

This article was downloaded by:

On: 22 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713454007>

Effects of crocetin on the matrix metalloproteinases in cardiac hypertrophy induced by norepinephrine in rats

X. -C. Shen^a; Y.-Lu^a; Z. -Y. Qian^a

^a Department of Pharmacology, China Pharmaceutical University, Nanjing, China

To cite this Article Shen, X. -C. , Y.-Lu and Qian, Z. -Y.(2006) 'Effects of crocetin on the matrix metalloproteinases in cardiac hypertrophy induced by norepinephrine in rats', Journal of Asian Natural Products Research, 8: 3, 201 – 208

To link to this Article: DOI: 10.1080/10286020412331286452

URL: <http://dx.doi.org/10.1080/10286020412331286452>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Effects of crocetin on the matrix metalloproteinases in cardiac hypertrophy induced by norepinephrine in rats

X.-C. SHEN, Y.-LU and Z.-Y. QIAN*

Department of Pharmacology, China Pharmaceutical University, Nanjing 210009 China

(Received 23 February 2004; revised 20 April 2004; in final form 29 April 2004)

The effects of crocetin on the cardiac hypertrophy induced by long-term treatment with norepinephrine (NE) in rats have been investigated. The activities of matrix metalloproteinases (MMP-2 and MMP-9) have been assayed by gelatin SDS-PAGE zymography. The expressions of MMP-2 and MMP-9 were detected by RT-PCR. ATPase activity and hydroxyproline contents were measured with a commercial kit. The results show that crocetin blocked the development of left ventricular hypertrophy induced by NE, decreased the level of collagen in myocardium, enhanced both the Na⁺–K⁺ ATPase activity in cardiac tissue and the Ca²⁺–Mg²⁺ ATPase activity in mitochondria and inhibited significantly the activity of MMP-2 and the expressions of MMP-2 and MMP-9. These results suggest that crocetin may prevent cardiac hypertrophy induced by NE in rats.

Keywords: Crocetin; Norepinephrine; Cardiac hypertrophy; Matrix metalloproteinase

1. Introduction

Crocetin (figure 1), a carotenoid, is a primary active ingredient in extraction from *Crocus sativus* L (Saffron) [1]. Saffron, a traditional Chinese medicine, has long been used to treat coronary heart disease and hyperlipemia and can promote blood circulation by removing blood stasis. Previous studies indicate that crocetin could inhibit protein kinase A (PKA) and protein kinase C (PKC) [2]. In our studies it exhibited a strong anti-oxidative effect [3], the ability of anti-myocardial ischemia induced by deligating coronary artery in rats and dogs. It also significantly inhibited the cardiac hypertrophy induced by constriction of the abdominal aorta [4] and decreased the activities of MMP-2 and MMP-9 [5].

Chronic heart failure (CHF) is a cardiovascular disorder with multiple-etiology, high-prevalence and poor-prognosis. The levels of catecholamines circulating in the heart are associated with both heart failure [6] and its prognosis [7]. The enhanced activity of the sympathetic nervous system results in increased release of norepinephrine (NE) from the sympathetic nerve endings in the myocardium. Recent studies have shown that NE stimulates the production of intracellular reactive oxygen species (ROS) in cultured neonatal

*Corresponding author. Tel.: +86-025-83271322. Fax: +86-025-83271335. E-mail: sxc9860@sina.com

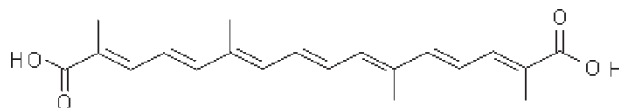


Figure 1. Chemical structure of crocetin.

rat cardiomyocytes [8]. The abnormal ROS can play an important role in signal transduction of the neonatal rat cardiomyocyte hypertrophy [9].

