Dietary capsaicin-mediated attenuation of hypertension in a rat model of renovascular hypertension

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Yukiko SEGAWA

Contents

Abstract

Background Capsaicin, a pungent component of chili pepper, has been reported to decrease blood pressure (BP) and to cause vasorelaxation via nitric oxide (NO) production. However, it is still unclear how dietary capsaicin effects on renovascular hypertension. To examine this, we observed the effects of dietary capsaicin on BP in 2-kidney, 1-clip renovascular hypertension (2K1C) rats, and investigated the participation of NO in the mechanism.

Methods Rats with 2K1C or sham-operated (SHAM) rats were treated with a 0.006% capsaicin diet (CAP) or a control diet (CTL) for 6 weeks. Systolic BP (SBP) was measured by tail-cuff method once a week. At the end, mean arterial BP (MAP) was measured in the rats under anesthesia. These observations were performed also in the rats taking a NO synthase (NOS) inhibitor, Nω-Nitro-L-arginine methyl ester hydrochloride (LN). After rats were euthanized, thoracic aortas were collected and used for western blot analyses to evaluate the phosphorylated ratio of endothelial NOS (eNOS), protein kinase A (PKA) and B (Akt), in order to explore a mechanism of the effects on BP by dietary capsaicin.

Results SBP and MAP in 2K1C rats were significantly higher than in SHAM

rats when fed CTL, but not when fed CAP. Those in 2K1C-CAP rats were significantly lower than in 2K1C-CTL rats. LN suppressed the effect by dietary capsaicin. The ratios of phosphorylated (p-) eNOS/eNOS and p-Akt/Akt, but not p-PKA/PKA, were significantly increased in rats fed CAP compared with rats fed CTL.

Conclusion Dietary capsaicin may alleviate 2K1C renovascular hypertension, probably via enhancing phosphorylation of Akt and eNOS.

List of Abbreviations

Akt: protein kinase B

Ang II: angiotensin II

ANOVA: measures analysis of variance

BP: blood pressure

CA: carotid arteries

EC: endothelial cell

eNOS: endothelial nitric oxide synthase

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

LN: Nω-Nitro-L-arginine methyl ester hydrochloride

MA: mesenteric arteries

MAP: mean arterial blood pressure

NO: nitric oxide

PKA: protein kinase A

PVDF: polyvinylidene difluoride

SBP: systolic blood pressure

SHR: spontaneously hypertensive rats

SN: sympathetic nervous

TRPV1: transient receptor potential vanilloid type 1

WKY: Wistar Kyoto rats

2K1C: 2-Kidney, 1-Clip hypertension model

Introduction

Hypertension is a major risk factor for arteriosclerosis, stroke and heart disease.[1-5] The higher the blood pressure (BP), the greater the burden on blood vessels of main internal organs such as the brain, kidneys, and heart.[6-8] According to a recent survey by the World Health Organization, individuals over 25 years of age diagnosed with hypertension exceeded one billion in 2008.[9] Further, it is estimated that a 2-mmHg decrease in average BP would prevent 20,000 deaths in the Japanese population with cardiovascular diseases.[10] Therefore, strategies to prevent from hypertension are very important. Exercise and a well-balanced dietary lifestyle are considered to be effective in both prevention and alleviation of hypertension, even if the causes of hypertension vary.[11–18] Epidemiological studies have revealed the relationship of BP reduction to the intake of large amounts of fruits and vegetables.[19-23] Based on such evidences, a diet plan based on Dietary Approaches to Stop Hypertension, known as the DASH diet, is recommended.[24-26] Obese and hypertensive patients following such a diet have shown weight loss and a decrease in BP.[24-26] In addition, some foods and food ingredients including capsaicin have been found to be effective against hypertension.

Capsaicin is a major pungent component of the chili pepper, which has been

widely used around the world as a spice. It is reported to have various physiological effects including increasing energy metabolism by increasing sympathetic nervous (SN) system activity.[27-29] Further, an epidemiological study reported that those who prefer spicy meals containing capsaicin have lower BP than others.[30]

Capsaicin activates the transient receptor potential vanilloid type 1 (TRPV1) channel, a six-transmembrane domain protein which presents in primary sensory afferent nerves.[31] TRPV1 is activated by high temperature (> 43°C), protons, and some lipid metabolites, in addition to capsaicin.[31] In terms of BP, it has been reported that activation of TRPV1 due to acute administration of capsaicin causes vasodilation in several animals.[32, 33] Wang Y et al. reported that an intravenous infusion of capsaicin decreased mean arterial blood pressure (MAP) in Dahl salt-sensitive rats.[34] In our previous study, BP of rats decreased due to an intravenous administration of capsaicin at a concentration of 0.13–1.3 nmol/kg BW/min.[35] Moreover, it has been reported that capsaicin caused a nitric oxide (NO)-mediated blood vessel relaxation response in a dose-dependent manner in experiments using porcine coronary arteries.[36] Therefore, it is plausible that capsaicin intake decreases BP through stimulation of TRPV1.

NO, a potent vasodilator, is produced from L-arginine via the action of

endothelial NO synthase (eNOS). eNOS regulates endothelial function and vascular tone by continuously producing NO at the endothelium.[37-41] NO produced by eNOS is reported to blunt the vasoconstrictor action of angiotensin II (Ang II) and to alleviate endothelial cell (EC) damage and oxidative stress.[42] Capsaicin and its derivatives have been reported to induce NO production.[43,44] Additionally, capsaicin was reported to cause NO production through stimulation of eNOS.[45,46] Thus, it is plausible that capsaicin decreases BP due to the effect of NO produced via eNOS activation. It is known that eNOS is activated by various factors, through activation of several pathways including protein kinase B (PKB; Akt), protein kinase A (PKA), heat shock proteins, AMP-activated protein kinase and extracellular signal-regulated kinase.[47-51]

The 2-Kidney, 1-Clip hypertension model (2K1C) is a renovascular hypertension animal model that activates the renin-angiotensin system by narrowing the unilateral renal artery using a clip.[52-54] Increased EC injury and oxidative stress is also observed in this model.[55,56] Although chronic ingestion of capsaicin has been reported to attenuate hypertension induced by a high-salt diet in mice and spontaneously hypertensive rats (SHR),[57-59] to the best of our knowledge, there is no study which has demonstrated whether the capsaicin induces antihypertensive effects to renovascular hypertension.

