Original Article

Olmesartan Ameliorates Insulin Sensitivity by Modulating Tumor Necrosis Factor- α and Cyclic AMP in Skeletal Muscle

Koichi YAMAGUCHI, Nobuyuki URA, Hideyuki MURAKAMI, Nobuhiko TOGASHI, Masaya HYAKUKOKU, Katsuhiro HIGASHIURA, and Kazuaki SHIMAMOTO

We have reported that tumor necrosis factor (TNF)- α in skeletal muscle is one of the determinants of insulin resistance and that the renin-angiotensin system may be related to the regulation of TNF- α in skeletal muscle. Recent studies have suggested the involvement of cyclic adenosine monophosphate (cAMP) in the regulation of TNF- α in vascular smooth muscle cells or monocytes. The aim of this study was to determine the relationship between cAMP and TNF- α in skeletal muscle in connection with the renin-angiotensin system. Six-week-old male Sprague-Dawley rats were fed either normal rat chow or fructose-rich chow for 6 weeks. For the last 2 weeks of a 6-week period, the rats were treated with a vehicle or with an angiotensin II type 1 receptor antagonist (olmesartan medoxomil, 0.1 mg/kg/day). TNF- α levels in the soleus muscle were significantly higher and cAMP levels in the soleus muscle were significantly lower in fructose-fed rats than in control rats. Olmesartan increased cAMP and reduced TNF- α simultaneously in fructose-fed rats. There was a significant negative correlation between levels of cAMP and TNF- α . Moreover, a cAMP analogue reduced TNF- α levels in the soleus muscle. These results indicate that the increase in TNF- α *via* suppression of cAMP may affect the induction of insulin resistance. In addition, the facts that olmesartan increased cAMP and decreased TNF- α suggest that a part of the TNF- α regulation by angiotensin II might consist of modulation of cAMP through Gi protein activation in skeletal muscle. (*Hypertens Res* 2005; 28: 773–778)

Key Words: insulin resistance, cyclic adenosine monophosphate, tumor necrosis factor- α , angiotensin II

Introduction

Recently, insulin resistance (IR) and hyperinsulinemia have been shown to be common findings in patients with essential hypertension, diabetes mellitus, or hyperlipidemia (1). These impairments of glucose metabolism are thought to be associated with each other and to play a role in the development of arteriosclerosis and cardiovascular diseases (2, 3). IR has been reported in several animal models of hypertension, including spontaneously hypertensive rats (4) and fructose-fed rats (FFR). In FFR, it has been shown that a diet of fruc-

tose induces a decrease in insulin sensitivity and an increase in blood pressure (BP) (5). Skeletal muscle is thought to be a major regulator of systemic insulin sensitivity. Euglycemic hyperinsulinemic glucose clamp studies have demonstrated that skeletal muscle accounts for over 80% of glucose disposal under hyperinsulinemic conditions in humans. Lesions of atherosclerosis occur mainly in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain or extremities, resulting in infarction. Recently, Ross hypothesized that one of the mechanisms by which atherosclerosis occurs involves cytokines derived from monocytes and T lymphocytes (6).

From the Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan.

Address for Reprints: Nobuyuki Ura, M.D., Ph.D., Second Department of Internal Medicine, Sapporo Medical University School of Medicine, S1W16, Chuo-ku, Sapporo 060–8543, Japan. E-mail: ura@sapmed.ac.jp

Received March 25, 2005; Accepted in revised form July 25, 2005.

Table 1. Characteristics of Animals at 12 Weeks of Age

Characteristics	SD (n=8)	FFR (n=14)	FFR+olmesartan (n=9)
BW (g)	388±10	378±9	383±6
SBP (mmHg)	132±2	144±3*	136±4
PR (bpm)	318±7	331±6	330±16
FBG (mmol/l)	4.8±0.2	4.7±0.2	4.7±0.2

Values are means±SEM. SD, Sprague-Dawley rats; FFR, fructose-fed rats; FFR+olmesartan, fructose-fed rats treated with olmesartan for 2 weeks. BW, body weight; SBP, systolic blood pressure; PR, pulse rate; FBG, fasting blood glucose. * $p<0.05$ vs. SD, FFR+olmesartan.

