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Role of Endogenous Hydrogen Peroxide during Angiotensin Type 1 Receptor Blockers Administration in Pacing-induced Metabolic Coronary Vasodilatation in Dogs in Vivo *

Toyotaka Yada, Hiroaki Shimokawa, Osamu Hiramatsu, Masami Goto, Yasuo Ogasawara and Fumihiko Kajiya

Abstract—Background: **We have previously demonstrated that endothelium-derived hydrogen peroxide (H_2O_2) is an endothelium-derived hyperpolarizing factor (EDHF) in canine coronary microcirculation in vivo. However, the role of H_2O_2/EDHF during angiotensin type 1 receptor blockers (ARB) administration in metabolic coronary dilatation in vivo remains to be examined. We examined whether H_2O_2 during ARB administration is involved in pacing-induced metabolic coronary vasodilatation in dogs in vivo and if so, whether such beneficial effects of ARB administration acutely improve coronary vasodilatation in diabetes mellitus (DM).**

Methods: **Canine subepicardial coronary small arteries (CSA, ≥100 μm) and arterioles (CA, <100 μm) in left anterior descending artery area were continuously observed by an intravital microscope under cyclooxygenase blockade (ibuprofen, 12.5 mg/kg, intravenous infusion, iv). Experiments were performed during paired right ventricular pacing under the following 4 conditions (n=5 each); (i) control, (ii) DM (alloxan 40 mg/ kg, iv, 1 week prior to study), (iii) DM+ARB (olmesartan, 10 μg/kg/min, 10 min, intracoronary infusion, ic)+L-NMMA (NOS inhibitor, 2 μmol/min, ic) and (iv) DM+ARB+catalase (H_2O_2 discomposer, 1000 U/ml, 5 min, ic).**

Results: **Cardiac tachypacing (60 to 120 bpm) caused coronary vasodilatation in both-sized arteries under control conditions. DM significantly decreased the vasodilatation compared with control in CSA and there was a residual vasodilatation for the loss of NO in CA, whereas DM+ARB+L-NMMA improved the vasodilatation compared with DM alone in CA and was significantly decreased by DM+ARB+catalase in CA.**

Conclusions: **These results indicate that H_2O_2 during ARB administration is involved in pacing-induced metabolic coronary vasodilatation in DM in vivo and that there are substantial compensatory interactions between NO and H_2O_2.**

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T. Yada is with the Department of Medical Engineering, Kawasaki Medical School, Kurashiki, 7010192 JAPAN (corresponding author to provide phone: 81-86-462-1111; fax: 81-86-463-2975; e-mail: yada@me.kawasaki-m.ac.jp).

H. Shimokawa was with the Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, 9808574 JAPAN (e-mail: shimo@cardio.med.tohoku.ac.jp).

O. Hiramatsu is with the Department of Medical Engineering, Kawasaki Medical School, Kurashiki, 7010192 JAPAN (e-mail: hiramatsu@me.kawasaki-m.ac.jp).

M. Goto is with the Department of Medical Engineering, Kawasaki Medical School, Kurashiki, 7010192 JAPAN (e-mail: goto@me.kawasaki-m.ac.jp).

Y. Ogasawara is with the Department of Medical Engineering, Kawasaki Medical School, Kurashiki, 7010192 JAPAN (e-mail: ogasawara@me.kawasaki-m.ac.jp).

F. Kajiya is with the Department of Medical Engineering, Kawasaki Medical School, Kurashiki, 7010192 JAPAN (e-mail: kajiya@me.kawasaki-m.ac.jp).

I. Introduction

Cardiac tachycardia by pacing or exercise increases myocardial oxygen consumption and increases coronary blood flow by several vascular regulating factors.[1-3] Shear stress play a crucial role in modulating vascular tone by endothelium-derived releasing factor (EDRFs), including nitric oxide (NO), prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF). [4,5] Flow-induced vasodilatation is mediated by either NO, [6,7] PGI_2, [8] both of them, [9] or EDHF. [10] Matoba et al. have previously identified that endothelium-derived hydrogen peroxide (H_2O_2) is a primary EDHF in mesenteric arteries of mice and humans, [11,12] and Morikawa et al. have recently confirmed that endothelial Cu,Zn-superoxide dismutase (SOD) plays an important role as an EDHF synthase in mice. [13] Miura et al. demonstrated that endothelium-derived H_2O_2 is involved as an EDHF in the flow-induced vasodilatation of isolated human coronary arterioles in vitro. [14] We have recently confirmed that endogenous H_2O_2 plays an important compensatory role during coronary autoregulation [15] and reperfusion injury in vivo [16] through the interactions with NO and adenosine.