In the present study, we evaluated BP in 2K1C rats fed a capsaicin diet. Because the capsaicin diet was observed to decrease BP in the hypertension model, we also investigated whether NO participates in the inhibitory effect of capsaicin on high BP, and whether the Akt/eNOS and/or the PKA/eNOS pathways contribute to the antihypertensive effect of capsaicin in the 2K1C model.

Materials and Methods

Animals and treatment

Four-week-old male Sprague–Dawley rats were obtained from Japan SLC, Inc. (Shizuoka, Japan), and were maintained in a temperature and moisture control room (21 ± 1°C, 60 ± 10% in a 12 h light/dark cycle). The rats had free access to standard chow (CE-2; CLEA Japan, Inc., Tokyo, Japan) and tap water during preliminary breeding. All procedures were performed in accordance with the Kobe Women's University animal guideline with the approval from the Institutional Animal Care and Use Committee of Kobe Women's University.

<Experiment I>

At 6 weeks of age, the rats underwent either sham operation (SHAM) or 2K1C surgery (2K1C). 2K1C surgery was performed by clipping the left renal artery using a silver clip with an internal diameter of 0.254 mm, as described previously.[60] Sham operation involved the same surgical procedure as 2K1C surgery except for the clip placement. These surgeries were performed under anesthesia induced using three types of mixed anesthetic agents (0.15 mg/kg medetomidine, 2 mg/kg midazolam, and 2.5 mg/kg butorphanol, i.p.). Three days before surgery, the rats were randomly divided into

two groups. They received either a control diet (CTL) or a capsaicin diet (CAP) which was a control diet containing 0.006% capsaicin (FUJIFILM-Wako Pure Chemical Corporation, Ltd. Osaka, Japan) via a pair-feeding procedure for 3 days. Then, each group of rats was randomly divided into a SHAM or a 2K1C group, and underwent the corresponding surgery. The groups were as follows: (1) SHAM-CTL, (2) SHAM-CAP, (3) 2K1C-CTL, and (4) 2K1C-CAP.

Systolic BP (SBP) of conscious rats, and body weight (BW) of all rats was measured every week. In addition, 6 weeks after the surgery, mean arterial BP (MAP) was measured under anesthesia as described below.

<Experiment II>

Additionally, we observed SBP in Wistar Kyoto rats (WKY) or SHR fed a control diet (CTL) or a capsaicin diet (CAP) for 7 weeks from the age of 5 weeks. The groups were as follows: (1) WKY-CTL, (2) WKY-CAP, (3) SHR-CTL, and (4) SHR-CAP.

BP evaluation

SBP was evaluated as described previously.[61,62] Briefly, it was measured by the

tail-cuff method using MK-1030 NIBP Monitor (Muromachi Kikai Co., Ltd. Tokyo, Japan) once per week during the experiment. Before the measurements, the rats were housed in a warm box for 10 min at 38°C to easily detect arterial pulsation in the tail and to stabilize their SBP. An average of 10 measurements was used for analysis.

At the end of the protocol, MAP of each rat was measured under anesthesia induced using three types of mixed anesthetic agents as described above. BP measurement was carried out by modifying a method described previously.[63] Briefly, MAP was monitored using a Power Lab System (AD Instruments, Belle Vista, Australia) with a signal amplifier (AD Instruments) hooked up to a BP transducer (AR611G; Nihon Kohden Corp., Tokyo, Japan), which was connected to a catheter inserted into the left femoral artery. The BP data used was collected during the last 3 min of a 10-min stabilization period.

Sample collection

After the measurement of MAP, rats were subject to blood collection and then euthanized via exsanguination under deep anesthesia using the mixed anesthetic agents described above. Afterwards, the thoracic aorta was removed from the rats immediately and stored frozen at −80°C until use in western blot analysis.

Western blot analysis

Western blot analyses were performed using a previously reported method with modifications.[64] The thoracic aortas were homogenized in ice-cold Pierce RIPA Lysis and Extraction Buffer® with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Tokyo, Japan). The homogenate was centrifuged at 14,000 g for 20 min at 4°C, and the supernatant was collected. The protein concentration was determined using the Bicinchoninic acid method with the Pierce BCA protein assay kit® (Thermo). Equal amounts of protein samples were mixed with an adequate volume of 4X Bolt™ LDS Sample Buffer (Life Technologies, Tokyo, Japan) and heated for 10 min at 70°C. The samples were separated via electrophoresis in 7–15% Bolt® Bis-Tris Plus Gels (Life Technologies) in 20X Bolt™ MOPS SDS Running Buffer (Life Technologies). The proteins were then transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories, Tokyo, Japan). The PVDF membranes were incubated in 5% skim milk in Tris-buffered saline solution with 0.1% Tween 20 (TBST) or PVDF blocking reagent (Toyobo, Osaka, Japan) for 1 h at room temperature (RT) and washed in TBST. Subsequently, the membranes were incubated with primary antibodies against eNOS (diluted 1:1000), p-eNOS (1:500), Akt (1:1000), p-Akt (1:1000), PKA (1:1000), p-PKA (1:1000), TRPV1 (1:1000) and

glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:1000) overnight at 4°C. The antibodies against TRPV1 were purchased from Alomone Labs (Jerusalem, Israel). All other antibodies were purchased from Cell Signaling (Tokyo, Japan). The membranes were then washed and incubated for 1 h at RT with horseradish peroxidase-conjugated secondary antibodies (1:10,000; Jackson Immuno Research Laboratories, West Grove, PA, USA) in Can Get Signal (Toyobo) or 5% bovine serum albumin (FUJIFILM-Wako) in TBST. The protein bands were visualized using the ECL system (Bio-Rad), and the protein expression levels were determined using the cooled CCD camera system Lumino Graph I (ATTO, Tokyo, Japan). Densitometry of the protein bands was performed using the software Image J (National Institute of Health, Bethesda, MD, USA). The protein expression levels were normalized to the housekeeping protein GAPDH.

