Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 464 (2003) 63–67
www.elsevier.com/locate/ejphar

Involvement of calcitonin gene-related peptide in the depressor effects of losartan and perindopril in rats

Xu-Ping Qin[a], Feng Ye[a], Duan-Fang Liao[b], Yuan-Jian Li[a,*]

[a] *Department of Pharmacology, Xiang-Ya School of Medicine, Central South University, Changsha, Hunan 410078. PR China*
[b] *The Institute of Pharmacy and Pharmacology, Nanhua University, Hengyan, Hunan 421001, PR China*

Received 27 May 2002; received in revised form 21 January 2003; accepted 24 January 2003

Abstract

Previous investigations have indicated that calcitonin gene-related peptide (CGRP) plays an important role in the regulation of cardiovascular function, and that the development of hypertension may be related to the reduction of sensory vasodilator nerve actions. In the present study, we examined the effect of perindopril, an angiotensin-converting enzyme inhibitor, and losartan, an angiotensin II receptor antagonist, on the plasma level and synthesis of CGRP in 2 kidneys, 1-clip hypertensive rats (2K1C, Goldblatt). In the hypertension group, systolic blood pressure and mean artery pressure were raised, and the level of CGRP in plasma was slightly raised compared with control groups. Chronic treatment with losartan or perindopril significantly increased the plasma concentration of CGRP and the expression of CGRP mRNA in dorsal root ganglia in the 2K1C, Goldblatt hypertensive rats. These results suggest that the 2K1C, Goldblatt hypertensive model has a compensatory increase of sensory nerve actions, and that the depressor effects of perindopril or losartan may be related to stimulation of the synthesis and release of CGRP in the 2K1C Goldblatt hypertensive rats.

Keywords: CGRP (Calcitonin gene-related peptide); Perindopril; Losartan; Renohypertension

1. Introduction

Calcitonin gene-related peptide (CGRP), a 37-amino-acid peptide, is distributed widely in vascular tissues of both the central nervous system and the periphery. CGRP is a potent vasodilator and plays an important role in modulation of the total peripheral resistance of the systemic circulation through local reflex mechanisms. Recently, it has been shown that CGRP concentration in the plasma is decreased in patients with essential hypertension and in spontaneously hypertensive rats, suggesting that the alteration in the level of CGRP may be related to the development of hypertension (Xu et al., 1989; Wang et al., 1999; Yamada et al., 1998). However, the expression of CGRP varies considerably in different animal models of hypertension (Supowit et al., 1993, 1995; Katki, et al., 2001). To our knowledge, the hypothesis of capsaicin-sensitive sensory nerves has not yet been tested in the 2-kidneys, 1-clip (2K1C, Goldblatt) rat hypertensive model.

The renin–angiotensin system is an important promotive factor to the development of renovascular hypertension in the 2K1C, Goldblatt rat hypertensive model (Amiri and Garcia, 1997; An et al., 1999; Kagiyama et al., 2001). Angiotensin II, besides direct vasoconstriction, regulates neurotransmission in sympathetic nerves. Recently, it has been shown that angiotensin II is also capable of regulating capsaicin-sensitive sensory nerve actions in isolated mesenteric artery in spontaneously hypertensive rats (Kawasaki et al., 1998). Angiotensin-converting enzyme inhibitors and the angiotensin II receptor antagonists are widely used for treatment of hypertension. There is evidence that angiotensin-converting enzyme inhibitors can potentiate vasodilator responses mediated by capsaicin-sensitive sensory nerves (Kawasaki, 1992). In the present study, therefore, we examined whether the depressor effects of perindopril and losartan are involved in endogenous CGRP action in 2K1C Goldblatt rats.

* Corresponding author. Tel.: +86-731-4805-441; fax: +86-731-4471-289.
E-mail address: LiYJ@public.cs.hn.cn (Y.-J. Li).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01370-0

2. Materials and methods

2.1. Animal preparation and experimental protocol

Male Sprague–Dawley rats (220–250 g) were obtained from the Animal Center of Xiang-Ya School of Medicine. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 86-23, revised 1986). The animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The left renal artery was separated via an abdominal approach in the experimental groups and placed in a 0.3-mm silver clip. The sham-operated group underwent the same procedure, but without clipping of the renal artery. The animals were kept in cages in a room on a 12:12-h light/dark cycle, and on tap water and standard rat chew ad libitum. Systolic blood pressure was recorded between 9:00 and 12:00 a.m., once a week, using the tail-cuff method.