It is known that vascular α-adrenergic receptor is modulated by the endothelium in dogs [17] while cardiac β-adrenergic receptor is modulated by K_{Ca} channels in pigs [18] and H_2O_2 in mice. [19] We also have recently demonstrated that endothelium-derived H_2O_2 plays an important cardioprotective role in the canine coronary microcirculation in vivo, including metabolic coronary vasodilatation. [20] Angiotensin Type 1 Receptor Blocker (ARB) exerts anti-inflammatory and anti-oxidative stress effects in the rat and mouse hearts, [21] respectively.

We examined whether H_2O_2 during ARB administration is involved in pacing-induced metabolic coronary vasodilatation in dogs in vivo and if so, whether such beneficial effects of ARB administration acutely improve coronary vasodilatation in diabetes mellitus (DM).

II. Methods

A. Animal Preparation

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Anesthetized mongrel dogs of either sex (15-25 kg in body weight, n=20) were ventilated with a ventilator. We continuously monitored aortic pressure and left ventricular pressure (LVP) with a catheter and blood flow of the left anterior descending coronary artery (LAD) with a transonic flow probe.

B. Measurements of Coronary Diameter by Intravital Microscope

We continuously monitored coronary vascular responses by an intravital microscope with a needle-probe (magnification of x200-300) in vivo, as previously described. [22] Canine subepicardial coronary small arteries (CSA, ≥100 μm) and arterioles (CA, <100 μm) in LAD area were continuously observed by an intravital microscope. Measurements were performed during LAD area.

C. Plasma Levels of 8-Hydroxydeoxyguanosine

Blood samples were obtained at baseline and cardiac pacing from the catheterized coronary sinus. Measurement of plasma 8-OHdG was performed with ELISA.

D. Measurements of Cu,Zn-SOD Levels

Measurements for myocardial levels of Cu, Zn-SOD were performed by ELISA method. Samples were obtained after experiments.

E. Measurements of H_2O_2 Levels

H_2O_2 in myocardium was determined by quantitative measurement with an Amplex Red by ELISA method. Myocardial samples were obtained after experiments.

F. Experimental Protocols

After the surgical procedure and instrumentation, at least 30 min were allowed for stabilization while monitoring hemodynamic variables. Coronary vasodilator responses were examined before and after cardiac pacing (60 to 120 b.p.m.) under the following 4 conditions with cyclooxygenase blockade (ibuprofen, 12.5 mg/kg, IV) in order to evaluate the role of ARB in combination of H_2O_2 and NO without PGI_2 in a different set of animals; (a) control condition, (b) DM (alloxan 40 mg/kg iv, 1 week prior to study), (c) DM+ARB (olmesartan, 10 μg/kg/min, ic)+L-NMMA (NOS inhibitor, 2 μmol/min, ic) and (d) DM+ARB+catalase (H_2O_2 discomposer, 1000 U/ml, 5 min, ic). The basal coronary diameter was defined as that before pacing. We continuously observed the diameter change in subepicardial CSA and CA with an intravital microscope before and at 2min after pacing.

G. Drugs

Olmesartan was obtained from Daiichi-Sankyo Pharmaceutical Co (Tokyo, Japan). Other drugs were obtained from Sigma Chemical Co. and were diluted in a physiological saline immediately before use.