BP evaluation with administration of L-NAME <Experiment III>

Because capsaicin was found to reduce BP in 2K1C rats, we tested whether NO is involved in the mechanism of the effect of capsaicin using the NO synthase inhibitor, Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, FUJIFILM-Wako). SHAM and 2K1C rats fed a CTL or CAP diet were treated with vehicle (Veh) or L-NAME (LN)

in tap water; in total, BP changes in eight groups of rats were evaluated. Handling and feeding of the animals were performed as described above except for the administration of LN in half of the rats from surgery until the end of the protocol. Further, in this experiment, we fed a CAP diet to the CAP groups beginning a week before surgery. L-NAME was dissolved in tap water at a dose of 0.3 g/L just before use, and given freely to the LN group of rats. The Veh groups were given tap water freely.

Statistical analysis

All data are expressed as mean ± standard error (SE). If statistical significance was detected using three-way or two-way repeated measures analysis of variance (ANOVA) or one-way ANOVA, pairwise comparisons were carried out using the two-tailed Student's t-test. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using the IBM Statistical Package for Social Science Statistics Version 23.0 (IBM Japan, Ltd, Tokyo, Japan).

Results

BW in SHAM and 2K1C rats

BW was not significantly different among the groups of SHAM and 2K1C rats fed a CTL or CAP diet drinking tap water in Experiment I (Fig. 1A). On the other hand, in Experiment III, observing the effects of LN, significant differences were detected in terms of week, animal, diet, drink, and each interaction using four-way ANOVA. BW was significantly increased in 2K1C-CTL-Veh animals compared with SHAM-CTL-Veh, 2K1C-CAP-Veh, and 2K1C-CTL-LN animals throughout the protocol. However, there were no significant differences in weekly BW except for between 2K1C-CTL-Veh and 2K1C-CAP-Veh animals, or between 2K1C-CTL-Veh and 2K1C-CTL-LN animals in the third week. BW was significantly reduced in 2K1C-CTL-LN animals compared with SHAM-CTL-LN animals during all the procedures but not weekly. Further, BW was significantly lower in SHAM-CAP-Veh animals than in SHAM-CAP-LN and 2K1C-CAP-Veh animals (Fig. 1B).

<Experiments I and II>

Effects of dietary capsaicin intake on SBP and MAP in 2K1C rats and SHR

In Experiment I, as shown in Fig. 2A, SBP was significantly higher in 2K1C-CTL animals than in SHAM-CTL animals beginning 3 weeks after surgery ($P < 0.05$). SBP in 2K1C-CAP animals was significantly lower than in 2K1C-CTL ($P < 0.05$). On the other hand, there were no significant differences in SBP between SHAM-CTL and SHAM-CAP animals throughout the protocol. In addition, no differences were found in SBP between SHAM-CAP and 2K1C-CAP animals.

Fig. 2B demonstrates that MAP in 2K1C-CTL animals was significantly higher than that in SHAM-CTL animals ($P < 0.05$). MAP in 2K1C-CAP animals was significantly lower than that in 2K1C-CTL animals ($P < 0.05$), while there were no significant differences in MAP between SHAM-CTL and SHAM-CAP animals.

As with 2K1C rats, SBP was markedly reduced in the CAP-diet group compared with the CTL-diet group in SHR in Experiment II (Fig. 2C). On the other hand, there was no significant difference between the CAP-diet group and the CTL-diet group in WKY rats (Fig. 2C).

These findings suggested that dietary capsaicin decreases BP in the hypertensive animal models, 2K1C rats and SHR, but not in the normotensive controls SHAM rats and WKY rats.

Involvement of eNOS, TRPV1, Akt, and PKA in the effects of hypertension attenuation due to dietary capsaicin in 2K1C rats

The p-eNOS/eNOS ratio was significantly elevated in SHAM and 2K1C rats fed the CAP compared with those fed the CTL diet ($P < 0.05$, Fig. 3). As shown in Fig. 4, the ratio of p-Akt/Akt significantly increased in the CAP animals compared with the CTL animals ($P < 0.05$), while there were no significant alterations in p-PKA/PKA ratio among all the groups. In addition, TRPV1 expression did not show significant differences among all the groups (Fig. 4).

<Experiment III>

Effects of LN treatment on BP suppression due to dietary capsaicin intake in 2K1C rats

As in Experiment I (Fig. 2(A)), Fig. 5A shows that SBP was markedly higher in 2K1C-CTL-Veh animals than in SHAM-CTL-Veh animals, and that it was significantly reduced in 2K1C-CAP-Veh animals compared with 2K1C-CTL-Veh animals throughout the procedure in Experiment III ($P < 0.05$). However, there was no significant reduction in 2K1C-CAP-LN animals compared with 2K1C-CTL-LN animals. Although SBP in 2K1C-CAP-Veh animals was not significantly different from that in

SHAM-CAP-Veh animals, SBP in 2K1C-CAP-LN animals was significantly higher than that in SHAM-CAP-LN animals ($P < 0.05$, Fig. 5A). An increase in SBP was observed in all L-NAME-treated animal groups compared with Veh groups ($P < 0.05$). SBP in the 2K1C-LN groups was significantly higher than that in the SHAM-LN groups with either diet ($P < 0.05$).

As in Experiment 1 (Fig. 2B), Fig. 5B demonstrated that 2K1C-CTL-Veh animals showed a significant increase in MAP compared to SHAM-CTL-Veh animals, but MAP in 2K1C-CAP-Veh animals was significantly reduced compared with 2K1C-CTL-Veh animals ($P < 0.05$). Both 2K1C-CTL and 2K1C-CAP animals treated with LN showed marked increases in MAP compared to the Veh animals. No significant difference was observed in MAP between 2K1C-CTL-LN and 2K1C-CAP-LN animals. In addition, MAP in 2K1C animals was significantly elevated compared with that in SHAM animals, with CTL or CAP diet in the LN group ($P < 0.05$). MAP in SHAM-CTL-LN animals was higher than that in SHAM-CTL-Veh animals ($P < 0.05$). There was no significant difference between SHAM-CTL-LN and SHAM-CAP-LN animals.