Matrix metalloproteinases (MMPs) belong to a family of proteolytic enzymes that has been implicated as playing a key role in extracellular matrix (EXM) degradation in several disorders [10]. Experimental animal hearts [11,12] and human hearts [13] with advanced heart failure or myocardial remodeling have also shown increases in activity of MMPs or messenger ribonucleic acid (mRNA) levels. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are particularly relevant in myocardial remodeling [14]. Mohamad reported that the patients of myocardial remodeling had a significant increase in zymographic activity of MMP-2 and MMP-9 [15]. Rouet-Benzineb suggests that MMP-2 and MMP-9 may be involved in disorganization of the contractile apparatus in dilated cardiomyopathy [16]. Concomitantly, activity and mRNA of MMP-2 had been increased in cardiac hypertrophy induced by long-term NE treatment in rats [17]. The inhibition of MMPs activities can attenuate the degree of cardiac hypertrophy [18,19]. In the present studies, we investigated the effects of crocetin on cardiac hypertrophy, activities and mRNA expressions of MMP-2 and MMP-9 in NE-induced cardiac hypertrophy in rats.

2. Results and discussion

2.1 Ventricular mass

Table 1 shows the NE-induced cardiac hypertrophy evaluated by left ventricular index and heart index, which refer to the ratios of left ventricular weight or heart weight and body weight. They were increased in hypertrophic heart induced by NE compared with a normal group. After treatment with crocetin the indexes were decreased significantly ($p < 0.05$, $p < 0.01$).

2.2 Collagen content and ATPase activity

The hydroxyproline assay showed that the level of hydroxyproline was significantly higher in the model group than that of normal group ($p < 0.01$), and it was much lower in

Table 1. Cardiac weight index after treatment with vehicle (control), captopril, and crocetin in rats.

Group	Dose (mg kg ⁻¹)	n	HW/BW	LVW/BW
Normal	–	9	2.88 ± 0.26	2.07 ± 0.15
Model	–	8	3.26 ± 0.23 ^a	2.40 ± 0.16 ^a
Captopril	60	9	2.95 ± 0.19 ^b	2.16 ± 0.16 ^b
Crocetin I	50	9	2.97 ± 0.24 ^c	2.18 ± 0.19 ^c
Crocetin II	25	9	3.03 ± 0.14 ^c	2.24 ± 0.11 ^c

BW: body weight; LVW: left ventricular weight; HW: heart weight. ^a $p < 0.01$ vs. normal, ^b $p < 0.01$ vs. model, ^c $p < 0.05$.

Table 2. Effects of crocetin on the level of hydroxyproline and ATPase activity on cardiac hypertrophy induced by NE.

Group	Dose (mg kg ⁻¹)	Na^+, K^+ -ATPase Ca^{2+}, Mg^{2+} -ATPase [$\mu\text{mol Pi (mg prot)}^{-1} \text{h}^{-1}$]		Hydroxyproline [$\mu\text{g (mg prot)}^{-1}$]
		Cardiac tissue	Mitochondrium	
Normal	–	0.994 ± 0.71	1.04 ± 0.61	0.373 ± 0.19
Model	–	0.232 ± 0.19 ^a	0.137 ± 0.10 ^a	0.783 ± 0.04 ^a
Captopril	60	0.839 ± 0.50 ^b	0.327 ± 0.31 ^c	0.631 ± 0.09 ^c
Crocetin I	50	0.927 ± 0.53 ^b	0.265 ± 0.21 ^c	0.636 ± 0.04 ^b
Crocetin II	25	0.625 ± 0.32 ^c	0.650 ± 0.54 ^c	0.628 ± 0.09 ^c

^a $p < 0.01$ vs. normal. ^b $p < 0.01$ vs. model. ^c $p < 0.05$.

crocetin-treated groups than that of model group. The ATPase activity of extracts from cardiac tissue in model group was much lower than that in a sham operation group ($p < 0.01$). After exposure of rats to crocetin and captopril, ATPase significant activity was evaluated ($p < 0.05$) (Table 2).

2.3 MMPs activity analysis

Figure 2 indicates that the activities of MMP-2 and MMP-9 were elevated significantly in the model group ($p < 0.01$), and treatment with crocetin, remarkably, decreased MMP-2 activity significantly but not that of MMP-9.

2.4 Levels of MMP-2 and MMP-9 mRNA in cardiac hypertrophy

NE induced up-regulation of MMP-2 and MMP-9 mRNA expression (figure 3). Crocetin and captopril down-regulated the mRNA expressions of both MMP-2 and MMP-9.

