เคอร์คูมินลดการตอบสนองของหลอดเลือดต่อการกระตุ้นระบบประสาท ซิมพาเทติกในหลอดเลือดมีเซนเตอริกของหนูความดันเลือดปกติและ ความดันเลือดสูง

พวงรัตน์ ภักดีโชติ[1*], ยุพา คู่คงวิริยพันธุ์[1], วาริณี เบิกบาน[4], ปาริฉัตร ประจะเนย์[2], ปณต ตั้งสุจริต[3], วิไลวรรณ กฤษณะพันธ์[1], วีรพล คู่คงวิริยพันธุ์[3]

[1]ภาควิชาสรีรวิทยา, [2]ภาควิชากายวิภาคศาสตร์, [3]ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น 40002
[4]คณะแพทย์แผนไทยและแพทย์ทางเลือก มหาวิทยาลัยราชภัฏอุบลราชธานี จังหวัดอุบลราชธานี

Curcumin Decreases Vascular Responses to Sympathetic Nerve Stimulation in Mesenteric Vascular Beds of Normotensive and Hypertensive Rats

Poungrat Pakdeechote[1*], Upa Kukongviriyapan[1], Warinee Berkban[4], Parichat Prachaney[2], Panot Tangsucjarit[3], Veerapol Kukongviriyapan[3], Wilaiwan Khrisanapant[1]

[1]*Department of Physiology,* [2]*Department of Anatomy,* [3]*Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, 40002*
[4]*Faculty of Thai traditional and alternative medicine, Ubon Ratchathani Rajabhat University (UBRU), Ubon Ratchathani, Thailand, 34000*

หลักการและวัตถุประสงค์: เคอร์คูมินมีฤทธิ์ป้องกันระบบหัวใจและหลอดเลือดเนื่องจากมีฤทธิ์ต้านออกซิเดชันและต้านการอักเสบ การศึกษาครั้งนี้มีสมมติฐานว่าเคอร์คูมินอาจมีผลปรับการตอบสนองของหลอดเลือดต่อการกระตุ้นระบบประสาทซิมพาเทติกที่มาเลี้ยงหลอดเลือดในหนูความดันเลือดปกติและหนูความดันเลือดสูง

วิธีการศึกษา: หนูทดลองพันธุ์สแปรกดอลเลย์ (Sprague-Dawley rats) เพศผู้ (200-225 กรัม) ถูกเหนี่ยวนำให้เกิดภาวะความดันเลือดสูงด้วยสารแอลเนม (N[G]-nitro-L-arginine methyl ester, L-NAME) ในน้ำดื่ม (40 มก./กก./วัน 3 สัปดาห์) ในขณะที่หนูความดันเลือดปกติได้รับน้ำกลั่น จากนั้นหลอดเลือดมีเซนเตอริกถูกแยกออกมา เซลล์ชั้นเอนโดทีเลียมถูกกำจัดด้วยสารโซเดียมดีออกซีโคเลท (sodium deoxycholate) และให้สารแคปไซซิน (capsaicin) (เพื่อกำจัดสารสื่อประสาทจากระบบรับความรู้สึก) การหดตัวตอบสนองของหลอดเลือดต่อการกระตุ้นด้วยไฟฟ้า (5-40 เฮิรตซ์) และต่อสารนออะดรีนาลีน (noradrenaline) (1 ไมโครโมล-1 มิลลิโมล) หรือ ฟีนิลเอฟรีน (phenyephrine)

Background and objective: Curcumin exhibits cardiovascular protective effects regarding to its antioxidant and anti-inflammatory effects. This study hypothesized that curcumin would modulate vascular responses to sympathetic nerve stimulation in normotensive and hypertensive rats.