Tumor necrosis factor (TNF)- α is a multipurpose cytokine that modulates immune functions, induces apoptotic cell death (7) and lysis of tumor cells (8), and stimulates production of other cytokines (9). Recent studies have suggested that TNF- α mediates IR by suppressing insulin signal transduction; TNF- α induces enhancement of serine phosphorylation of insulin receptor substrate (IRS)-1, resulting in inhibition of IRS-1 tyrosine phosphorylation by insulin. TNF- α is expressed in both adipose tissue and skeletal muscle, and many animal models of obesity and IR have been shown to have significantly higher TNF- α mRNA and protein levels than those in lean controls (9). We recently reported that IR and hypertension in FFR were concomitant with an increase in TNF- α in skeletal muscle and that an angiotensin II type 1 receptor blocker (ARB) decreased skeletal muscle TNF- α and improved IR in FFR (10). However, the relationship between IR and the mechanism of the regulation of TNF- α is not clear.

Recent studies have shown that cyclic adenosine monophosphate (cAMP) inhibits the release of TNF- α in human vascular smooth muscle, monocytes and hepatocytes (11–13). However, there have been no reports of the involvement of cAMP in the regulation of TNF- α in skeletal muscles. To evaluate the effects of angiotensin II on insulin resistance in skeletal muscle, TNF- α and cAMP were investigated simultaneously.

Methods

Study-1: Measurement of BP, Pulse Rate (PR) and Insulin Sensitivity in FFR

Six-week-old male Sprague-Dawley rats (Charles River Japan Inc., Yokohama, Japan) were used for the experiments. The care of the animals was in strict accordance with the guiding principles of the Physiological Society of Japan. Before any manipulation, all rats were fed standard rat chow containing 60% vegetable starch, 5% fat and 24% protein (Oriental Yeast Co., Tokyo, Japan). They were maintained on a 12-h light/dark cycle and chow *ad libitum*. The rats were

Fig. 1. Effect of olmesartan on insulin sensitivity (M value) of FFR. Values are the means±SEM. SD, Sprague-Dawley rats (n=8); FFR, fructose-fed rats (n=14); FFR+olmesartan, fructose-fed rats treated with olmesartan for 2 weeks (n=9).

acclimated to handling before randomization and then divided into two groups at the start of the study: those fed standard chow (SD group, n=8) or those fed fructose-rich chow containing 60% fructose, 5% fat and 24% protein (#78463; Teklad, Madison, USA) (FFR group, n=23) for 6 weeks. The latter group of rats was treated either with 0.1 mg/kg/day of olmesartan medoxomil, an ARB, in 2.5% gum arabic solution (FFR+olmesartan, n=9) or with a vehicle (2.5% gum arabic solution) (FFR, n=14), and controls were treated with the same vehicle, by gavage, for the last 2 weeks.

BP and PR Measurement

Systolic blood pressure (SBP) and PR were measured in all conscious rats using the indirect tail-cuff method. The averages of six such recordings were taken as the individual SBP and PR. Results obtained using this method were highly correlated with direct measurements.

Euglycemic Hyperinsulinemic Glucose Clamp Technique

At the end of the treatment period, rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). The left common carotid artery and the left jugular vein were exposed and then cannulated with a polyethylene tube (PE50; Becton Dickinson and Co., Sparks, USA) for collecting blood samples and for administration of the infusate. After overnight fasting (approximately 12 h), each rat was placed in a foam plastic jacket that allowed movement of all four limbs and forward vision. At the start of the clamp, fasting blood glucose (FBG) was measured, and the initial load of insulin (25 mU/kg of Humalin R, U-40; Shionogi Pharmaceutical Co., Osaka, Japan) was infused by a bolus, followed by an infusion of insulin at a rate of 4 mU/kg/min for 154 min. During the clamp, 12.5% glucose solution was infused as needed to maintain blood glucose at the preinfusion level. Ten microliters of arterial blood was sampled at 7-min intervals for deter-