Many reports have shown that stimulation of myocardial β - and α -adrenergic receptors can result in the development of cardiac hypertrophy in rats [20,21]. ATPases of cardiac cells are one of the most important enzymes in maintaining the fluxes of vital cations by hydrolysis of the terminal high-energy phosphate of ATP [22]. Intracellular sodium concentration is overloaded following the decrease of Na^+, K^+ -ATPase. This overloading activates sodium–calcium exchanger of cardiac myocyte membrane, and calcium ions enter cardiac myocyte. Decreased activity of Ca^{2+}, Mg^{2+} -ATPase mainly results in disequilibrium of calcium and magnesium both infra- and exo-cell, and calcium aggregates in the cell. Decreased activity of ATPase leads to intracellular calcium overloading, which activates many signal conducting systems and kinase activation. In the end, it can cause cardiac hypertrophy. Decreased cardiac mechanical performance is reported to be highly related to decreased ATPase activity in several models of cardiac hypertrophy. Our results demonstrate that crocetin can increase the cardiac ATPase activity, and then improve the cardiac contractility.

In the process of the pathology of cardiac hypertrophy, the changes of phenotype of cardiac myocyte, including the augmentation of the cellular volume, the alteration of the type of contraction proteins, the proliferation of the myocardium interstitial cell, the hyperplasia of fibrous tissue, all result in piling up of the collagen and cellulose, and ultimately induce the derangement of myocardium constitution [23]. Our experimental results indicate that the myocardium hydroxyproline increased in cardiac hypertrophy, and that crocetin decreased it.