Methods: Male Sprague-Dawley rats (200-225 g) were induced hypertension by administering N[G]-nitro-L-arginine methyl ester (L-NAME) (40 mg/kg/day, 3 weeks) in drinking water while normotensive rats were given distilled water. Mesenteric vascular beds from both normotensive and hypertensive rats were isolated. Chemical removal of vascular endothelium by sodium deoxycholate was performed and preparations were pretreated with capsaisin (0.1 μM), to deplete sensory neurotransmitters. Contractile responses to electrical field stimulation (EFS 5-40 Hz, 90V, 1 ms for 30s, at 5-min intervals) and exogenous noradrenaline (NA) (1 μmol-

*Corresponding author: Poungrat Pakdeechote Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, 40002 e-mail: ppoung@kku.ac.th

(1 ไมโครโมล-1 มิลลิโมล) ถูกทดสอบในสภาวะที่มีเคอร์คูมิน (curecumin) (10⁻⁶ โมลาร์)

<u>ผลการศึกษา</u>: หนูความดันเลือดสูงมีความดันเลือดซิสโทลิก สูงเมื่อเปรียบเทียบกับหนูความดันเลือดปกติ (178±5 vs. 121±2 มิลลิเมตรปรอท, p<0.001) เคอร์คูมินลดการตอบ สนองของหลอดเลือดต่อระบบประสาทซิมพาเทติก สาร นออะดรีนาลีน สารฟีนิลเอฟรีน ในหลอดเลือดจากหนูทุกกลุ่ม อย่างมีนัยสำคัญทางสถิติ (p<0.05)

<u>สรุป</u> : เคอร์คูมินมีฤทธิ์ยับยั้งการหดตัวของหลอดเลือด เนื่อง จากการกระตุ้นระบบประสาทซิมพาเทติกทั้งใน หลอดเลือด หนูความดันเลือดปกติและความดันเลือดสูง โดยฤทธิ์ยับยั้งนี้ น่าจะเกี่ยวข้องกับการยับยั้งที่หลังจุดประสาน (postjunctional site)

1 mmol) or phenylephrine (PE) (1 μmol-1 mmol) were examined in the presence of curcumin (10⁻⁶ M).

Results: Hypertensive rats showed high systolic blood pressure compared to normotensive rats (178±5 vs. 121±2 mmHg, p<0.001). Curcumin significantly attenuated sympathetic nerve mediated-responses (p<0.05, n=6,) and contractile responses to exogenous NA and PE in all preparations (p<0.05).

Conclusions: Curcumin exhibits an inhibitory effect on sympathetic neurogenic vasoconstrictor responses in normotensive and hypertensive rat mesenteric vascular beds. This inhibitory effect is likely to involve the postjunctional site inhibition.

Key Words: curcumin, sympathetic nerve stimulation, mesenteric vascular beds

Introduction

Vascular diameter is influenced by a number of factors including vasodilator metabolites, circulating hormones, mediators released by the vascular endothelium and neurotransmitters released from sympathetic and sensory nerves. Noradrenaline (NA) is the principal sympathetic neurotransmitter released from synaptic vesicles of the sympathetic varicosity during nerve stimulation[1]. NA diffuses across the synaptic cleft and binds to α_1-adrenoceptors to produce vasoconstriction[2,3]. In addition, sensory nerve fibers also innervate some blood vessels generally releasing neuropeptides (Calcitonin gene-related peptide, CGRP) that cause vasorelaxation[4].

Curcumin is a major active compound derived from the spice turmeric[5] and used as an alternative medicinal agent. The beneficial effects of curcumin including, wound healing[6], antioxidant and antiinflammatory[7] have been reported. Furthermore, curcumin and its derivative also exhibit a cardiovascular protective effect in L-NAME hypertensive rats. For example, curcumin partially prevents the development of hypertension induced by chronic L-NAME administration relating to its vascular remodeling effects[8]. Nakmareong and coworkers found the antihypertensive and antioxidant effects of curcumin

and tetrahydrocurcumin in L-NAME hypertensive rats[9]. Nevertheless, little is known with regarding to the effect of curcumin on perivascular nerves mediated responses in normal and hypertensive rats. The aim of this study was to examine whether curcumin could modulate vascular responses to sympathetic nerve stimulation in normotensive and L-NAME induced hypertensive rats.

Materials & methods

Animals

Male Sprague-Dawley rats (220-225 g) were obtained from the Animal Care Unit of the Faculty of Medicine, Khon Kaen University (Khon Kaen, Thailand). All animals were maintained in a temperature controlled room at 24°C with a 12-hour dark/light cycle. The animals were given free access to standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and distilled water (DW) or L-NAME (40 mg/kg/day) in drinking water. All animal procedures were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 20/2551).