Discussion

The present study demonstrates that BP in 2K1C rats fed a capsaicin diet was reduced compared with that in 2K1C rats fed a control diet Fig. 2 (A, B), suggesting that capsaicin alleviates the BP elevation in 2K1C rats. Our previous study found that BP was decreased due to an intravenous administration of low-concentration capsaicin in normotensive rats.[35] In the present study, we demonstrate the antihypertensive effect of dietary capsaicin in 2K1C rats. These findings may contribute to developing methods of dietary prevention of renovascular hypertension in humans using capsaicin. Because chili peppers containing capsaicin are used for cooking all over the world and are inexpensive, relevant methods of their use may be easy to adopt in daily life.

The antihypertensive effect of acute administrations of capsaicin on BP was observed in Dahl salt-sensitive rats.[34] In chronic administration, rise in BP induced by a high-salt diet was reported to be prevented by a capsaicin diet for 12 weeks and 10 months in mice.[57,58] In addition, administration of capsaicin to SHR for 7 months showed a suppression of BP increase.[59] Besides these experiments with long-term administration of capsaicin, in the present study, we demonstrated that a capsaicin diet intake for 3 weeks or more alleviated an increase in BP in 2K1C rats and SHR (Fig. 2).

Further, these data suggested that dietary capsaicin prevents multiple types of hypertension. However, the amount of capsaicin suitable for human has not been clear. The amount of capsaicin used in this study cannot be taken because it is too large amount when converted to humans. In addition, species differences between rats and humans should be considered. On the other hand, a partial antihypertensive effect may be obtained without taking the full amounts used in this study. Further studies are necessary to use the present findings for preventing hypertension in humans.

In the present study, a significant decrease in BP was obtained 3 weeks after initiating a capsaicin diet in SHR, unlike the findings of a previous study (Fig. 2C).[59] The capsaicin administration in our study began with a 0.006% capsaicin diet in six-week-old rats, while the previous study began with a 0.02% capsaicin diet at the age of 8 weeks. We infer that the significant antihypertensive effect observed due to the smaller capsaicin amount and earlier initiation of treatment in the present study may suggest effective usage of dietary capsaicin for preventing hypertension.

Fig. 5 shows that NO may be involved in one of the possible mechanisms of capsaicin's effect on the suppression of BP increase in 2K1C rats. In the Veh groups, Experiment III (Fig. 5) confirmed the results of Experiment I (Fig. 2), showing that indeed, capsaicin attenuated a BP increase in 2K1C rats. While capsaicin decreased SBP

in 2K1C rats nearly to the levels of those in SHAM rats in the Veh groups, it did not decrease SBP to the levels of those in SHAM rats in the LN groups. Moreover, in terms of MAP, a capsaicin diet resulted in no significant difference in 2K1C rats in the LN group. Thus, LN weakened or eliminated the BP-decreasing effects of capsaicin in 2K1C rats (Fig. 5), suggesting that NO may participate in the mechanisms of the antihypertensive effect of capsaicin in 2K1C rats.

The 2K1C renovascular hypertensive model is characterized by an increase of BP via an elevation of Ang II expression, which results from ischemia in the clipped kidney and shear stress in the non-clipped kidney.[52-54] Further, kidney clipping causes oxidative stress and EC damage.[55] NO has the effect of alleviating Ang II-induced vasoconstriction responses[42] and thus, capsaicin may have alleviated Ang II vasoconstriction via NO production in 2K1C rats. Additionally, it is possible that active oxygen was eliminated due to NO production caused by capsaicin, preventing EC damage in the 2K1C rats. Therefore, it is possible that ingestion of a capsaicin diet suppressed a rise in BP in 2K1C rats via NO production.

TRPV1 channels are stimulated by capsaicin, subsequently producing NO.[45,46] It was reported that relaxation due to activation of TRPV1 channels by capsaicin could

be attenuated by LN or removal of vascular ECs.[45] Furthermore, it was reported that a 6- or 7-month administration of capsaicin significantly increased endothelial-dependent vasodilation, and NOS inhibition by LN administration suppressed the vasodilation resulting due to chronic administration of dietary capsaicin.[59,65] Therefore, we infer that NO derived from vascular ECs via stimulation of TRPV1 by capsaicin is involved in the mechanisms of the effect of chronic capsaicin intake in alleviating hypertension in 2K1C rats. In the present study, TRPV1 protein expression was not changed, although it has been reported to increase by capsaicin ingestion in previous studies.[59,65] In our study, capsaicin was administered for 7 weeks, while it was taken for 6 or 7 months in the previous studies. It is possible that TRPV1 expression level did not increase due to the short administration period in the present study. Additionally, it has been reported that capsaicin infusion formed TRPV1-Akt-eNOS complex, and subsequently caused vasorelaxation.[45] Thus, capsaicin might decrease BP via the increase in TRPV1-Akt-eNOS complex but not in TRPV1 protein itself in the present study.

The p-eNOS/eNOS ratio but not eNOS was significantly higher in the thoracic aorta in rats in the CAP groups compared with those in the CTL groups (Fig. 3). These observations suggest that capsaicin may induce phosphorylation of eNOS. In SHR or

stroke-prone SHR (SHRsp), a capsaicin diet was reported to improve EC-dependent vasorelaxation via increment in p-eNOS in mesenteric arteries (MA) or carotid arteries (CA).[59,65] In mice as well, p-eNOS was reported to increase in EC cells, MA, and CA due to capsaicin administration.[59,65] These findings may support the present observations. Because the increment in p-eNOS induces NO production, it may have contributed to the mechanism via which capsaicin intake suppressed BP elevation in 2K1C rats.