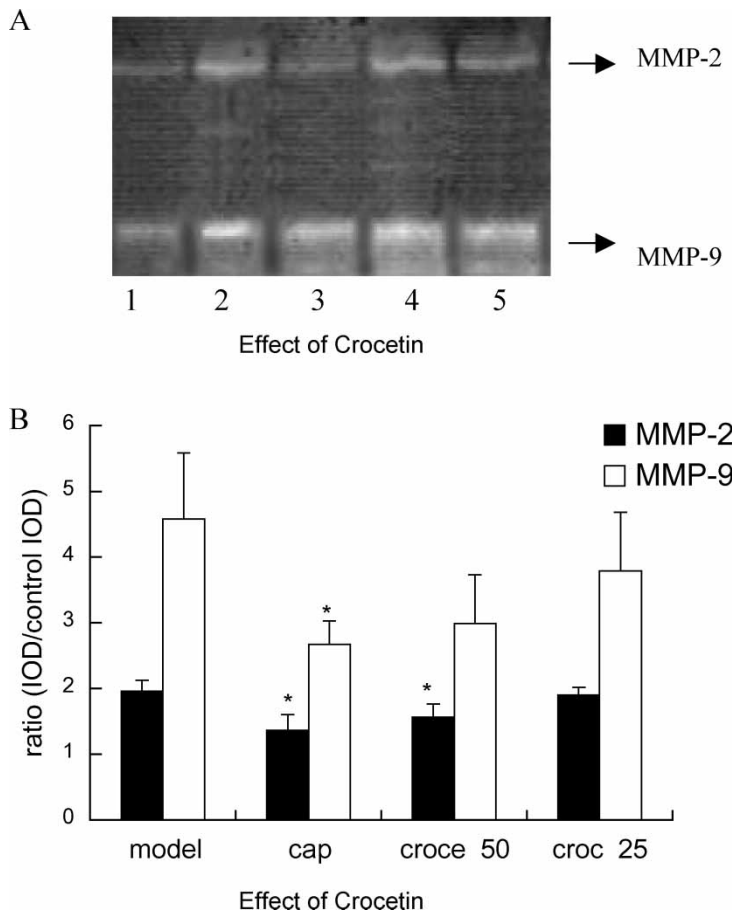


Figure 2. Gelatin SDS-PAGE zymogram analysis of MMP-2 and MMP-9 activity in NE-induced rat cardiac hypertrophy left ventricular tissue. Left ventricular tissue extracts were analyzed for MMP-2 and MMP-9 activity in different groups using SDS-PAGE zymograms containing gelatin. (A) Representative gelatin-containing zymogram illustrating MMP-2 and MMP-9 activity in left ventricular tissue extracts collected from different groups. Each lane was loaded with 8 μg protein extract from the LV [lane: 1, normal; 2, model; 3, captopril; 4, crocetin (25 mg kg^{-1}); 5, crocetin (50 mg kg^{-1})]. (B) Effect of crocetin on activity of MMP-2 and MMP-9 in NE-induced cardiac hypertrophy tissue. Ratios of $\text{IOD}_{\text{MMP-2}}/\text{IOD}_{\text{normal MMP-2}}$ and $\text{IOD}_{\text{MMP-9}}/\text{IOD}_{\text{normal MMP-9}}$; $n = 4$, mean \pm s. * $p < 0.05$ vs. model.

Subsequently, crocetin suppressed the increased collagen of interstitial cell and ameliorated myocardium reconstitution.

Matrix metalloproteinases (MMPs) are members of a family of zinc-dependent enzymes that degrade molecules of the extracellular matrix [20,23]. They have been implicated in contributing to myocardial remodeling in animal models and human with congestive heart failure and cardiomyopathy [24]. MMPs are suggested to play an important role in tissue remodeling under both normal and pathological conditions [24]. MMPs activity is indicated as having a positive correlation with cardiac hypertrophy. Treatment with MMP inhibitors may prevent myocardial remodeling. The present studies revealed that crocetin prevented cardiac hypertrophy by attenuating activities of MMP-2 and MMP-9 and expressions of MMP-2 and MMP-9.

In summary, crocetin may depress fibrillar collagen production, expressions of MMP-2 and MMP-9, and activities of MMP-2 and MMP-9 in NE-induced cardiac hypertrophy in rats.