Fig. 2. A: Comparison of TNF- α levels in the soleus muscle. B: Comparison of cAMP levels in the soleus muscle. Values are the means $\pm$ SEM. SD, Sprague-Dawley rats ($n=7$); FFR, fructose-fed rats ($n=9$); FFR+olmesartan, fructose-fed rats treated with olmesartan for 2 weeks ($n=7$).

mination of blood glucose. The average of the rate of glucose infusion for the last 35 min was taken as the index of insulin sensitivity (M value) of each rat.

Study-2: Measurement of TNF- α and cAMP in the Soleus Muscle in FFR

Biochemical Measurements of TNF- α and cAMP

Based on the results of Study-1, we prepared another three groups (SD, $n=7$; FFR, $n=9$; FFR+olmesartan, $n=7$), for measurement of TNF- α and cAMP. Rats were anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg) and perfused with 150 ml of sterile saline through the apex of the left ventricle, and then the soleus muscles were dissected immediately. The soleus muscles were weighed and frozen in liquid nitrogen. TNF- α and cAMP were investigated simultaneously.

To measure TNF- α , the muscles were homogenized with a Polytron (Kinematica, Luzern, Switzerland) in ice-cold 0.1 mol/l Tris-HCl buffer (pH 7.6) containing 1 mol/l NaCl, 2% low endotoxin, fatty acid free bovine serum albumin (BSA; Sigma Co., St. Louis, USA), 2 mmol/l ethylenediaminetetraacetic acid (EDTA disodium salt), 80 trypsin inhibitory units of aprotinin/l and 0.02% NaN_3 (buffer A) at 15% wet tissue wt/vol. The solution was centrifuged at $13,500 \times g$ for 30 min, and the supernatant was subjected to a TNF- α assay (14). TNF- α was measured with a rat ELISA kit (Biosource International, Camarillo, USA).

To measure cAMP, the remaining soleus muscles were homogenized with 0.5 ml acidic ethanol (1 mol/l HCl:ethanol, 1:100). Homogenates were transferred to eppendorf tubes and centrifuged at $12,000 \times g$ for 15 min. The supernatant solutions were combined and evaporated to dryness under a

stream of nitrogen at 55°C. The residue was dissolved in 50 mmol/l Tris and 4 mmol/l EDTA buffer (pH 7.5) and then frozen at -20°C until assayed. The volume of buffer (0.1 to 2.0 ml) for dissolving the residue was selected so that a 50- μ l aliquot of the sample would fall within the range of maximum sensitivity (0.5 to 4.0 pmol) for the cAMP assay. cAMP was assayed using a commercial protein-binding kit (Amersham, Arlington Heights, USA). Except for bovine insulin (Calbiochem, San Diego, USA), all chemicals were obtained from Sigma (15).

Study-3: Effects of cAMP on Regulation of TNF- α in the Soleus Muscle

Six-week-old male Sprague-Dawley rats were anesthetized with sodium pentobarbital and perfused with 150 ml of sterile saline through the apex of the left ventricle, and then the soleus muscles were cut longitudinally into small strips (25–35 mg/strip) under sterile conditions (16). After dissection, the soleus muscle strips were immediately pre-incubated in 1 ml of Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, Life Technologies, Inc., Rockville, USA) containing 2% BSA and then incubated in 1 ml DMEM containing 2% BSA with 0.1 mmol/l dibutyl cAMP (dBcAMP) as a cAMP analogue, or without dBcAMP, for 4 h. The medium was then subjected to a TNF- α assay.