At the end of the fourth week, systolic blood pressure was very stable, and 18 rats (systolic blood pressure ≥ 160 mm Hg) were randomly divided into 3 groups. The drug-treated group was given losartan (20 mg/kg) or perindopril (3 mg/kg), and the drugs were dissolved in tap water (30–40 ml/day rat).

At the end of the experiment, the left carotid artery was cannulated under anesthesia, and mean blood pressure was measured with a pressure transducer and recorded on a polygraph (model LMS-2B, Chendu China).

2.2. Radioimmunoassay

At the end of the experiment, blood samples (2 ml) were collected from carotid artery. The plasma was obtained by centrifuging at $3500 \times g$ for 10 min at 4 °C. CGRP-like immunoactivity in the plasma was measured using antisera raised against rat CGRP, ^{125}I-labeled CGRP and rat CGRP standard.

2.3. RNA preparation and reverse-transcription polymerase chain reaction (RT-PCR)

After collection of blood samples, lumbar dorsal root ganglia were rapidly removed and homogenized in Trizol reagent. Total RNA isolation and semiquantitative RT-PCR were performed according to standard techniques. The specific primer pairs and the size of the expected products were as follows (forward and reverse, respectively): α-CGRP, 5′-AAGTTCTCCCCTTTCCTGGT-3′ and 5′-GGTGGGCACAAAGTTGTCCT-3′ (318 bp); β-actin, 5′-GAGACCTTCAACACCCCAGCC-3′ and 5′-TCGGGGCATCGGAACCGCTCA-3′ (422 bp) (Peng et al., 2002).

The PCR amplification profiles consisted of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 45 s. The linear exponential phases for α-CGRP and β-actin PCR were 28 and 25 cycles, respectively. Equal amounts of corresponding α-CGRP and β-actin RT-PCR products were loaded on 1.7% agarose gels. Optical densities of ethidium bromide-stained DNA bands were quantitated and the results were expressed as α-CGRP/β-actin ratios.

2.4. Reagents

Losartan was kindly provided by Merck (USA). Perindopril was produced by Les Laboratory Servier, 92200 Neuilly Sur Seine-France. Primers for PCR were synthesized by Sangon (Shanghai, P.R. China). Trizol reagent was obtained from GIBCO BRL (USA). The RT-PCR kits were purchased from Division of TaKaRa (Dalian, P.R. China). Radioimmunoassay kits for measurement of CGRP were purchased from the Immunity Institute of Dongya (Beijing, P.R.China).

2.5. Statistical analysis.

Data are expressed as means ± S.E.M. Statistical significance was determined by analysis of variance, followed by the Newman–Keuls–Student's *t*-test for multiple comparisons. The acceptable level of significance was $P < 0.05$.

3. Results

3.1. Blood pressure

There were no differences in the baseline value of systolic blood pressure among groups. One week after operation, systolic blood pressure in the operated group was significantly raised compared with the sham-operated

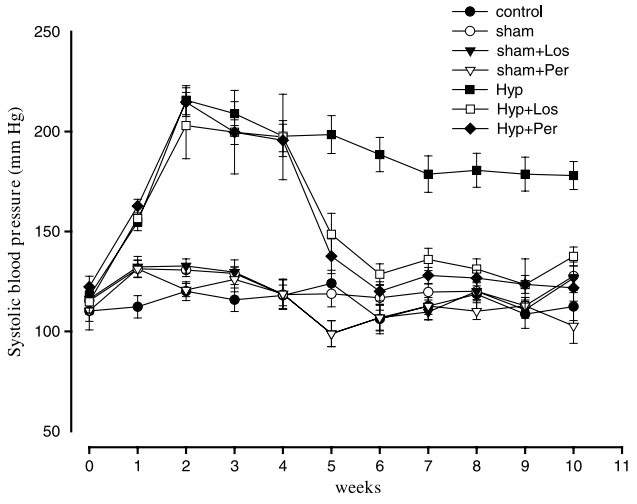

Fig. 1. Effect of perindopril or losartan on systolic blood pressure. Control: non-operated group; Sham: sham-operated group; Hyp: hypertension group; Los: losartan (20 mg/kg); Per: perindopril (3 mg/kg); All values were expressed as means ± S.E.M. ($n = 6$).