Although the increase in eNOS phosphorylation was observed in both 2K1C-CAP and SHAM-CAP rats, neither SBP nor MAP in SHAM-CAP rats was decreased (Figs. 2, 3). If more NO is produced through increase of eNOS phosphorylation, BP should also have changed in SHAM rats ingesting capsaicin. However, capsaicin was not observed to reduce BP in SHAM rats in our studies. In other studies as well, to the best of our knowledge, there are no reports of chronic capsaicin ingestion decreasing BP in normotensive rats. A study reported that endothelium-dependent vasodilation and eNOS activation were increased due to an chronic ingestion of capsaicin in normotensive rats, but no effect on BP was observed.[65] Thus, NO production due to dietary capsaicin may not achieve BP reduction in

normotensive rats, although the mechanism is unclear. One of the possible mechanisms is compensation via the SN system. Previous studies have found that capsaicin causes an increase in BP through the activation of the SN in rats and humans.[66-70] These contrasting effects of capsaicin intake, of increasing BP via SN activation and lowering BP via NO may be mutually compensatory resulting in homeostasis in normotensive rats. Thus, BP in SHAM rat fed a capsaicin diet may not have changed. On the other hand, capsaicin intake might not have led to further activation of the SN and only led to reducing BP via NO in 2K1C rats, because the SN was probably already activated in the hypertensive rats.[71] Other mechanisms maintaining homeostasis could exist; some counteracting effects against the hypotensive effect of capsaicin may be present to maintain BP in normotensive rats. These hypotheses have not been tested in this study. Thus, further studies are needed to resolve this issue.

Fig.4 shows that a capsaicin diet significantly increased the p-Akt/Akt ratio compared with that in control groups, regardless of whether they were SHAM or 2K1C rats. However, the p-PKA/PKA ratio was not different among the groups. Thus, capsaicin diet may have caused eNOS phosphorylation via Akt, while it may not have induced phosphorylation of PKA in the thoracic aorta of these rats. Acute injection of

capsaicin is reported to phosphorylate eNOS via phosphorylation of Akt in aortas of wild type mice.[45] It was recently reported that TRPV1 stimulated by capsaicin cause an increase in the phosphorylation of eNOS via Akt activation, inhibiting inflammation.[46] Moreover, the Akt/eNOS pathway is reported to attenuate high BP in pulmonary arterial hypertensive rats and SHR.[72-74] On the basis of these reports as well as the present observation, capsaicin is supposed to have activated Akt/eNOS pathway via stimulating TRPV1, although TRPV1 protein was not increased in the present data.

BW was different between some groups in Experiment III, although all animal ingested the same amount of diet every week by pair-feeding (Fig. 1(B)). However, the difference of BW observed in Experiment III (Fig. 1(B)) did not influence our conclusion, because the results of BP in Veh groups in Experiment III were similar to the results in Experiment I (Fig. 1(A)) and because BW was decreased but BP was still significantly elevated in 2K1C-CTL-LN compared with 2K1C-CTL-Veh, although weight loss decreases BP in Experiment III.

In summary, our results show, first, that chronic administration of capsaicin for 3 weeks and more suppressed BP elevation in 2K1C hypertensive rats as well as in SHR. Second, the alleviation of BP elevation due to dietary capsaicin was inhibited via LN administration. Third, phosphorylation of eNOS and Akt were increased in the thoracic

aorta due to capsaicin intake in 2K1C rats. These results suggest that a capsaicin diet attenuates BP elevation via NO activation, probably through increasing phosphorylation of Akt and subsequently that of eNOS in 2K1C rats. On the other hand, capsaicin intake may not decrease BP in normotensive rats, although it induced NO activation and increased phosphorylation of eNOS and Akt, the mechanism of which needs further studies to be elucidated.

Conclusion

The capsaicin diet attenuates BP elevation via NO activation, perhaps through increasing phosphorylation of Akt and subsequently that of eNOS in 2K1C rats.