Statistical Analysis

All data are expressed as the means $\pm$ SEM. Data were statistically analyzed with one-way analysis of variance (ANOVA) followed by Fisher's PLSD test for multiple comparisons. Regression analyses were used to compare the relationship

Fig. 3. Correlation between cAMP and TNF- α levels in the soleus muscle. SD, Sprague-Dawley rats; FFR, fructose-fed rats; FFR+olmesartan, fructose-fed rats treated with olmesartan for 2 weeks.

between TNF- α and cAMP. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Study-1

As shown in Table 1, SBP in FFR was significantly higher than that in the control rats, and olmesartan significantly lowered SBP in FFR. There were no significant intergroup differences in body weight, PR, and FBG. As shown in Fig. 1, the M value was significantly lower in FFR than in the control rats (18.4 ± 1.3 mg/kg/min and 12.6 ± 1.4 mg/kg/min in the control and FFR groups, respectively, $p<0.05$). Olmesartan significantly improved the M values of FFR (16.9 ± 1.7 mg/kg/min in the FFR+olmesartan group, $p<0.05$).

Study-2

As shown in Fig. 2a, TNF- α in the soleus muscle increased significantly in FFR compared to that in control rats (242 ± 81 pg/g wet tissue and 503 ± 41 pg/g wet tissue in the control and FFR groups, respectively, $p<0.05$). Olmesartan significantly lowered TNF- α in the soleus muscle (220 ± 23 pg/g wet tissue in the FFR+olmesartan group, $p<0.05$).

As shown in Fig. 2b, cAMP in the soleus muscle decreased significantly in FFR compared to that in control rats (195 ± 48 fmol/ μ g protein and 138 ± 19 fmol/ μ g protein in the control and FFR groups, respectively, $p<0.05$). Olmesartan significantly increased cAMP in the soleus muscle (186 ± 66 fmol/ μ g protein in the FFR+olmesartan group, $p<0.05$). The cAMP level was significantly correlated with the TNF- α level in the soleus muscle (Fig. 3).

Fig. 4. Comparison of TNF- α concentrations in the soleus muscles of SD rats. Values are the means $\pm$ SEM ($n=4$). dBcAMP, soleus muscle treated with dBcAMP for 4 h.

Study-3

dBcAMP significantly decreased the secretion of TNF- α from the soleus muscle in SD rats (2.5 ± 2.8 pg/ml/4 h and 34.1 ± 3.3 pg/ml/4 h for incubation with or without 0.1 mmol/l dBcAMP, respectively, $p<0.001$) (Fig. 4).

Discussion

This study confirmed previously reported results showing that feeding healthy rats fructose-rich chow results in IR and hypertension. Skeletal muscle TNF- α is associated with IR (9). Treatment with olmesartan resulted in recovery of the M value in FFR. We demonstrated that olmesartan significantly increased cAMP and lowered skeletal muscle TNF- α . The cAMP level was significantly correlated with the TNF- α level in FFR. Several possible mechanisms of IR have been proposed. These mechanisms include sympathetic nerve and renin-angiotensin system (RAS) activation and an increase in TNF- α in fat tissue or muscle. Recent studies have suggested that TNF- α mediates IR by suppressing insulin signal transduction; TNF- α induces enhancement of serine phosphorylation of IRS-1, resulting in inhibition of IRS-1 tyrosine phosphorylation by insulin. TNF- α is expressed in both adipose tissue and skeletal muscle, and many animal models of obesity and IR have been shown to have significantly higher TNF mRNA and protein levels than those in lean controls (9). We recently reported that IR and hypertension in FFR are concomitant with an increase in TNF- α in skeletal muscle (10). We have also reported that TNF- α significantly suppressed [3 H]2-deoxyglucose (2-DOG) uptake by insulin and decreased IRS-1 tyrosine phosphorylation in L6 myotubes derived from rat skeletal muscle (17). Also, a TNF- α converting enzyme inhibitor that inactivates TNF- α has been shown to improve IR in FFR (18).