Table 1
Effect of losartan or perindopril on systolic blood pressure (mm Hg)

	Pre-operation	Post-operation	
		Pretreatment	Posttreatment
Control	110.2 ± 5.2	118.0 ± 5.6	112.4 ± 7.0
Sham	115.3 ± 6.7	119.4 ± 7.4	127.7 ± 7.0
Sham + losartan	116.2 ± 6.8	118.6 ± 7.3	126.7 ± 6.0
Sham + perindopril	110.3 ± 9.6	117.8 ± 7.4	102.7 ± 8.7
Hypertension (Hyp)	117.7 ± 4.2	197.7 ± 7.8[a]	178.0 ± 7.0
Hyp + losartan	114.8 ± 5.6	197.3 ± 21.4[a]	137.5 ± 4.7[b]
Hyp + perindopril	122.3 ± 4.5	195.6 ± 8.1[a]	121.8 ± 6.7[b]

Values are means ± S.E.M ($n = 6$).
[a] $P < 0.01$ vs. Sham or Control.
[b] $P < 0.01$ vs. Hyp.

or the non-operated group. At the end of the second week, systolic blood pressure in the operated groups reached a maximal level, then gradually decreased and remained at a higher and stable level during the experiment. Treatment with losartan or peridoperil for 1 week markedly decreased systolic blood pressure and its depressor effect was maintained during the experiment (Fig. 1; Table 1).

Similarly, mean blood pressure in the hypertension groups was significantly elevated compared with the sham-operated or non-operated group. Treatment with losartan or perindopril significantly decreased mean blood pressure (Fig. 2).

3.2. Plasma concentrations of CGRP

The plasma concentration of CGRP in the hypertensive rats was slightly increased compared with the sham-operated or non-operated group ($P < 0.05$). However, the plasma concentration of CGRP in the losartan- or perindopril-treated group was significantly increased compared with that in the hypertension group (Fig. 3). Treatment with losartan or perindopril had no effect on plasma concentrations of CGRP in normotensive rats (Fig. 3).

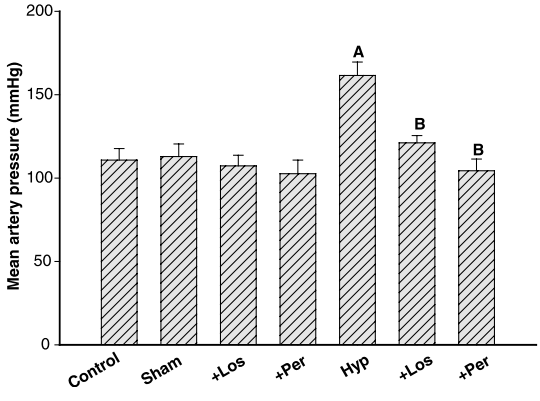

Fig. 2. Effect of losartan or perindopriln on mean artery pressure. Control: non-operated group; Sham: sham-operated group; Hyp: hypertension group; Los: losartan (20 mg/kg); Per: perindopril (3 mg/kg). Values are expressed as means ± S.E.M. ($n = 6$). [A]$P < 0.01$ compared with Sham. [B]$P < 0.01$ compared with Hyp.

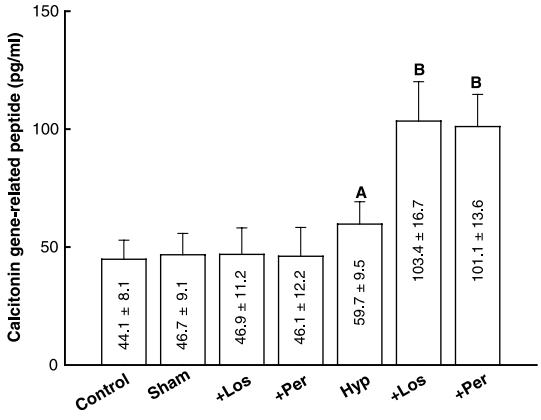

Fig. 3. Effect of perindopril or losartan on plasma concentrations of CGRP. Control: non-operated group; Sham: sham-operated group; Hyp: hypertension group; Los: losartan (20 mg/kg); Per: perindopril (3 mg/kg). Values are means ± S.E.M. ($n = 6$). [A]$P < 0.05$ compared with Sham. [B]$P < 0.01$ compared with Hyp.

3.3. CGRP mRNA expression

The expression of CGRP mRNA in dorsal root ganglia in the hypertension group was slightly increased compared with that in the sham-operated or non-operated group ($P < 0.05$). However, treatment with losartan or perindopril significantly increased the expression of CGRP mRNA compared with that in the hypertension group (Fig. 4).