Induction of L-NAME hypertension

Rats were induced hypertension by administering L-NAME (50 mg/kg/day) in drinking water for 3 weeks while control rats received DW[10]. Rats with systolic blood

pressure (SBP) higher than 160 mmHg (tail cuff measurement) were considered to be hypertension.

Mesenteric vascular bed preparations

After 3 weeks of treatment, rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) followed by exsanguination. The abdominal cavity was opened and the main branch of the superior mesenteric artery was identified, cleaned of connective tissue and cannulated with a blunted hypodermic needle (no.21). The superior mesenteric vein was cut and preparations were flushed gently with Kreb's solution (0.5 ml)[11]. Briefly, the mesenteric vascular bed was separated from the gut by carefully cutting close to the intestinal wall. The mesenteric bed preparation was placed on a stainless steel grid (7x5 cm) in a warm humid chamber (37°C) and perfused at a constant flow rate of 5 ml/min, using a peristaltic pump (07534-04, Cole-Palmer Instrument, Illinois, USA.). Kreb'solution is composed of the following (mM): NaCl 118, NaHCO$_3$ 25, KCl 4.8, KH$_2$PO4 1.2, MgSO$_4$.7H$_2$O 1.2, CaCl$_2$ 1.25 and glucose 11.1. The solution was maintained at 37 $^{\circ}$C and continually gassed with a 95% O2 and 5% CO2 gas mixture. Mesenteric vascular responses were detected as changes in perfusion pressure (mmHg). Mean perfusion pressure was monitored using a pressure transducer and the data recorded using BIOPAC System (Inc., California, USA.). The preparation was allowed to equilibrate for 30 min before experimentation.

Chemical denuation of vascular endothelium

The vascular endothelium was chemically removed using sodium deoxycholate (SD) 1.8 mg/ml in saline for 30 s[12]. SD produces a transient increase in perfusion pressure (20-30 mmHg). The preparation was maintained by a 30 min washout period. After chemical removal of vascular endothelium, preparations were pretreated with capsaisin (10-7 M) for 20 minutes, followed by a 15 minutes washout period, to deplete sensory neurotransmitters and to desensitize vanilloid receptors[3]. A bolus injection of acetylcholine (ACh) (1 nmol) through rubber tubing proximal to the tissue to confirm the endothelial functions was also performed. Acetylcholine produces vasodilation by activation of nitric oxide production from endothelial cells.

Experimental protocols

Effects of curcumin on contractile responses to sympathetic nerve stimulation in mesenteric vascular beds isolated from normal and L-NAME induced hypertensive rats

Contractile responses to electrical field stimulation (EFS 5-40 Hz, 90V, 1 ms for 30s, at 5-min intervals) were performed. A second frequency response curve was generated after a further 30 minutes and served as a time control. In a further two groups of experiment, contractile responses to EFS (5-40 Hz, 90V, 1 ms for 30s, at 5-min intervals) were recorded in the presence of curcumin (0.1 μM) after obtaining an initial control frequency response curve.

Effects of curcumin on contractile response to exogenous a$_1$ adrenergic receptor agonists in mesenteric vascular beds isolated from normal and L-NAME induced hypertensive rats

After capsaicin pretreatment, dose-response curves (in 3-fold increments) were generated to either noradrenaline (1 μmol-1 mmol) or phenylephrine, a selective α_1-adrenergic receptor agonist (1 μmol-1 mmol), under baseline perfusion conditions.

Statistical analysis

Data are presented as mean $\pm$ S.E.M. Statistical comparisons between concentration curves were made using two-way analysis of variance (ANOVA) with a Duncan's multiple range post hoc test. Other comparisons were made using unpaired *t*-test. A value of p<0.05 was taken to indicate statistical significance.

Results

L-NAME induced-hypertensive rats showed higher SBP compared to normotensive rats (178$\pm$5 vs. 121$\pm$2 mmHg, p<0.001, n=12, unpaired *t*-test). Perfusion pressure of hypertensive preparations was significantly higher than that of normotensive preparations (37$\pm$2 vs. 26$\pm$2 mmHg, p<0.05, n=12, unpaired *t*-test).