H. Statistical Analysis

Results are expressed as means±SEM. Vascular responses of CSA and CA were analyzed by one-way analysis of variance followed by Scheffe's post hoc test for multiple comparisons. Differences in the vasodilatation of subepicardial coronary microvessels before and during pacing were examined by a multiple regression analysis using a model, in which the change in coronary diameter was set as a dependent variable (y) and vascular size as an explanatory variable (x), while the statuses of control and other inhibitors were set as dummy variables (D1, D2) in the following equation: $y=a0 + a1x + a2D1 + a3D2$, where a0 through a3 are partial regression coefficients. [15] Significance tests were made as simultaneous tests for slope and intercept differences. The power of this analysis is greater than that of using the animal as the unit of analysis, giving smaller P values. The criterion for statistical significance was at $P<0.05$.

III. Results

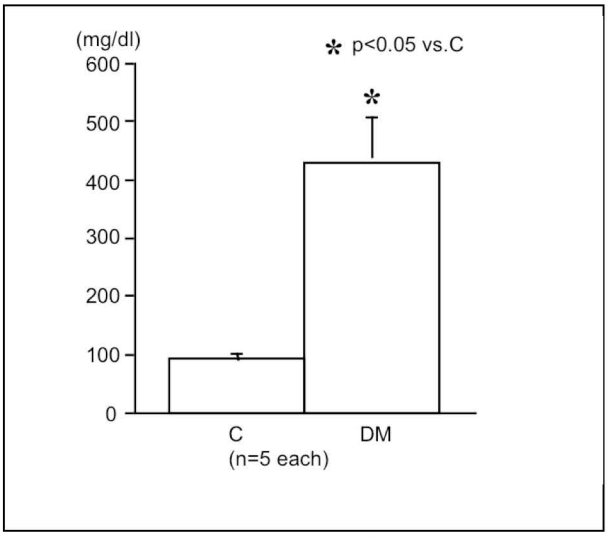

Figure 1. Blood glucose

DM had elevated plasma glucose levels compared with control (C) (Figure 1).

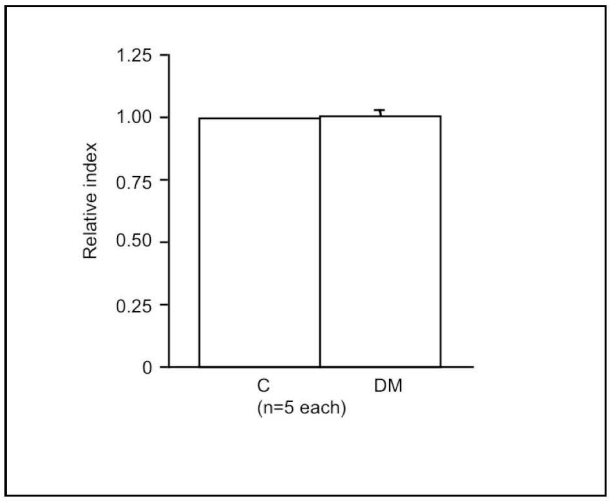

Figure 2. Cu, Zn-SOD (relative index /control)

There is no significant difference of Cu, Zn-SOD between control (C) and DM (Figure 2).