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Figures

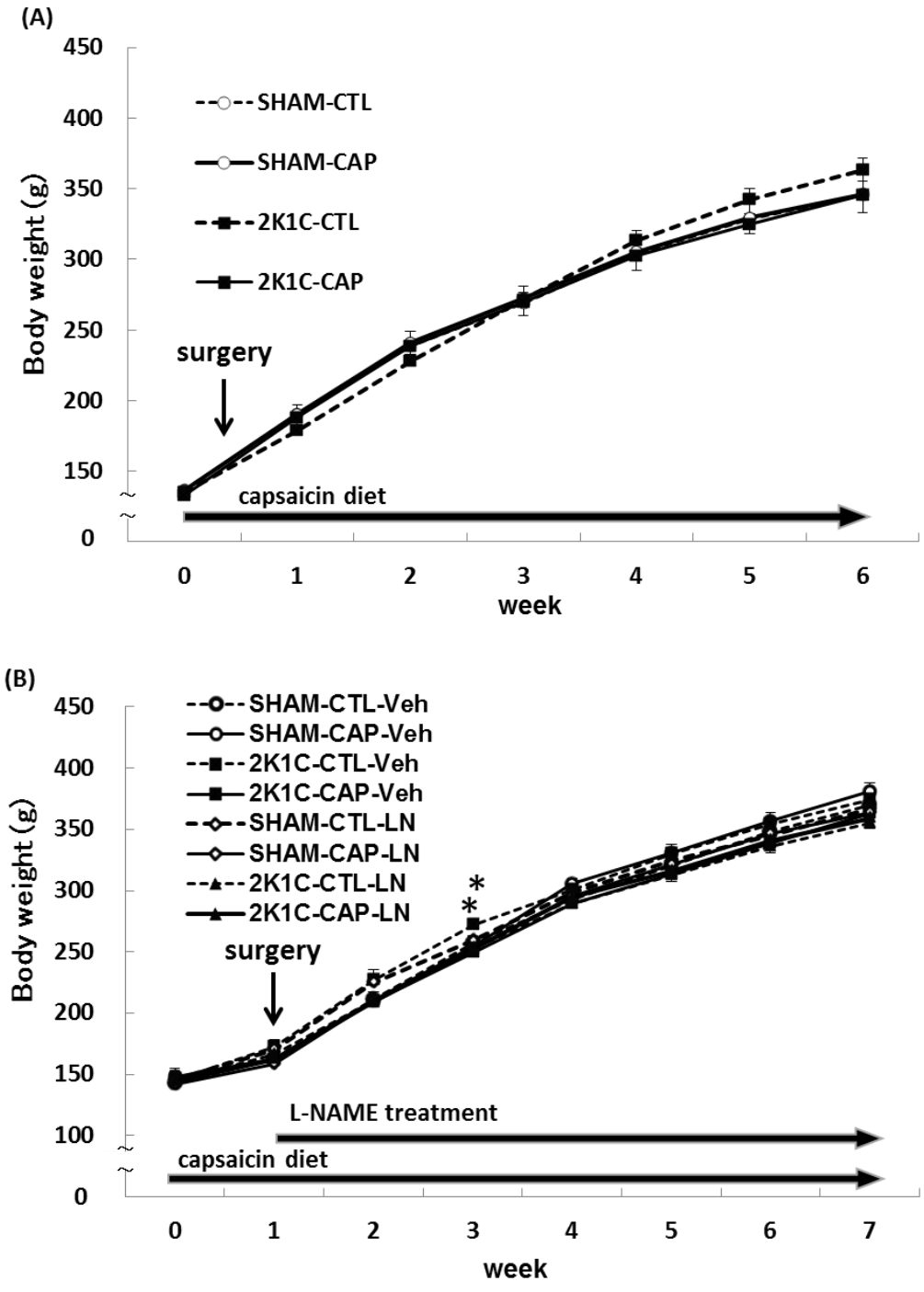


Fig. 1. Body weight (BW) in SHAM or 2K1C rats fed a control diet with capsaicin

(A) and BW in SHAM or 2K1C rats fed a control diet with capsaicin drinking tap water or LN in tap water (B). Values are mean ± SE; n = 6–10. (A) Three-way ANOVA, $P < 0.05$ for time. (B) Four-way ANOVA, $P < 0.05$ for time, animal (SHAM vs. 2K1C), diet (CTL vs. CAP), drink (Veh vs. LN) and each interaction. $^{*}P < 0.05$ vs. 2K1C-CTL-Veh. $P < 0.05$ 2K1C-CTL-Veh compared with SHAM-CTL-Veh, 2K1C-CAP-Veh, and 2K1C-CTL-LN throughout the protocol. $P < 0.05$ 2K1C-CTL-LN compared with SHAM-CTL-LN throughout the protocol. $P < 0.05$ SHAM-CAP-Veh compared with SHAM-CAP-LN and 2K1C-CAP-Veh. Abbreviations: SHAM, sham-operated control rats; 2K1C, two-kidney, one-clip hypertensive rats; CTL, control diet; CAP, capsaicin diet. Veh, vehicle (tap water); LN, Nω-Nitro-L-arginine methyl ester hydrochloride.

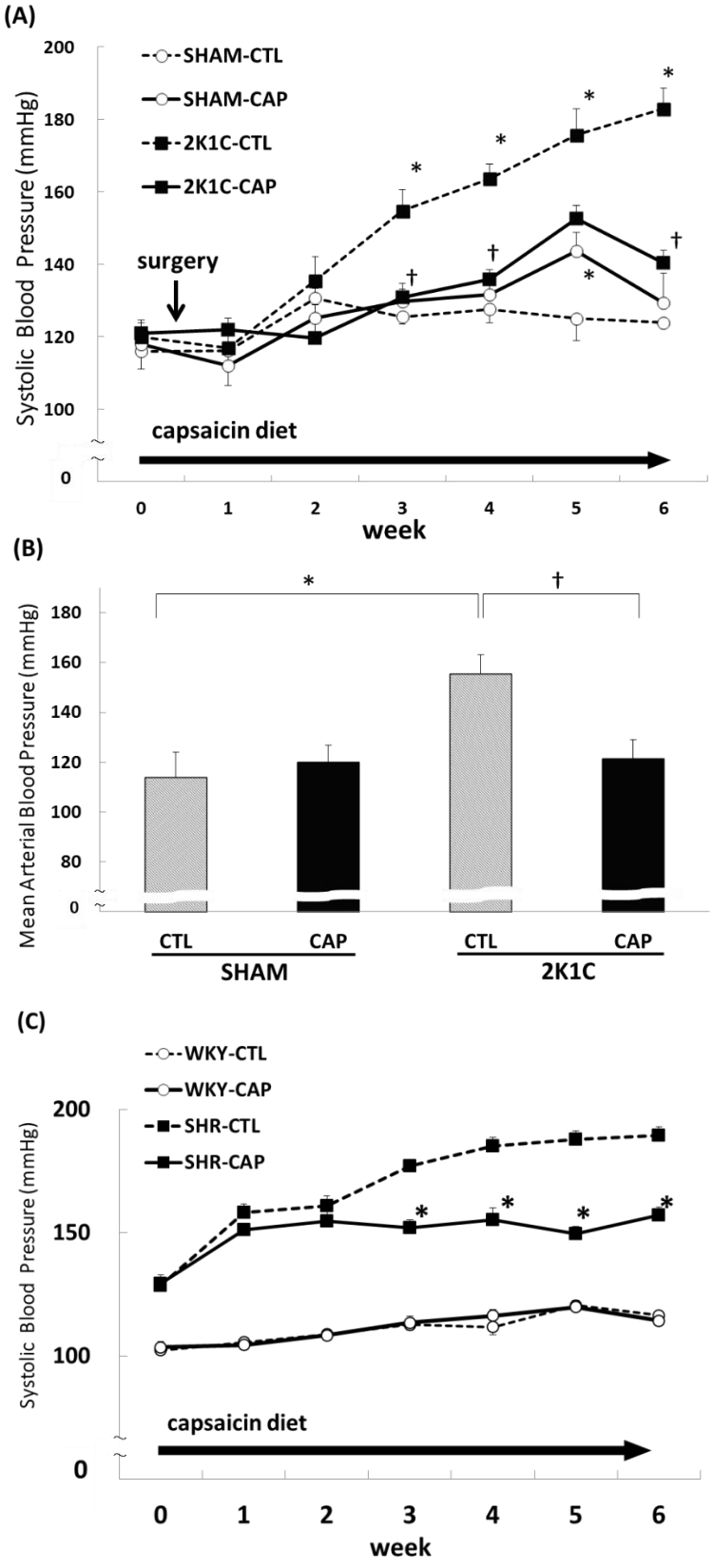

(A)
SHAM-CTL
SHAM-CAP
2K1C-CTL
2K1C-CAP
surgery
capsaicin diet
Systolic Blood Pressure (mmHg)
week
(B)
Mean Arterial Blood Pressure (mmHg)
CTL
CAP
CTL
CAP
SHAM
2K1C
(C)
WKY-CTL
WKY-CAP
SHR-CTL
SHR-CAP
capsaicin diet
Systolic Blood Pressure (mmHg)
week

