastolic BP (Supplementary Fig. 3A–H, n=6).

Concentration-dependent effects of mouse chemerin fragment

Figure 4 showed that the effects of mChemerin-9 (1–30 nmol/head, i.c.v., n=4) but not mChemerin-8 (n=4) and -7 (n=6) were concentration-dependent.

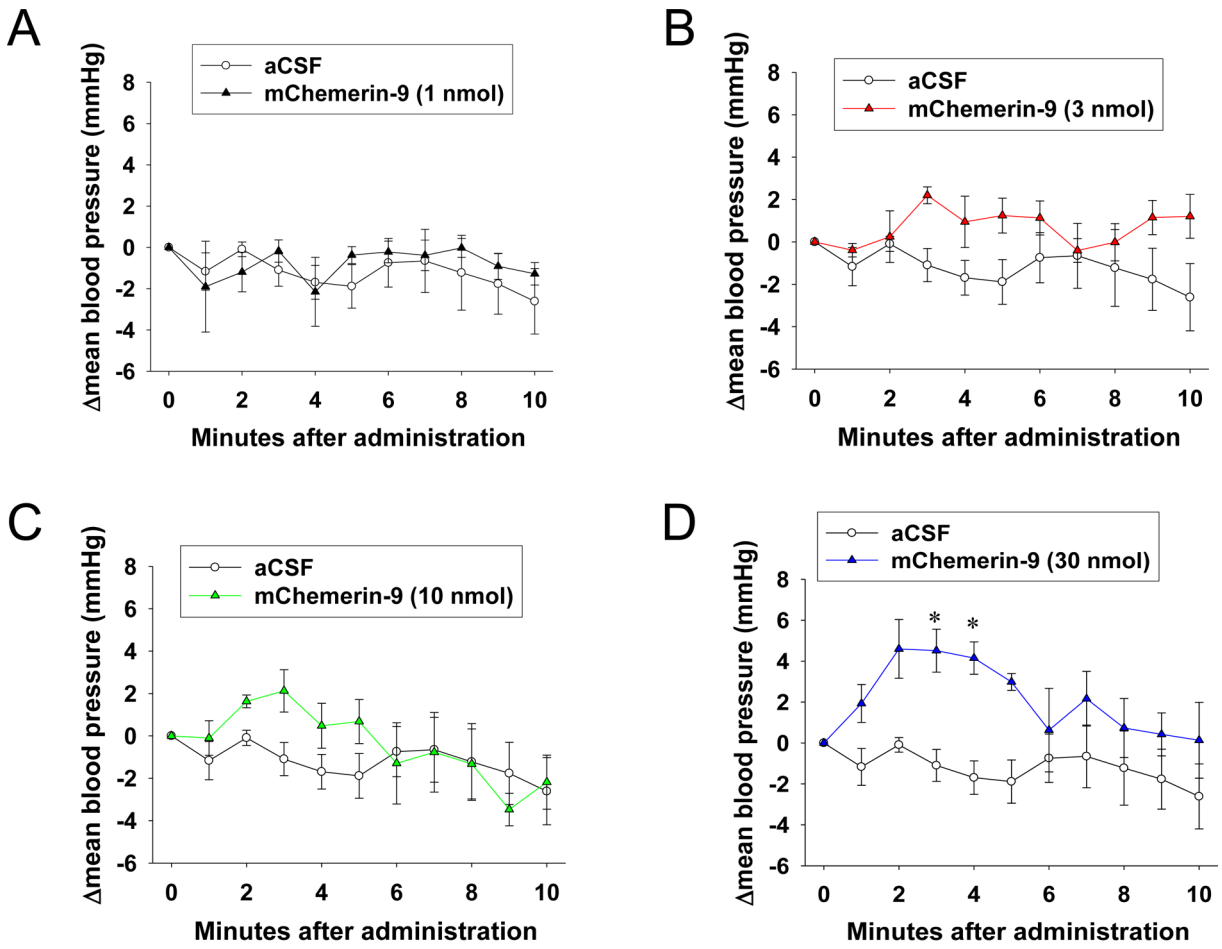

Fig. 1. Effects of intracerebroventricular (i.c.v.) injection of mouse chemerin-9 (mChemerin-9) on blood pressure in Wistar rats. After artificial cerebro-spinal fluid (aCSF, i.c.v.), vehicle or mChemerin-9 (1–30 nmol/head, i.c.v.) was cumulatively administered to Wistar rats, systemic blood pressure was measured by a cannulation method under an isoflurane anesthesia. Quantitative results of Δmean blood pressure [difference from base line (at 0 min)] were shown as mean ± S.E.M. (**A**: 1 nmol/head, **B**: 3 nmol/head, **C**: 10 nmol/head, **D**: 30 nmol/head, n=4). **P*<0.05 vs. aCSF.