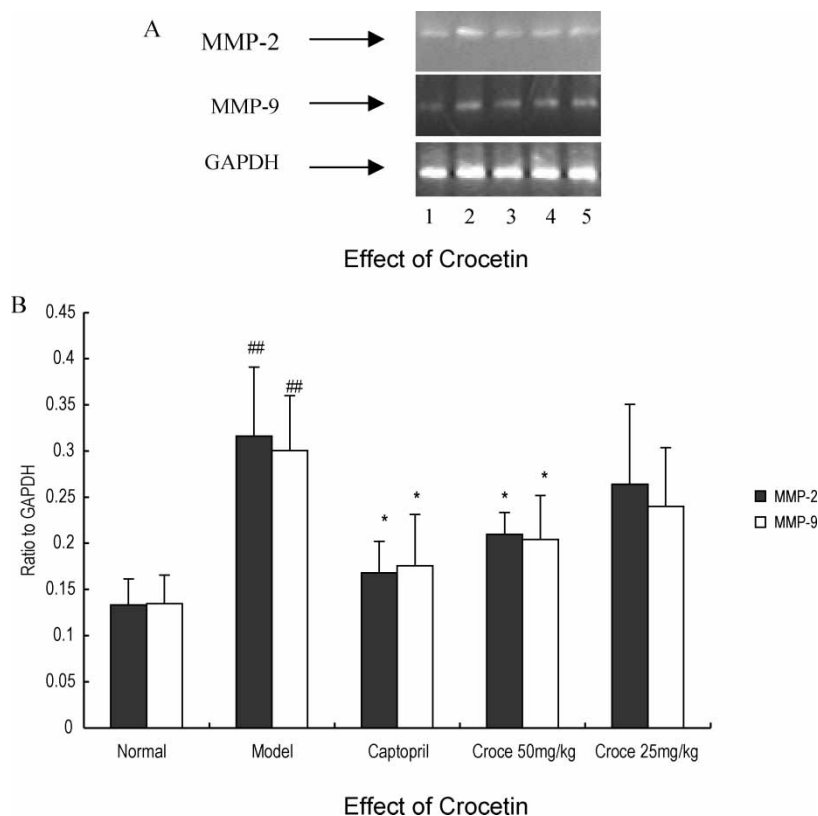


Figure 3. Effect of crocetin on mRNA expressions of MMP-2 and MMP-9 by RT-PCR. Expression of MMP-2, MMP-9 and GAPDH mRNA were determined by RT-PCR. PCR products were run and separated by 1.7% agarose gel. (A). Representative mRNA expressions for MMP-2 and MMP-9 determined by the RT-PCR method in the left ventricular tissues from NE-induced cardiac hypertrophy. [lane: 1, normal; 2, model; 3, captopril; 4, crocetin (50 mg kg^{-1}); 5, crocetin (25 mg kg^{-1})]. (B). Effect of crocetin on MMP-2 and MMP-9 expressions in cardiac hypertrophy induced by NE in rats. Ratios of $A_{\text{MMP-2}}/A_{\text{GAPDH}}$ and $A_{\text{MMP-9}}/A_{\text{GAPDH}}$ were used to express the levels of MMP-2 and MMP-9 mRNA in cardiac tissue, respectively. $n = 4$, mean \pm s, $*p < 0.05$ vs. model, $\#p < 0.05$ vs. normal.

The detailed mechanisms of crocetin in the prevention and treatment of cardiac hypertrophy remain to be investigated further.

3. Experimental

3.1 Drugs and reagents

Crocetin ($\text{C}_{20}\text{H}_{24}\text{O}_4$, >90% purity as determined by HPLC) was isolated and purified in our laboratory. NE was purchased from the Shanghai Hefen Pharmaceutical Factory. ATPase, hydroxyproline assay kits were purchased from Nanjing Jiancheng Bioengineering Institute. AMV reverse transcriptase was purchased from Gibco. *Taq* polymerase was purchased from Sangon (Shanghai, China). Other reagents were all of AR grade.

3.2 Animals

Female Sprague–Dawley (SD) rats (180–220 g) were provided by the Experimental Animal Center of China Pharmaceutical University and raised under constant temperature and

humidity conditions; they were given free access to a standard chow and tap water before and during the experimental period.

3.3 Cardiac hypertrophy

Norepinephrine ($1.5 \text{ mg kg}^{-1} \cdot \text{d}^{-1}$) was injected intraperitoneally in ascorbic acid saline twice daily for 15 d [20]. The animals were divided into five groups: (1) control (normal saline), (2) NE, (3) NE + crocetin ($50 \text{ mg kg}^{-1} \cdot \text{d}^{-1} \times 15 \text{ d}$), (4) NE + crocetin ($25 \text{ mg kg}^{-1} \cdot \text{d}^{-1} \times 15 \text{ d}$), (5) NE + captopril ($60 \text{ mg kg}^{-1} \cdot \text{d}^{-1} \times 15 \text{ d}$). Crocetin and captopril were treated i.g. After the rats were sacrificed, their hearts were removed, and the free wall of the right ventricle was separated from the intra-ventricular septum, which remained as a part of the left ventricle (LV). The left ventricles were weighed after freezing in liquid nitrogen and stored at -70°C . The left ventricular index and heart index were calculated by the ratios between left ventricular weight or heart weight and body weight.

3.4 Homogenization of LV tissue

LV tissues were sliced into small pieces and thoroughly washed with potassium phosphate buffer (50 mmol L^{-1}) to avoid contamination from blood. Homogenization of the LV tissue was performed following the method described by Lee *et al.* [25]. The homogenates were used to determine ATPase activity, hydroxyproline contents. Protein concentration was determined by the Coomassie brilliant blue method, using bovine serum albumin as a standard.