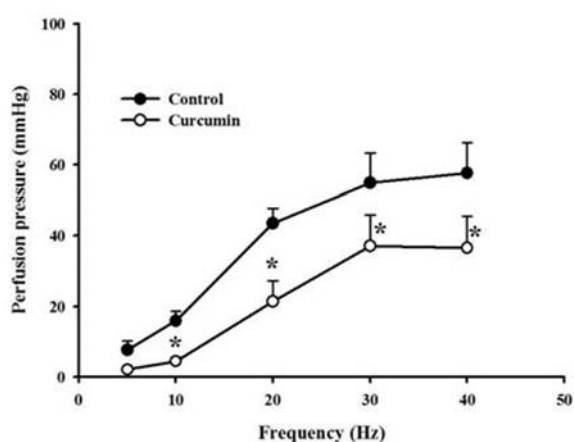**Effect of curcumin on nerve mediated vasoconstriction**

Electrical field stimulation produced an increase in perfusion pressure that was frequency-dependent. Two consecutive frequency response curves to electrical field stimulation were reproducible (time control). Curcumin

significantly attenuated contractile responses to sympathetic nerve stimulation in the perfused mesenteric vascular beds of both normal and L-NAME induced hypertensive rats (Figure 1A and 1B)
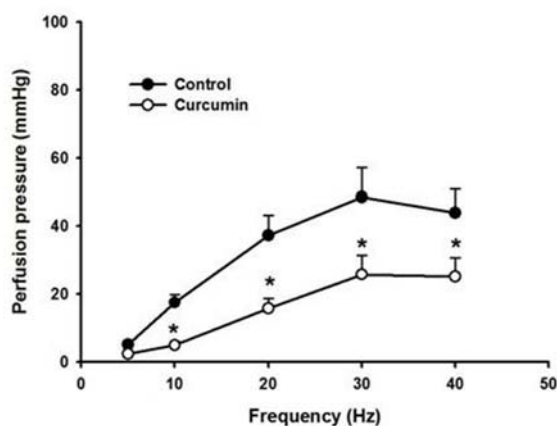
Effect of curcumin on contractile responses to exogenous noradrenaline and phenylephrine in the mesenteric vascular bed of normal and L-NAME hypertensive rats.

Dose-dependent pressor responses to exogenous noradrenaline and phenylephrine were not affected by time (time control). Curcumin significantly reduced contractile responses to exogenous noradrenaline or phenylephrine in the mesenteric vascular bed of normal preparations (figure 2A and 2B). This inhibitory action of curcumin was also found in hypertensive rat preparations (figure 3A and 3B).
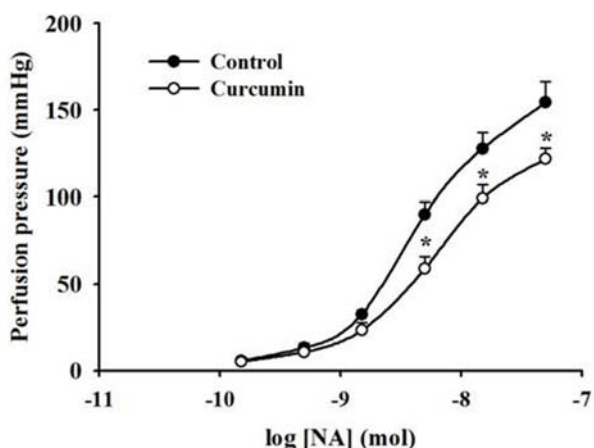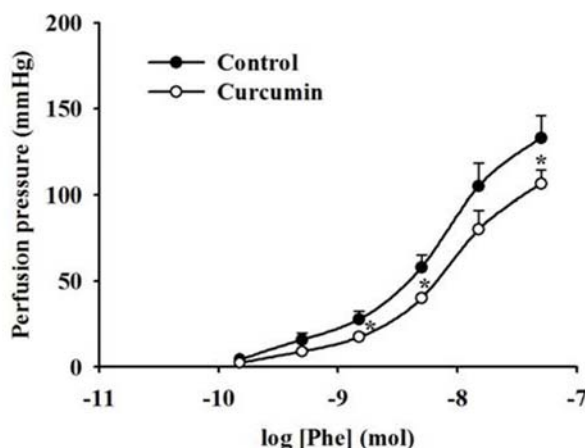