DISCUSSION

In the present study, we performed acute i.c.v. injection of mChemerin-9 (148F-156S; FLPGQFAFS), mChemerin-8 (148F-155F; FLPGQFAF), and mChemerin-7 (148F-154A; FLPGQFA), and examined the effects on systemic BP. The major findings are that mChemerin-9 but not mChemerin-8 and -7 induced a pressor response, which was concentration-dependent (Figs. 1–4). In summary, we for the first time demonstrated that mChemerin-9 that corresponds to the C-terminal nine amino acids of mouse chemerin156S increased systemic BP, and also that chemerin fragments showed different effects on systemic BP dependent on the C-terminal cleavage site.

While several *in vitro* studies on the activity of various chemerin cleavage products have been performed [1, 16, 19], *in vivo* effects of chemerin fragments remained to be determined. In the present study, we examined the effects of acute i.c.v. injection of chemerin fragments on systemic BP. We confirmed that mChemerin-7 that correspond to the C-terminus of weakly active mouse chemerin154A did not affect systemic BP (Fig. 3). Thus, it is suggested that the *in vivo* activity of mouse chemerin154A is not high, similar to the *in vitro* activity. On the other hand, we confirmed that acute i.c.v. injection of mChemerin-9 that corresponds to the C-terminus of active mouse chemerin156S induced a pressor response (Fig. 1). Thus, our data suggested that the effects of chemerin fragments were different dependent on the C-terminal cleavage site, not only *in vitro* but also *in vivo*. In addition, we also found that the *in vivo* activity of mChemerin-8 (Fig. 2) was not corelated with the in *vitro* activity of mouse chemerin155F.

It was reported *in vitro* that EC_{50} of mouse chemerin156S was ~5.1 nM, while that of mChemerin-9 was ~42 nM [14]. Similarly, EC_{50} of full length hChemerin157S was the lowest (~4.5 nM), followed by EC_{50} of hChemerin-9 (~7.1 nM) [16]. Cerebral spinal fluid volume in rat was approximately 280 µL [2]. In the present study, we administered rats with mChemerin-9 fragment maximally at 30 nmol. Thus, it is estimated that final concentration was approximately 100 µM, resulting in higher concentration compared with EC_{50} of mChemerin-9. It was reported that the concentration of chemerin in blood of healthy subjects was ~190 ng/mL, while it increased