Fig. 2. Systolic blood pressure (A) and mean arterial blood pressure at the end of the protocol (B) in SHAM or 2K1C rats fed a diet with or without CAP for 6 weeks after surgery, and systolic blood pressure in WKY rats or SHR fed a diet with or without CAP for 7 weeks (C). MAP was measured through a catheter in the left common femoral artery under anesthesia. Values are mean ± SE; n = 6–12. (A) Three-way ANOVA, $P < 0.05$ for time, animal (SHAM vs. 2K1C), diet (CTL vs. CAP), time × animal, and animal × diet. $^{*}P < 0.05$ vs. SHAM-CTL, $^{\dagger}P < 0.05$ vs. 2K1C-CTL. (B) Two-way ANOVA, $P < 0.05$ for animal (SHAM vs. 2K1C) and animal × diet (CTL vs. CAP). $^{*}P < 0.05$ vs. SHAM-CTL, $^{\dagger}P < 0.05$ vs. 2K1C-CTL. (C) Three-way ANOVA, $P < 0.05$ for time, diet (CTL vs. CAP). * $P < 0.05$ vs. SHR-CTL. Abbreviations: SBP, systolic blood pressure; MAP, mean arterial blood pressure; SHAM, sham-operated control rats; 2K1C, two-kidney, one-clip hypertension model; CTL, control diet; CAP, capsaicin diet; WKY, Wister Kyoto rats; SHR, spontaneously hypertensive rats; SE, standard error; ANOVA, analysis of variance.

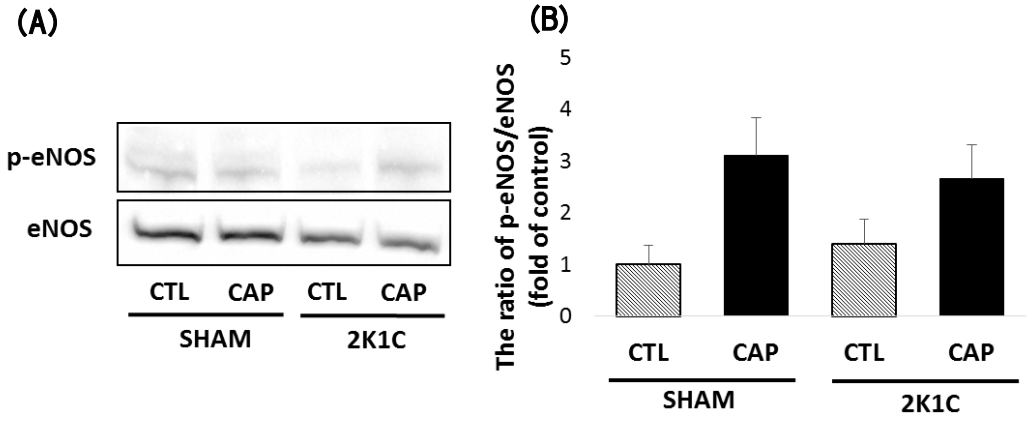
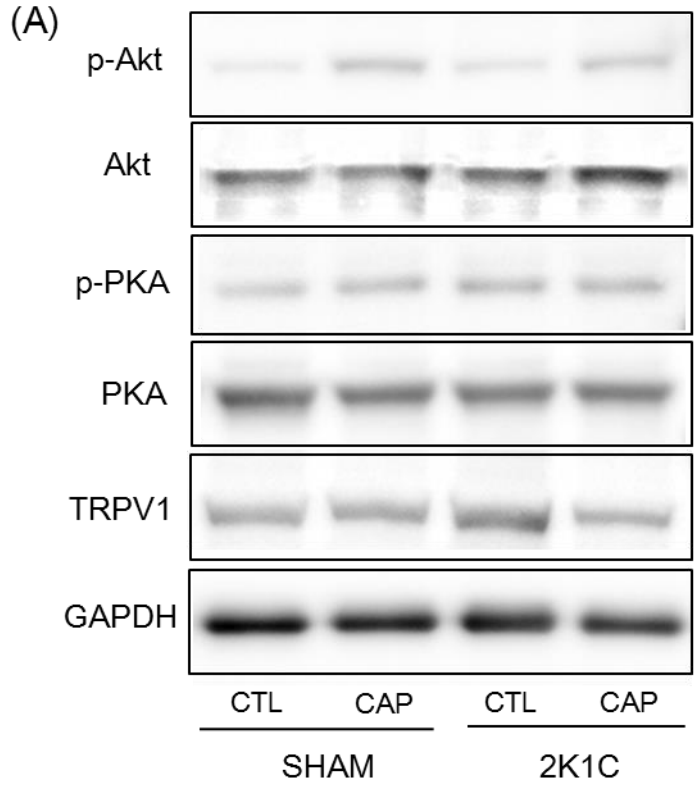


Fig. 3. Representative western blots for p-eNOS and eNOS (A) and p-eNOS/eNOS ratio (B) in the thoracic aorta of SHAM or 2K1C rats fed a diet with or without CAP. Values are mean (± SE) folds over CTL, n = 6. Two-way ANOVA, $P < 0.05$ for diet (CTL vs. CAP). eNOS, endothelial nitric oxide synthase; p-eNOS, phosphorylated endothelial nitric oxide synthase. See legend of Fig. 1 for other abbreviations.