3.5 SDS-PAGE zymography

Extracellular proteins of approximately 25 mg frozen tissue were extracted with a 20-fold volume of extraction buffer [10 mM Tris-HCl pH 7.5, 150 mM NaCl, 20 mM CaCl_2 , $1 \mu\text{M}$ ZnSO_4 , 0.01% (v/v) Triton X-100, 1.5 mM NaN_3 , 0.5% PMSF] overnight at 4°C after homogenation. These extracts contained approximately 1.5 mg ml^{-1} protein. Myocardial matrix metalloproteinase activity was measured as previously described [26–28]. Briefly, gelatin [0.1% (w/v)] was added to a Laemmli acrylamide polymerization mixture. In a 1:2 preparation the extracts were mixed with substrate gel sample buffer [10% (w/v) SDS, 4% (w/v) sucrose, 0.25 mM Tris-HCl pH 6.8 and 0.1% (w/v) bromphenol blue]. Approximately $8 \mu\text{g}$ of mixture was loaded immediately without boiling. The gels were run at 15 mA per gel through stacking phase (4%) and at 20 mA per gel for the separating phase (10%), maintaining a running buffer temperature 4°C . After SDS-PAGE, the gels were washed twice with 2.5% Triton X-100 solution for 30 min each, with water and then incubated overnight at 37°C in a 0.05 mol L^{-1} Tris-HCl buffer, pH 7.5, containing 5 mmol L^{-1} CaCl_2 . The gels were then stained for 60–90 min in 0.25% Coomassie brilliant blue R250 in acetic acid–methanol–water (1:4.5:4.5 by volume) and subsequently destained with 10% methanol and 7% acetic acid. Enzyme activity of MMP-2 and MMP-9 can be visualized in the gelatin-containing zymograms as clear bands against a blue background. The relative clearing of each sample was quantitated by determining the inverse optical density, using Labwork 4.0 image acquisition and analysis software.

3.6 Reverse transcription-PCR

Total RNA was isolated according to the manufacturer's instructions. MMP-2 and MMP-9 were measured by reverse transcription polymerase chain reaction (RT-PCR). For MMP-2,

the sense primer was 5'-CCC AGA AAA GAT TGA CGC-3', and the antisense primer was 5'-CGA CAG CAT CCA GGT TAT-3'. The PCR product size was 314 bp, and the primer was designated by Primer 5.0 according to MMP-2 cDNA (Genebank NM-031054). For MMP-9, the sense primer was 5'-CGT GGC CTA CGT GAC CTATG-3', and the antisense primer was 5'-GGA TAG CTC GGT GGT GTC CT-3'; the PCR product size was 592 bp [29]. As an inner control, rat cDNA for glyceraldehyde 3-phosphate dehydrogenase (GADPH) mRNA was produced by RT-PCR according to Ref. [30]. Some 5 µg of total RNA was reverse transcribed into cDNA and then amplified using RT-PCR procedures. The amplification profile involved denaturation at 94°C for 5 min and at 94°C for 30 s, annealing at 58°C for 30 s (MMP-2 and GAPDH), 68°C for 30 s (MMP-9), and extension at 72°C for 30 s, followed by extension 72°C for 10 min; the number of necessary cycles was 30. The PCR products were separated by electrophoresis on a 1.7% agarose gel—the gel was stained in ethidium bromide after the electrophoresis was over. Identity was confirmed using Labwork 4.0 image acquisition and analysis software. The values are expressed as ratios of MMP-2/GAPDH, MMP-9/GAPDH.

3.7 Statistical analysis

All data were expressed as $\bar{x} \pm s$ and analyzed by the *t*-test.

Acknowledgements

The authors are grateful to Professor Jing-jing Liu for his technical help and to Ph.D Hao Hong for valuable comments on the manuscript.