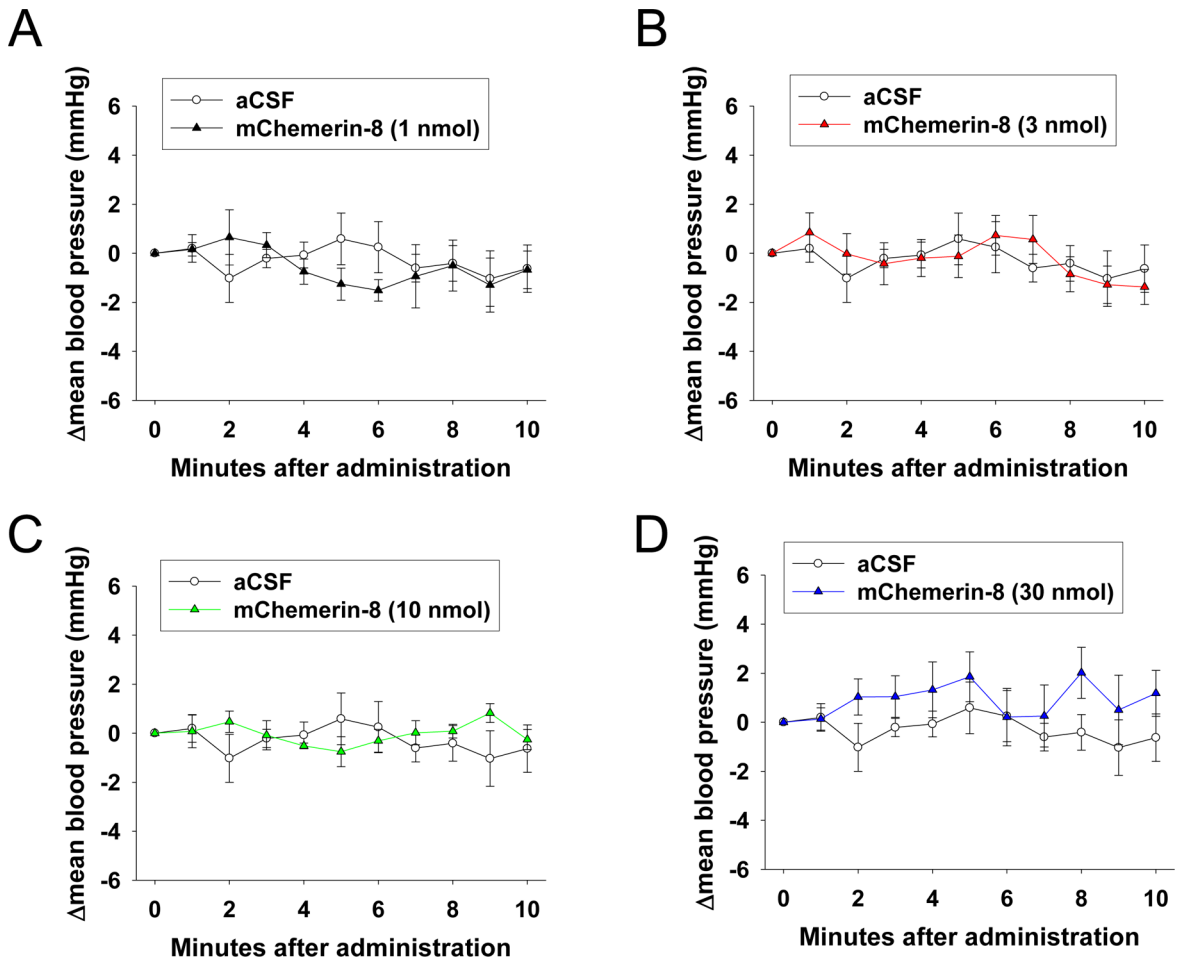

Fig. 2. Effects of intracerebroventricular (i.c.v.) injection of mouse chemerin-8 (mChemerin-8) on blood pressure in Wistar rats. After artificial cerebro-spinal fluid (aCSF, i.c.v.) or mChemerin-8 (1–30 nmol/head, i.c.v.) was cumulatively administered to Wistar rats, systemic blood pressure was measured by a cannulation method under an isoflurane anesthesia. Quantitative results of Δmean blood pressure were shown as mean ± S.E.M. (**A**: 1 nmol/head, **B**: 3 nmol/head, **C**: 10 nmol/head, **D**: 30 nmol/head, n=4).

to ~350 ng/mL in obese subjects [13]. Since local chemerin concentration is expected to be much higher in the certain tissues of obese subjects, the concentration of chemerin fragments used in this study was presumably valid.

We showed that acute i.c.v. injection of mChemerin-9 (30 nmol/head) induced a significant pressor response, while we previously reported that acute i.c.v. injection of lower concentration of hChemerin-9 (10 nmol/head) induced a significant pressor response [17]. We also performed i.c.v. injection of rat chemerin-9 that corresponds to the C-terminal nine amino acids of rat chemerin157S, and found that it did not affect systemic blood pressure (Supplementary Fig. 4). This was presumably caused by species-dependent differences in N-terminal amino acids (N-terminal of hChemerin-9; $Y^{149}F^{150}$ vs. $F^{148}L^{149}$ in mChemerin-9 vs. $F^{149}F^{150}$ in rat chemerin-9). As mentioned above, the C-terminal cleavage site has been considered to be important for the activity of chemerin fragments [16]. It was further suggested that N-terminal cleavage site is also important for the activity.

In conclusion, we for the first time demonstrated that mChemerin-9 that corresponds to the C-terminal nine amino acids of mouse chemerin156S increased systemic BP in rats, and also that chemerin fragments showed different effects on systemic BP dependent on their C-terminal cleavage site.

CONFLICT OF INTEREST. The authors have nothing to disclose.