Figure 1   Effect of curcumin on contractile response to EFS (5-40 Hz, 90V, 1 ms for 30s, at 5-min intervals) in the perfused mesenteric vascular bed of normal (A) and L-NAME induced hypertensive rats (B) (n=6/group). * p <0.01 vs control (ANOVA).
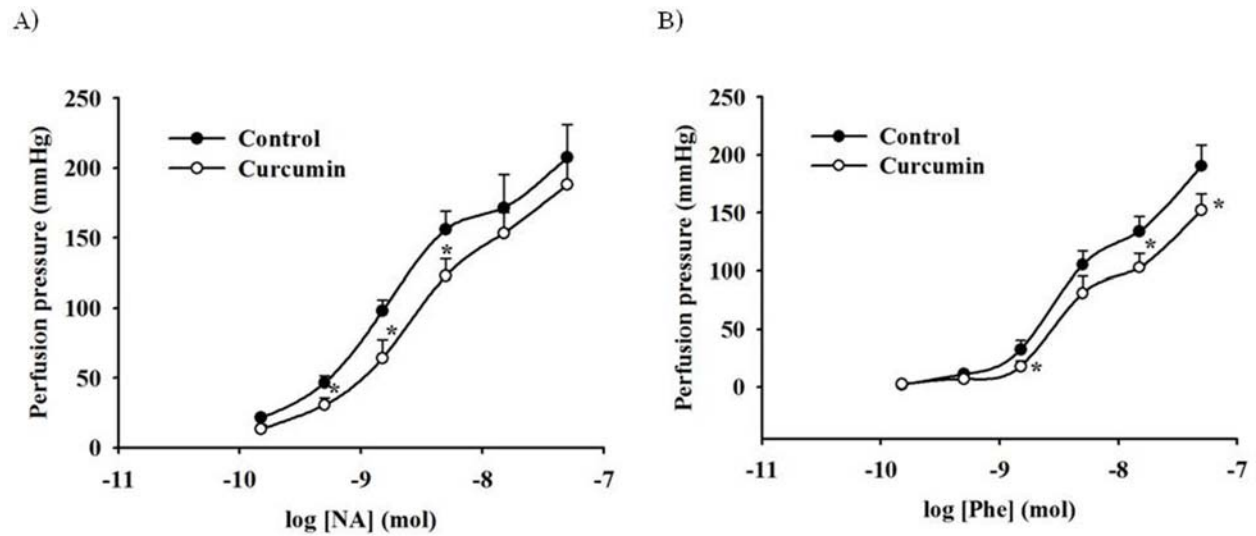

Figure 2   Effect of curcumin on contractile responses to exogenous noradrenaline (NA) (A) and phenylephrine (Phe) (B) in the perfused mesenteric vascular bed of normal rats (n=6/group). * p <0.05 vs control (ANOVA).

Figure 3 Effect of curcumin on contractile responses to exogenous noradrenaline (NA) (A) and phenylephrine (Phe) (B) in the perfused mesenteric vascular bed of L-NAME induced hypertensive rats (n=6/group). * $p<0.05$ vs control (ANOVA).

Discussion

The main findings of this study are that vasoconstriction response to sympathetic nerve stimulation was attenuated by curcumin in the rat perfused mesenteric vascular bed from both normal and hypertensive rats. In addition, dose-dependent pressor responses to NA and phenylephrine were significantly decreased in the presence of curcumin in all preparations. The mechanism whereby curcumin inhibited vasoconstriction induced by sympathetic nerve stimulation is unknown. However, curcumin should affect at the postsynaptic sites.