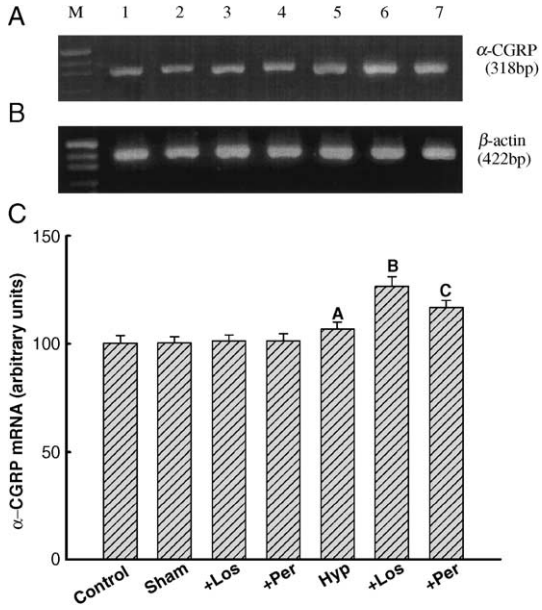

Fig. 4. Effect of perindopril or losartan on the expression of α-CGRP mRNA in DRG. Total cellular RNA samples isolated from DRG. The α-CGRP mRNA (A) and β-actin (B) were determined by quantification of blot analysis of RT-PCR. Optical densities of ethidium bromide-stained DNA bands were quantitated and the results were expressed as α-CGRP mRNA/β-actin ratios. The results are shown in the bar graph above the photo of RT-PCR products. (C) M: PUC 19 DNA/*Msp*I (*Hpa*II); 1: Control; 2: Sham; 3: Sham + losartan; 4: Sham + perindopril; 5: hypertension (Hyp); 6: Hyp + losartan; 7: Hyp + perindopril. Values are means ± S.E.M. ($n = 6$). [A]$P < 0.05$ compared with Sham; [B]$P < 0.05$, [C]$P < 0.01$ compared with Hyp.

Losartan or perindopril did not affect the expression of CGRP mRNA in normotensive rats (Fig. 4).

4. Discussion

Previous investigations have indicated that CGRP may play an important role in modulation of peripheral vascular tone. Systemic administration of CGRP produces a dose-dependent vasodilation in normotensive and hypertensive rats (Kawasaki et al., 1991; Shen et al., 2001). It has been found that vasodilator nerve actions in the mesenteric artery as well as plasma concentrations of CGRP were decreased in spontaneously hypertensive rats (Xu et al., 1989; Kawasaki, 1992). The decreased level of CGRP was also seen in patients with essential hypertension (Tang et al., 1989; Shi et al., 1990). CGRP has two isoforms, named α- and β-CGRP, which are encoded in different genes. Recently, it has been reported that in α-CGRP/calcitonin gene knockout mice systemic blood pressure was higher than in wild-type mice (Gangula et al., 2000). These findings suggest that the development of hypertension is related to a reduction of sensory nerve actions. Our results had shown that the cardiovascular actions of CGRP are mainly mediated by the α-CGRP isoform (Peng et al., 2002).

CGRP is synthesized in the cell bodies of primary sensory neurons and transported axonally to mainly peripheral but also to central nerve terminals, where it is stored in large, dense-cored secretory granules. It has been shown that synthesis and release of CGRP were decreased in spontaneously hypertensive rats (Supowit et al., 1993;). In contrast, an increase in CGRP production was observed in deoxycorticosterone acetate salt hypertensive rats and in subtotal nephrectomy salt rats models (Supowit et al., 1995; Supowit et al., 1997). Also, acute administration of the CGRP receptor antagonist CGRP-(8–37) increased the blood pressure in several models of hypertension such as deoxycorticosterone acetate salt, subtotal nephrectomy salt rats, and L-NAME-induced hypertension during pregnancy (Supowit et al., 1995, 1997, 1998, 2000; Gangula et al., 1997). In the present study, plasma concentrations of CGRP and the expression of CGRP mRNA in dorsal root ganglia were increased in the 2K1C Goldblatt hypertensive rats. These results support the hypothesis that the increased production of CGRP may act as a compensatory vasodilator mechanism to partially counter the systemic blood pressure increase.