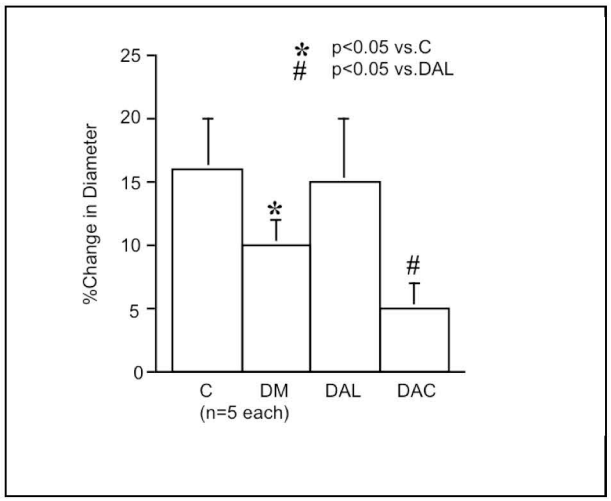

Figure 3. %Change in diameter on arterioles

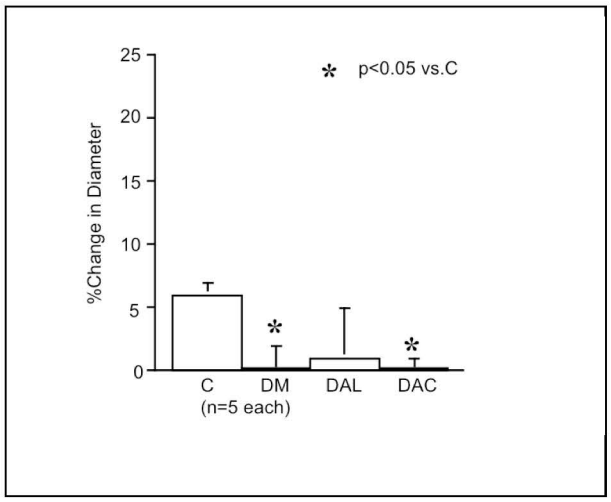

Figure 4. %Change in diameter on small arteries

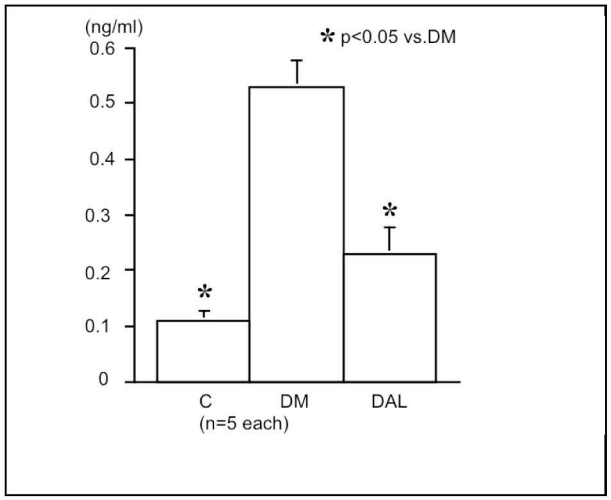

Figure 5. Plasma 8-OHdG

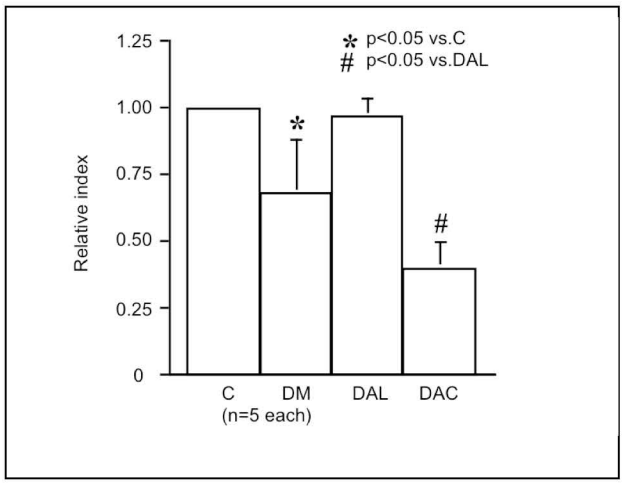

Figure 6. Myocardial H_2O_2 production (relative index /control)

Cardiac pacing in control (C) caused significant vasodilatation in CA but not in CSA (Figure 3 and 4). DM significantly decreased the coronary vasodilatation compared with C in both-sized arteries, whereas DAL significantly improved the vasodilatation compared with DM in CA and was significantly decreased by DAC in CA. DAL ameliorated oxidative stress compared with DM as assessed by plasma 8-OHdG, respectively (Figure 5). H_2O_2 production (relative index /control) in DAL was significantly increased compared with DM and was significantly decreased by DAC (Figure 6).

IV. Concluding Remarks

A. Major Findings

The major findings of the present study are that H_2O_2 during ARB administration is involved in pacing-induced metabolic coronary vasodilatation in DM in vivo and that there are substantial compensatory interactions between NO and H_2O_2. After administration with ARB, the plasma levels of 8-OHdG, an oxidative stress marker, were significantly decreased in DAL, suggesting acute anti-inflammatory effects of ARB.