We have shown that curcumin exhibits an inhibitory effect on sympathetic neurogenic vasoconstrictor responses in normotensive and hypertensive rat mesenteric vascular beds. The decrease in nerve-mediated vasoconstriction may involve a decrease NA release by a prejunctional mechanism or postjuctional inhibition[13]. To prove the mechanism(s) where curcumin be active to decrease nerve-mediated vasoconstriction, we found the inhibitory effect of curcumin on exogenous noradrenaline and phenylephrine-induced vasoconstriction. This latter observation could indicate that the sympathoinhibitory effect of curcumin was mediated by

the postjunctional site. There is evidence to support the direct effect of curcumin that it mediated vasodilation and vasoconstriction on peripheral arteriole via adrenergic receptors[14] since the molecular structure of curcumin is similar to noradrenaline, and α-adrenergic or β-adrenergic agonists and antagonists[14]. This inhibitory action of curcumin could not involve CGRP-nergic nerve and endothelium-dependent vasorelaxation, because capsaicin pre-treatment to deplete sensory nerve neurotransmitters[3] and endothelium removal[12] were performed in this study.

In conclusions, curcumin inhibits vascular responses to the sympathetic nerve stimulation in the rat perfused mesenteric vascular bed isolated from normal and hypertensive rats. The simplest explanation for this change is that curcumin may not have direct effect on the availability of NA. This inhibitory effect is likely to involve the postjunctional site inhibition.

References

1. Falck B, Torp A. New evidence for the localization of noradrenalin in the adrenergic nerve terminals. Med Exp Int J Exp Med 1962;6:169-72.

2. Starke K, Endo T, Taube HD. Pre- and postsynaptic components in effect of drugs with alpha adrenoceptor affinity. Nature 1975;254:440-1.

3. Pakdeechote P, Rummery NM, Ralevic V, Dunn WR. Raised tone reveals purinergic-mediated responses to sympathetic nerve stimulation in the rat perfused mesenteric vascular bed. Eur J Pharmacol 2007;563:180-6.

4. Kawasaki H, Takasaki K, Saito A, Goto K. Calcitonin gene-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance vessels of the rat. Nature 1988;335(6186):164-7.

5. Gupta SC, Prasad S, Kim JH, Patchva S, Webb LJ, Priyadarsini IK, et al. Multitargeting by curcumin as revealed by molecular interaction studies. Nat Prod Rep 2011;28:1937-55.

6. Kulac M, Aktas C, Tulubas F, Uygur R, Kanter M, Erboga M, et al. The effects of topical treatment with curcumin on burn wound healing in rats. J Mol Histol 2013;44:83-90.

7. Fu Y, Zheng S, Lin J, Ryerse J, Chen A. Curcumin protects the rat liver from CCl4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Mol Pharmacol 2008;73:399-409.

8. Hlavackova L, Janegova A, Ulicna O, Janega P, Cerna A, Babal P. Spice up the hypertension diet - curcumin and piperine prevent remodeling of aorta in experimental L-NAME induced hypertension. Nutr Metab (Lond) 2011;8:72.

9. Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, Kongyingyoes B, et al. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. Naunyn Schmiedebergs Arch Pharmacol 2011;383:519-29.

10. Pakdeechote P, Kukongviriyapan U, Berkban W, Prachaney P, Kukongviriyapan V, Nakmareong S. Mentha cordifolia extract inhibits the development of hypertension in L-NAME-induced hypertensive rats J Med Plant Res 2011;5:1175-83.

11. Pakdeechote P, Dunn WR, Ralevic V. Cannabinoids inhibit noradrenergic and purinergic sympathetic cotransmission in the rat isolated mesenteric arterial bed. Br J Pharmacol 2007;152:725-33.

12. Sugiyama T, Hatanaka Y, Iwatani Y, Jin X, Kawasaki H. Lafutidine facilitates calcitonin gene-related peptide (CGRP) nerve-mediated vasodilation via vanilloid-1 receptors in rat mesenteric resistance arteries. J Pharmacol Sci 2008;106:505-11.

13. Tsuru H, Tanimitsu N, Hirai T. Role of perivascular sympathetic nerves and regional differences in the features of sympathetic innervation of the vascular system. Jpn J Pharmacol 2002;88(1):9-13.

14. Dewar AM, Clark RA, Singer AJ, Frame MD. Curcumin mediates both dilation and constriction of peripheral arterioles via adrenergic receptors. J Invest Dermatol 2011;131:1754-60.