The renin–angiotensin system is important for the regulation of sodium, potassium and fluid balance, and significantly influences vascular tone and sympathetic nervous system activity. All these factors contribute to blood pressure homeostasis. Angiotensin-converting enzyme inhibitors and angiotensin II type I (AT_1) receptor antagonists are used extensively for treatment of hypertension. Perindopril, the angiotesin-converting enzyme inhibitor, reduces levels of angiotensin II and attenuates responses to angiotensin II, resulting in vasorelaxation. Losartan, a blocker of the angiotensin AT_1 receptor, blocks the binding of angiotensin II to angiotensin AT_1 receptors in blood vessels and other tissues, relaxes smooth muscle, thereby promoting vasodilatation by preventing effects of angiotensin II. Previous investigators have reported that captopril can prevent the decreased vasodilator response and CGRP release induced by peripheral nerve stimulation in the mesenteric artery of spontaneously hypertensive rats (Kawasaki, 1992; Kawasaki et al., 1998), and that the neurogenic vasodilation was significantly inhibited by rennin substrates, angiotensin I, and angiotensin II in the spontaneously hypertensive rats, an effect which was abolished by angiotesin II receptor antagonists (Kawasaki et al., 1998). The present results revealed that perindopril or losartan significantly decreased blood pressure concomitantly with an increase in levels of CGRP in plasma in this hypertensive model. These findings suggest that the depressor effects of angiotesin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists may be related to stimulation of synthesis and release of CGRP.

It is noteworthy that perindopril and losartan had no effect on the synthesis and release of CGRP in normotensive rats. A similar effect has also been reported by other investigators (Kawasaki, 2002). A possible explanation is that, in hypertensive rats, the increased activation of the rennin–angiotensin system inhibits the synthesis and release of CGRP, and treatment with angiotensin-converting enzyme inhibitors or angiotensin AT_1 receptor antagonists attenuates or abolishes the inhibitory effect of angiotensin II on the synthesis and release of CGRP.

The mechanism responsible for the increased synthesis and release of CGRP induced by angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists in hypertensive rats is unclear. It has been reported that angiotensin II is capable of inhibiting autonomic neurotransmission in a variety of arteries of experimental animals (Ronai, 1990; Ferguson and Randall, 1989). As mentioned above, rennin substrate, angiotensin I, and angiotensin II inhibited the vasodilator response to periarterial nerve stimulation in the isolated perfused mesenteric vascular beds, and it has been hypothesized that angiotensin II may act on a presynaptic site (probably angiotensin II receptors) of CGRP-containing nerves to decrease the neurogenic release of CGRP (Kawasaki, 1998). It is possible that angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists increase the synthesis and release of CGRP through attenuation or abolition of the inhibitory effect of angiotensin II on sensory nerves. However, further work is needed before one can draw a definitive conclusion about this matter.

In summary, the present results suggest that the 2K1C Goldblatt hypertensive model exhibits a compensatory increase of sensory nerve action, and that the depressor effects of losartan and perindopril may be related to stimulation of the synthesis and release of CGRP in this hypertensive model.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation China (No. 30070870 and No. 30171084) and the National Major Basic Research Program of China (No. G2000056905).

References

Amiri, F., Garcia, R., 1997. Renal angiotensin II receptor regulation in two-kidney, one clip hypertensive rats: effect of ACE inhibition. Hypertension 30, 337–344.

An, M.R., Chung, Y.J., Kang, D.G., Nam, S.C., Lee, J., 1999. Augmented expression of cardiac atrial natriuretic peptide system in hypertensive rats. J. Korean Med. Sci. 14, 497–501.

Ferguson, D.R., Randall, M.D., 1989. Neurotransmission in pig renal artery: the actions of angiotensin II and dopamine. Br. J. Pharmacol. 96, 279–282.

Gangula, P.R., Supowit, S.C., Wimalawansa, S.J., Zhao, H., Hallman, D.M., Dipette, D.J., Yallampalli, C., 1997. Calcitonin gene-related peptide is a depressor in N^G-nitro-L-arginine methylester-induced hypertension during pregnancy. Hypertension 29, 248–253.

Gangula, P.R., Zhao, H., Supowit, S.C., Wimalawansa, S.J., Dipette, D.J., Westlund, K.N., Gagel, R.F., Yallampalli, C., 2000. Increased blood pressure in alpha-calcitonin gene-related peptide/calcitonin gene knockout mice. Hypertension 35, 470–475.

Kagiyama, S., Varela, A., Phillips, M.I., Galli, S.M., 2001. Antisense inhibition of brain renin–angiotensin system decreased blood pressure in chronic 2-kidney, 1 clip hypertensive rats. Hypertension 37, 371–375.