References

- [1] W.-Y. Gao, D.-Y. Zhu. *Chin. Tradit. Herb Drugs* **30**, 389 (1999).
- [2] H.-W. Bing, M.P. Gideon. *Phytochemistry* **41**, 55 (1996).
- [3] T.Z. Liu, Y. Lu, C.Y. Bao, Z.Y. Qian. *J. China Pharm. Univ.* **33**, 505 (2002).
- [4] X.C. Shen, Z.Y. Qian. *Chin. Tradit. Herb Drugs* **34**, 535 (2003).
- [5] X.S. Shen, Z.Y. Qian. *Acta Pharmacol. Sin.* **39**, 172 (2004).
- [6] J.A. Thomas, B.A. Marks. *Am. J. Cardiol.* **41**, 233 (1978).
- [7] J.N. Cohn, T.B. Levine, M.T. Olivari, V. Garberg, D. Lura, G.S. Francis, A.B. Simon, T. Rector. *N. Engl. J. Med.* **311**, 819 (1984).
- [8] J.D. Luo, F. Xie, W.W. Zhang, X.D. Ma, J.X. Guan, X. Chen. *Br. J. Pharmacol.* **132**, 159 (2001).
- [9] J.D. Luo, W.W. Zhang, G.P. Zhang. *Acta Pharmacol. Sin.* **23**, 124 (2002).
- [10] S.D. Shapiro. *Curr. Opin. Cell Biol.* **10**, 602 (1998).
- [11] M.L. Coker, C.V. Thomas, M.J. Clair, J.W. Hendrick, R.S. Krombach, Z.S. Galis, F.G. Spinale. *Am. J. Physiol.* **274**, H1516–H1523 (1998).
- [12] J.P. Cleutjens, J.C. Kandala, E. Guarda, R.V. Guntaka, K.T. Weber. *J. Mol. Cell. Cardiol.* **27**, 1281 (1995).
- [13] C.V. Thomas, M.L. Coker, J.L. Zellner, J.R. Handy, A.J. Crumbley, F.G. Spinale. *Circulation* **97**, 1708 (1998).
- [14] L.C. Amandal, L.B.J. Gregory, P. Thomas, S.J. Joseph. *Circulation* **105**, 1983 (2002).
- [15] H.Y.E. Mohamad, T. Murat, C.S. Randall, B.R. Norman, Y.D. Yang, V. Geoffrey, P. Kimerly, C. Daniel, M. Patrick, B.Y. James. *Circulation* **105**, 1955 (2002).
- [16] P. Rouet-Benzineb, J.M. Buhler, P. Dreyfus, A. Delcourt, R. Dorent, J. Perennec, B. Crozatier, A. Hartf, C. Lafuma. *Eur. J. Heart Fail.* **1**, 337 (1999).
- [17] B. Wilfried, B.R. Alexander, D. Alexander, D. Monika, L. Monika, A.B. Hideo, G.Z. Heinz. *Cardiovasc. Res.* **52**, 265 (2001).
- [18] L.E. Rohde, A. Ducharme, L.H. Arroyo, M. Aikawa, G.H. Sukhova, A. Lopez-Anaya, K.F. McClure, P.G. Mitchell, P. Libby, R.T. Lee. *Circulation* **99**, 3063 (1999).
- [19] A. Ducharme, S. Frantz, M. Aikawa, E. Rabkin, M. Lindsey, L.E. Rohde, F.J. Schoen, R.A. Kelly, Z. Werb, P. Libby, R.T. Lee. *J. Clin. Investig.* **106**, 55 (2000).

- [20] Y. Xiang, J. Huang. *Acta Pharmacol. Sin.* **21**, 850 (2000).
- [21] J.N. Tsoporis, A. Marks, H.J. Kahn, J.W. Butany, P.P. Liu, D.O. Hanion. *J. Clin. Investig.* **102**, 1609 (1998).
- [22] C. Moisin, N. Balta, V. Filcescu, I.F. Dumitriu, G. Stoian, G. Petec. *Rom. J. Physiol.* **35**, 303 (1998).
- [23] J. Tang, A.R. Zhou. *J. Chin. Med. Univ.* **26**, 208 (1994).
- [24] C.M. Dollery, J.R. McEwan, A.M. Henney. *Circ. Res.* **77**, 863 (1995).
- [25] W.C. Lee, K.Y. Lin, Y.T. Chiu, J.H. Lin, H.C. Cheng, H.C. Huang, P.C. Yang, S.K. Liu, S.J. Mao. *FASEB J.* **10**, 1198 (1996).
- [26] A. Deten, A. Holzl, M. Leicht, W. Barth, H.G. Zimmer. *J. Mol. Cell. Cardiol.* **33**, 1191 (2001).
- [27] F.G. Spinale, M.L. Coker, L.J. Heung, B.R. Bond, H.R. Gunasinghe, T. Etoh, A.T. Goldberg, J.L. Zellner, A.J. Crumbley. *Circulation* **102**, 1944 (2000).
- [28] S.C. Tyagi, L. Matsubara, K.T. Weber. *Clin. Biochem.* **26**, 191 (1993).
- [29] K. Masutomo, N. Makino, M.S. Fushiki. *Mol. Cell. Biochem.* **224**, 19 (2001).
- [30] J. Zhang, M. Wang, Z.J. Wang, J.W. Zhou, Y.B. Zhang. *Pharm. Biotechnol.* **10**, 33 (2003).