Several studies have shown the effects of ARBs on IR in insulin-resistant animal models (10, 19). A possible mechanism by which ARBs ameliorate IR is the stimulation of

angiotensin type 2 (AT2) receptors and resultant increase in NO production. Olmesartan has been reported to prevent endothelin-1 induced hypertension and the formation of reactive oxygen species (20). Recently, Folli *et al.* suggested that angiotensin II impairs insulin signaling *via* the angiotensin type 1 (AT1) receptor by stimulating serine phosphorylation of the insulin receptor β subunit, IRS-1, and the regulatory subunit of phosphatidylinositol 3 (PI3) kinase (21). There has been some data indicating that angiotensin II increases TNF- α in many organs or cells other than skeletal muscle, such as cardiac fibroblasts (22), monocytes (23), and the kidneys (24). We recently reported that IR and hypertension in FFR were concomitant with an increase in TNF- α in skeletal muscle and that an ARB decreased skeletal muscle TNF- α and improved IR in FFR (10). Okada *et al.* demonstrated that olmesartan improves insulin sensitivity and overproduction of triglyceride in FFR independently of its hypotensive action (25). In our experiment, olmesartan decreased TNF- α and increased cAMP to almost the control levels. However, the precise mechanism of IR in FFR is still unknown, and our results cannot be explained by only angiotensin II blockade. Further investigation of these mechanisms is needed.

We demonstrated that olmesartan significantly increased cAMP and lowered skeletal muscle TNF- α . In fact, we showed that dBcAMP significantly decreased the secretion of TNF- α from the soleus muscle in SD rats. Also, lower levels of cAMP and higher levels of TNF- α were observed in FFR than in control rats. Meng *et al.* demonstrated that hypoxia decreases cellular cAMP levels and enhances macrophage secretion of TNF- α following LPS stimulation (26). It has also been reported that exogenous administration of dBcAMP attenuates TNF- α production (26), and that dBcAMP inhibits TNF- α production (27, 28). In the present *ex vivo* study we examined whether angiotensin II would decrease cAMP or increase TNF- α levels in the soleus muscles. Although TNF- α was increased after 2 to 4 h of incubation, cAMP was not detected either in the incubation medium containing angiotensin II or in that containing vehicle as a control. This was a limitation of our experiment, since cAMP was quickly degraded (29), and unlike in the previous *in vivo* study, there was no supply of ATP in the present study. Therefore, we could not detect any cAMP in the medium after 2 to 4 h of incubation, and further experiments will thus be needed to investigate whether cAMP is produced by the soleus muscle in the presence of angiotensin II.

In a previous study, angiotensin II decreased the forskolin-induced increase in cAMP in nonhypertrophic hearts of Dahl salt-sensitive rats (30). Angiotensin II has also been found to inhibit stimulation of cAMP by isoproterenol in rat heart myocytes (31). Finally, angiotensin II has been shown to inhibit both adenylate cyclase and cAMP accumulation in hepatocytes (32), the rat aorta (33), and the pituitary gland (34). The results of these studies raise the possibility that the adenylate cyclase inhibitory guanine nucleotide regulatory protein (Gi) plays a role in mediating responses to angiotensin

II (35). In A10 cells, angiotensin II has been shown to decrease the level of cAMP by enhancing the expression of Gi α (36).

The fact that olmesartan increased cAMP and decreased TNF- α suggests that a part of TNF- α regulation by angiotensin II might consist of modulation of cAMP through Gi protein activation in FFR. Based on the results of these studies, we assume that one of the mechanisms by which IR is induced by angiotensin II is an increase in TNF- α *via* suppression of cAMP.

In conclusion, the results of this study suggest that TNF- α in skeletal muscle is linked to IR and that olmesartan, an ARB, may improve IR in FFR either by increasing cAMP or decreasing TNF- α . There was a significant negative correlation between levels of cAMP and TNF- α in the soleus muscle. Also, dBcAMP significantly decreased the production of TNF- α in skeletal muscle. These results suggest that olmesartan ameliorates IR in FFR by modulating cAMP and TNF- α in the skeletal muscle, and that one of the mechanisms by which angiotensin II regulates TNF- α might be modulation of cAMP.