Katki, K.A., Supowit, S.C., Dipette, D.J., 2001. Role of calcitonin gene-related peptide and substance P in Dahl-salt hypertension. Hypertension 38, 679–682.

Kawasaki, H., 1992. Effects of chronic administration of antihypertensive drugs on vasodilation mediated by calcitonin gene-related peptide-containing vasodilator nerves in spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. 19, 569–573.

Kawasaki, H., 2002. Regulation of vascular function by perivascular calcitonin gene-related peptide-containing nerves. Jpn. J. Pharmacol. 88, 39–43.

Kawasaki, H., Saito, A., Goto, K., Takasaki, K., 1991. Age-related changes in calcitonin gene-related peptide (CGRP)-mediated neurogenic vasodilation of the mesenteric resistance vessel in SHR. Clin. Exp. Hypertens., A 13, 745–754.

Kawasaki, H., Takenaga, M., Araki, H., Fetagmi, K., Gomita, Y., 1998. Angiotensin inhibits neurotransmission of calcitonin gene-related peptide-containing vasodilater nerves in mesteric artery of spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 284, 508–515.

Peng, J., Lu, R., Deng, H.W., Li, Y.J., 2002. Involvement of α-calcitonin gene-related peptide in monophoshoryl lipid A-induced delayed preconditioning in rat hearts. Eur. J. Pharmacol. 436, 89–91.

Ronai, A.Z., 1990. Inhibition of neurotransmission by angiotensin I and II in rabbit isolated ear artery. Eur. J. Pharmacol. 179, 281–286.

Shen, Y.T., Pittman, T.J., Buie, P.S., Boldue, D.L., Kane, S.A., Koblan, K.S., Gould, R.J., Lynch Jr., J.J., 2001. Functional role of alpha-calcitonin gene-related peptide in the regulation of the cardiovascular system. J. Pharmacol. Exp. Ther. 298, 551–558.

Shi, X.Y., Yang, Y., Zhao, Y.T., 1990. Plasma calcitonin gene-related peptide level in patients with essential hypertention. Chung-Hua Nei K'o Tsa Chih - Chin. J. Intern. Med. 29, 616.

Supowit, S.C., Ramana, C.V., Westlund, K.N., Dipette, D.J., 1993. Calcitonin gene-related peptide gene expression in the spontaneously hypertension rat. Hypertension 21, 1010–1014.

Supowit, S.C., Gururaj, A., Ramana, C.V., Westlund, K.N., Dipette, D.J., 1995. Enhanced neuronal expression of calcitonin gene-related peptide in mineralocoricoid-salt hypertension. Hypertension 25, 1333–1338.

Supowit, S.C., Zhao, H., Hallman, D.M., Dipette, D.J., 1997. Calcitonin gene-related peptides is a depressor of deoxycorticosterone-salt hypertension in the rat. Hypertension 29, 945–950.

Supowit, S.C., Zhao, H., Hallman, D.M., Dipette, D.J., 1998. Calcitonin gene-related peptides is a depressor in subtotal nephrectomy hypertension in the rat. Hypertension 31, 391–396.

Supowit, S.C., Watts, S.C., Zhao, H., Wang, D., Dipette, D.J., 2000. Vascular reactivity to calcitonin gene-related peptides is enhanced in subtotal nephrectomy-salt hypertension. Hypertension 36, 701 (Abstract).

Tang, J.A., Xu, D., Yuan, X.Q., Meng, Z.H., Cheng, F.R., Chen, M.Z., Liu, G.Z., Liu, L.S., Zhang, Z.K., 1989. Calcitonin gene-related peptide in the pathogenesis and treatment of hypertension. Chin. Med. J. 102, 897–901.

Wang, C., Li, L., Lu, F., 1999. The change of plasma endothelin-1 and calcitonin gene-related peptide levels in patients with pregnancy induced hypertension. Zhonghua Fu-Chanke Zashi 34, 140–142.

Xu, D., Wang, W., Wang, J.P., Yuan, Q.X., Fiscus, R.R., Chang, J.K., Tang, J., 1989. Calcitonin gene-related peptide in normotensive and spontaneously hypertension rats. Peptides 10, 309–312.

Yamada, M., Ishikawa, T., Fujimori, A., Miyauchi, T., Goto, K., 1998. Enhanced depressor and hyperemic responses to calcitonin gene-related peptide in spontaneously hypertensive rats. Peptides 19, 697–701.