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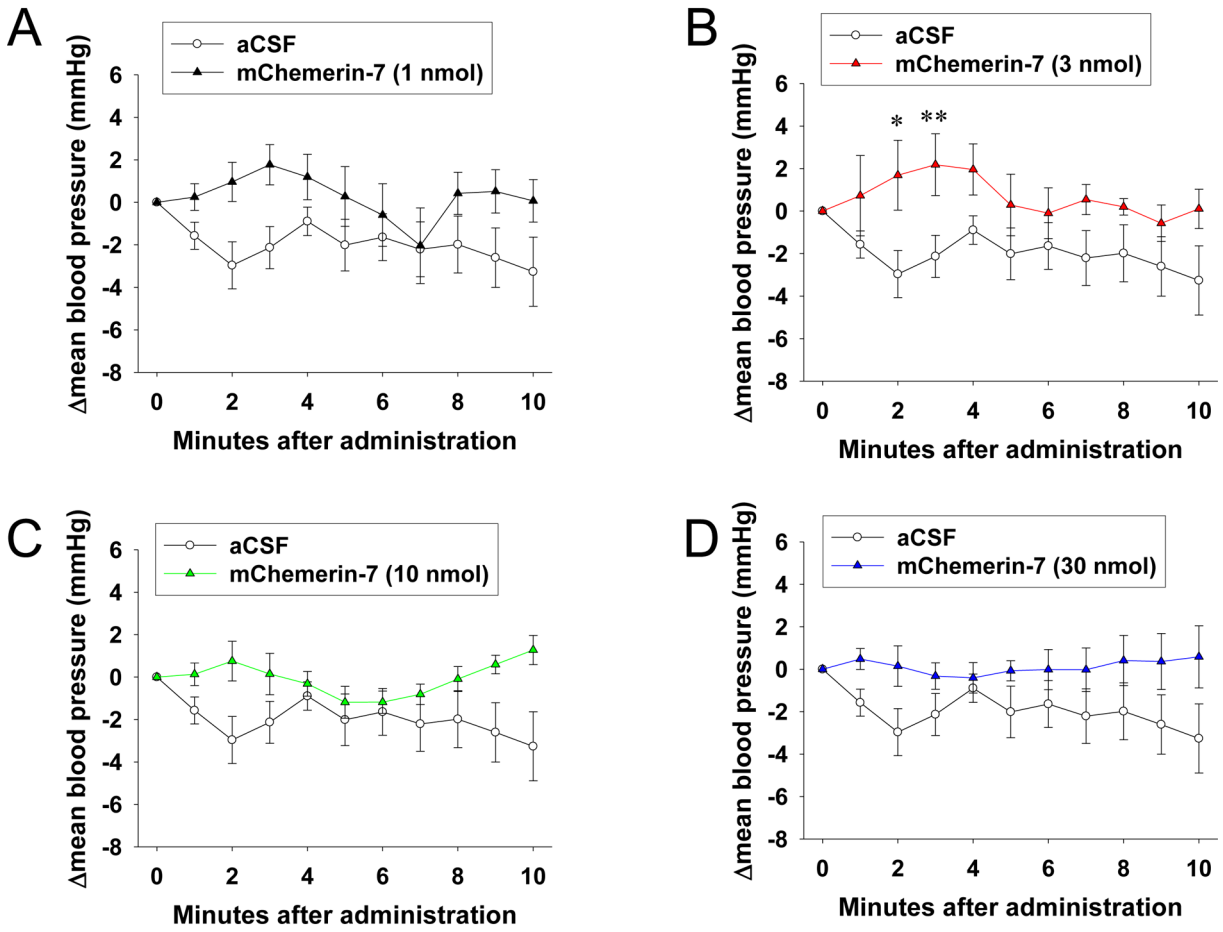

Fig. 3. Effects of intracerebroventricular (i.c.v.) injection of mouse chemerin-7 (mChemerin-7) on blood pressure in Wistar rats. After artificial cerebro-spinal fluid (aCSF, i.c.v.) or mChemerin-7 (1–30 nmol/head, i.c.v.) was cumulatively administered to Wistar rats, systemic blood pressure was measured by a cannulation method under an isoflurane anesthesia. Quantitative results of Δmean blood pressure were shown as mean ± S.E.M. (**A**: 1 nmol/head, **B**: 3 nmol/head, **C**: 10 nmol/head, **D**: 30 nmol/head, n=6). **P*<0.05, ***P*<0.01 vs. aCSF.

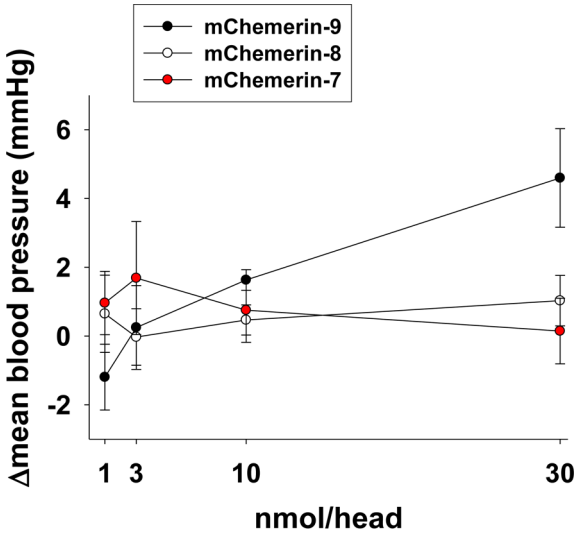

Fig. 4. Concentration-dependent effects of mouse chemerin fragment. After each chemerin fragment (mChemerin-9, -8, and -7; 1–30 nmol/head, intracerebroventricular) was cumulatively administered to Wistar rats, systemic blood pressure was measured by a cannulation method under an isoflurane anesthesia. Quantitative results of Δmean blood pressure for each chemerin fragment were shown as mean ± S.E.M. (mChemerin-9, -8: n=4, mChemerin-7: n=6).

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