References

1. Imura O: Insulin resistance and hypertension in Japanese. *Hypertens Res* 1996; **19** (Suppl 1): S1–S8.
2. Reaven GM: Insulin resistance, hyperinsulinemia, and hypertriglyceridemia in the etiology and clinical course of hypertension. *Am J Med* 1991; **90** (Suppl): 7S–12S.
3. DeFronzo RA: Insulin resistance, hyperinsulinemia, and coronary artery disease: a complex metabolic web. *J Cardiovasc Pharmacol* 1992; **20** (Suppl II): S1–S16.
4. Hulman S, Falkner B, Freyvogel N: Insulin resistance in the conscious spontaneously hypertensive rat: euglycemic hyperinsulinemic clamp study. *Metabolism* 1993; **42**: 14–18.
5. Hwang IS, Ho H, Hoffman BB, Reaven GM: Fructose-induced insulin resistance and hypertension in rats. *Hypertension* 1987; **10**: 512–516.
6. Ross R: Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
7. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N: Macrophage-induced angiogenesis is mediated by tumor necrosis factor- α . *Nature* 1987; **329**: 630–632.
8. Mahony SM, Tisdale MJ: Role of prostaglandins in tumor necrosis factor induced weight loss. *Br J Cancer* 1989; **60**: 51–55.
9. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science* 1993; **259**: 87–91.
10. Togashi N, Ura N, Higashiura K, Murakami H, Shimamoto K: The contribution of skeletal muscle tumor necrosis factor- α to insulin resistance and hypertension in fructose-fed rats. *J Hypertens* 2000; **18**: 1605–1610.
11. Zhang LM, Castresana MR, Shker U, Dalton ML, Leeper-Woodford SK, Newman WH: Increased intracellular cyclic adenosine 3',5'-monophosphate inhibits release of tumor