(B)

	SHAM		2K1C		ANOVA		
	CTL	CAP	CTL	CAP	animal	diet	interaction
p-Akt/Akt ratio	1.00±0.16	1.71±0.30	1.05±0.13	1.25±0.21	NS	<0.05	NS
p-PKA/PKA ratio	1.00±0.23	1.02±0.12	1.05±0.20	1.02±0.19	NS	NS	NS
TRPV1 protein	1.00±0.37	0.97±0.29	1.26±0.39	1.02±0.20	NS	NS	NS

Fig. 4. Representative western blots for p-Akt, Akt, p-PKA, PKA and TRPV1 (A) and Akt/Akt, p-PKA/PKA ratio and protein expression of TRPV1 (B) in thoracic aorta of SHAM or 2K1C rats fed a diet with or without CAP. (B) shows values as mean (± SE) folds over SHAM-CTL, and statistical evaluation by ANOVA. n = 6–10. $p < 0.05$, NS = not significance for ANOVA. There are no significant differences between any two values. Abbreviations: Akt, protein kinase B; p-Akt, phosphorylation protein

kinase B; PKA, protein kinase A; p-PKA, phosphorylation protein kinase A; TRPV1, transient receptor potential vanilloid 1. See legend of Fig. 1 for other abbreviations.

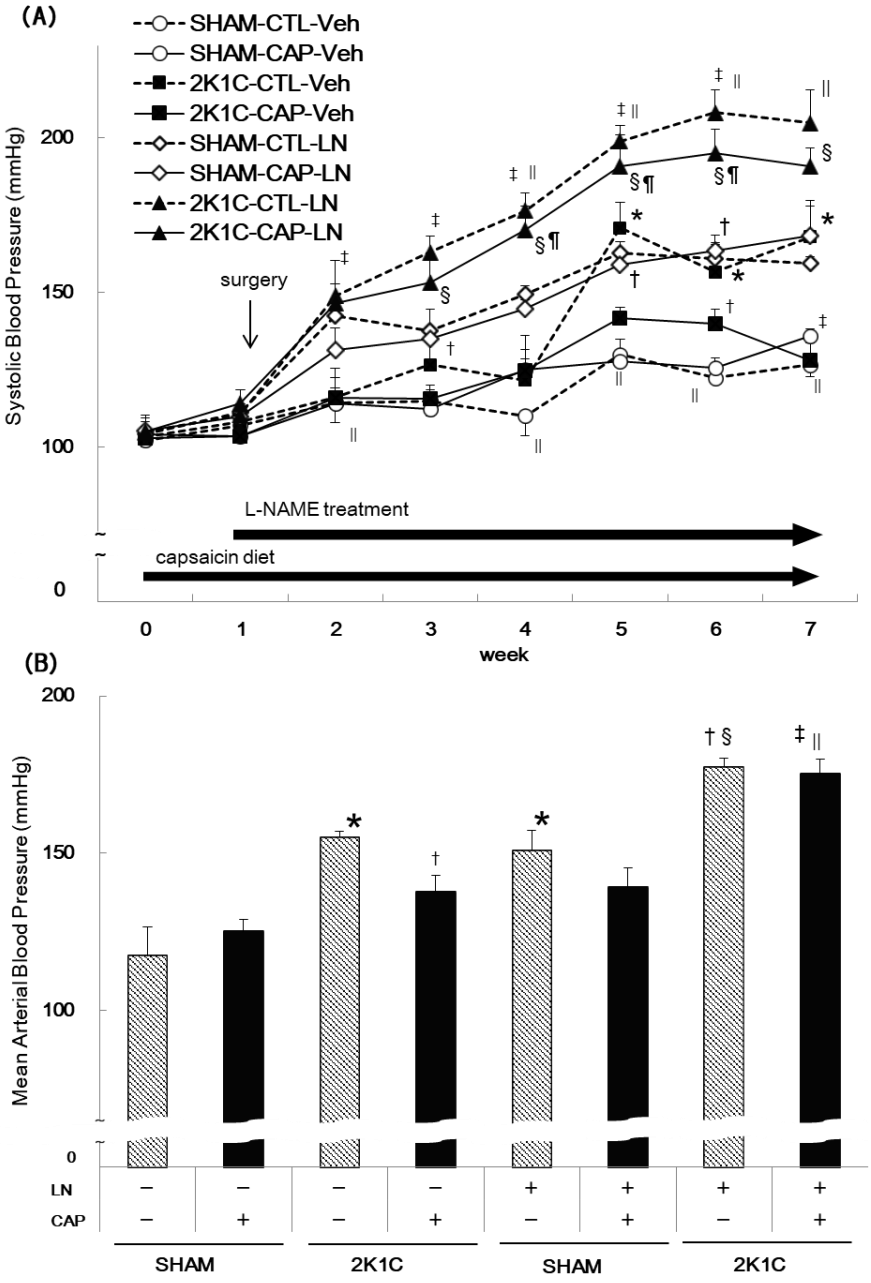


Fig. 5. Systolic blood pressure (A) and mean arterial blood pressure at the end of the protocol (B) in SHAM or 2K1C rats fed a diet with or without CAP and drinking tap water or LN in tap water for 6 weeks after surgery. MAP was

measured through a catheter in the left common femoral artery under anesthesia. Values are mean ± SE; n = 4–6. (A) Four-way ANOVA, $P < 0.05$ for time, animal (SHAM vs. 2K1C), diet (CTL vs. CAP), drink (Veh vs. LN) and each interaction. $^{*}P < 0.05$ vs. SHAM-CTL-Veh. $^{\dagger}P < 0.05$ vs. SHAM-CAP-Veh. $^{\ddagger}P < 0.05$ vs. 2K1C-CTL-Veh. $^{\S}P < 0.05$ vs. 2K1C-CAP-Veh, $^{\|}P < 0.05$ vs. SHAM-CTL-LN, $^{\P}P < 0.05$ vs. SHAM-CAP-LN. (B) Three-way ANOVA, $P < 0.05$ for time, animal (SHAM vs. 2K1C), drink (Veh vs. LN) and animal × diet ×drink. $^{*}P < 0.05$ vs. SHAM-CTL-Veh, $^{\dagger}P < 0.05$ vs. 2K1C-CTL-Veh, $^{\ddagger}P < 0.05$ vs. 2K1C-CAP-Veh, $^{\S}P < 0.05$ vs. SHAM-CTL-LN, $^{\|}P < 0.05$ vs. SHAM-CAP-LN. See legend of Fig. 1 for other abbreviations.

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