- necrosis factor- α from human vascular tissue and cultured smooth muscle cells. *Crit Care Med* 1997; **25**: 1855–1861.
12. Shames BD, McIntyre RC Jr, Bensard DD, *et al*: Suppression of tumor necrosis factor α production by cAMP in human monocytes; dissociation with mRNA level and independent of interleukin-10. *J Surg Res* 2001; **99**: 187–193.
 13. Jianrong Li, Sufang Yang, Billiar TR: Cyclic nucleotides suppress tumor necrosis factor-mediated apoptosis by inhibiting caspase activation and cytochrome C release in primary hepatocytes *via* a mechanism independent of Akt activation. *J Biol Chem* 2000; **275**: 13026–13034.
 14. Murase K, Odaka H, Suzuki M, Tayuki N, Ikeda H: Pioglitazone time dependently reduces tumor necrosis factor- α level in muscle and improves metabolic abnormalities in Wistar fatty rats. *Diabetologia* 1998; **41**: 257–264.
 15. Kirby CR, Woodman CR, Woolridge D, Tischler ME: Cyclic adenosine monophosphate accumulation and beta-adrenergic binding in unweighted and denervated rat soleus muscle. *Metabolism* 1992; **41**: 793–799.
 16. Crettaz M, Prentki M, Zaninetti D, Jeanrenaud B: Insulin resistance in soleus muscle from obese Zucker rats. *Biochem J* 1980; **186**: 525–534.
 17. Yamaguchi K, Higashiura K, Ura N, *et al*: The effect of TNF- α on tissue specificity and selectivity to insulin signaling. *Hypertens Res* 2003; **26**: 389–396.
 18. Togashi N, Ura N, Higashiura K, Murakami H, Shimamoto K: Effect of TNF- α -converting enzyme inhibitor on insulin resistance in fructose-fed-rats. *Hypertension* 2002; **39**: 578–580.
 19. Shiuchi T, Iwai M, Li HS, *et al*: Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. *Hypertension* 2004; **43**: 1003–1010.
 20. Yao L, Kobori H, Rahman M, *et al*: Olmesartan improves endothelin-induced hypertension and oxidative stress in rats. *Hypertens Res* 2004; **27**: 493–500.
 21. Folli F, Kahn R, Hansen H, Julie L, Feener E: Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. *J Clin Invest* 1997; **100**: 2158–2169.
 22. Yokoyama T, Sekiguchi, Tanaka T, *et al*: Angiotensin II and mechanical stretch induce production of tumor necrosis factor in cardiac fibroblasts. *Am J Physiol* 1999; **276**: H1968–H1976.
 23. Hahn AW, Jonas U, Buhler FR, Resink TJ: Activation of human peripheral monocytes by angiotensin II. *FEBS Lett* 1994; **347**: 178–180.
 24. Ferreri NR, Zhao Y, Takizawa H, McGiff JC: Tumor necrosis factor- α -angiotension interactions and regulation of blood pressure. *J Hypertension* 1997; **15**: 1481–1484.
 25. Okada K, Hirano T, Ran J, Adachi M: Olmesartan medoxomil, an angiotensin II receptor blocker ameliorates insulin resistance and decreases triglyceride production in fructose-fed rats. *Hypertens Res* 2004; **27**: 293–299.
 26. Meng X, Ao L, Shames BD, Harken AH: Inhibition of cyclic-3',5' nucleotide phosphodiesterase abrogates the synergism of hypoxia with lipopolysaccharide in the induction of macrophage TNF- α production. *J Surg Res* 2001; **101**: 210–215.
 27. Procopio DO, Teixeira MM, Travassos LR, Ferguson MA, Almeida IC, Gazzinelli RT: Differential inhibitory mechanism of cyclic AMP on TNF- α and IL-12 synthesis by macrophages exposed to microbial stimuli. *Br J Pharmacol* 1999; **127**: 1195–1205.
 28. Katakami Y, Nakao Y, Koizumi T, Katakami N, Ogawa R, Fujita T: Regulation of tumor necrosis factor production by mouse peritoneal macrophages: the role of cellular cyclic AMP. *Immunology* 1988; **64**: 719–724.
 29. Broadus AE, Kaminsky NI, Hardman JG, Sutherland EW, Liddle GW: Kinetic parameters and renal clearance of plasma adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in man. *J Clin Invest* 1970; **49**: 2222–2236.
 30. Sunga PS, Rabkin SW: Angiotensin II induced alteration of cyclic adenosine 3' 5'-monophosphate generation in the hypertrophic myocardium of Dahl salt-sensitive rat on a high-salt diet. *Can J Physiol Pharmacol* 1994; **72**: 591–594.
 31. Allen IS, Gaa ST, Rogers TB: Changes in expression of a functional Gi protein in cultured rat heart cells. *Am J Physiol* 1988; **255**: C51–C59.
 32. Crane JK, Campanile CP, Garrison JC: The hepatic angiotensin II receptor. *J Biol Chem* 1982; **257**: 4959–4965.
 33. Anand-Srivastava MB: Angiotensin II receptors negatively coupled to adenylate cyclase in rat aorta. *Biochem Biophys Res Commun* 1983; **117**: 420–428.
 34. Enjalbert A, Sladeczek F, Guillon G, *et al*: Angiotensin II and dopamine modulate both cAMP and inositol phosphate productions in anterior pituitary cells. Involvement in prolactin secretion. *J Biol Chem* 1986; **261**: 4071–4075.
 35. Pobiner BF, Hewlett EL, Garrison JC: Role of Ni coupling angiotensin receptors to inhibition of adenylate cyclase in hepatocytes. *J Biol Chem* 1985; **260**: 16200–16209.
 36. Palaparti A, Ge C, Anand-Srivastava MB: Angiotensin II enhances the expression of Gi α in A10 cells (smooth muscle): relationship with adenylyl cyclase activity. *Arch Biochem Biophys* 1999; **365**: 113–122.