B. Role of Endogenous H_2O_2 during Pacing in Vivo

We have previously demonstrated that endothelial Cu, Zn-SOD plays an important role in producing H_2O_2 as an EDHF synthase in mouse and human mesenteric arteries. [12,13] However, in the present study, there was no significant upregulation of Cu, Zn-SOD in the treatment groups, suggesting that Cu, Zn-SOD upregulation may not be involved in the beneficial effects of ARB. β-adrenergic stimulation is mediated by K_{Ca} channels in porcine coronary arteries [23] and rat mesenteric artery [24] in vitro, and H_2O_2 enhances adenylyl cyclase activity stimulated by isoproterenol in mouse vascular smooth muscle cells, [25] whereas endothelin-receptor stimulation inhibits the opening of K_{Ca} channels. [26] In the present study, catalase inhibited the pacing-induced metabolic coronary dilatation to the same extent in the presence of NO synthesis inhibition in vivo

(Figures 3). Activation of β-adrenoceptor may also contribute to the stimulation of EDHF in vivo, and the opening of K_{Ca} channels may compete with endothelin-receptor stimulation.

C. Role of ARB during Pacing in Vivo

In patients with DM, ARB improved endothelial dysfunction. [27] ARB can improve endothelial function by increasing NO bioavailability or decreasing oxidative stress. [28] It was reported that arteriolar dilatation during exercise was less sensitive to L-NAME but highly sensitive to catalase. [29] In the present study, DAL significantly improved the vasodilatation compared with DM in CA, and ARB reduced oxidative stress, as evidenced by reduced plasma levels of 8-OHdG in the coronary sinus. These results indicate that endothelium-derived H_2O_2 in ARB could play an important compensatory role in the presence of impaired NO-mediated vasodilatation by decreasing oxidative stress.

V. REFERENCES

[1] Y. Ishibashi, DJ. Duncker, J .Zhang, and RJ Bache, "ATP-sensitive K^+ channels, adenosine, and nitric oxide-mediated mechanisms account for coronary vasodilation during exercise, " *Circ Res* vol. 82, pp. 346-359, 1998.

[2] CJ. Jones, L. Kuo, Davis, MJ. DeFily, and WM. Chilian, "Role of nitric oxide in the coronary microvascular responses to adenosine and increased metabolic demand, " *Circulation* vol. 91, pp. 1807-1813, 1995.

[3] T. Yada, KN. Richmond, R. Van Bibber, K. Kroll, and EO. Feigl, "Role of adenosine in local metabolic coronary vasodilation, " *Am J Physiol* , vol. 276, pp. H1425- H1433, 1999.

[4] M. Feletou, and PM. Vanhoutte, "Endothelium-dependent hyperpolarization of canine smooth muscle, " *Br J Pharmacol* vol. 93, pp. 515-524, 1988.

[5] H. Shimokawa, "Primary endothelial dysfunction, " Atherosclerosis *J Mol Cell Cardiol* vol. 31 pp. 23-37, 1999.

[6] L. Kuo, MJ. Davis, and WM. Chilian, "Endothelium-dependent, flow-induced dilation of isolated coronary arterioles, " *Am J Physiol* vol. 259, pp. H1063-H1070, 1991.

[7] L. Kuo, WM. Chilian and MJ. Davis, "Interaction of pressure- and flow-induced responses in porcine coronary resistance vessels, , " *Am J Physiol* vol. 261, pp. H1706- H1715, 1991.

[8] A. Koller, D. Sun, and G. Kaley, "Role of shear stress and endothelial prostaglandins in flow- and viscosity-induced dilation of arterioles in vitro, " *Circ Res* vol. 72, pp. 1276-1284, 1993;.

[9] A. Koller, D. Sun, A. Huang, and G. Kaley, "Corelease of nitric oxide and prostaglandins mediates flow-dependent dilation of ratgracilis muscle arterioles, " *Am J Physiol* vol. 26, pp. H326- H332, 1994.

[10] Y, Takamura, H. Shimokawa, H. Zhao, H. Igarashi, K. Egashira, and A. Takeshita, "Important role of endothelium-derived hyperpolarizing factor in shear stress-induced endothelium-dependent relaxations in the rat mesenteric artery, " *J Cardiovasc Pharmacol* vol. 34, pp. 381-387, 1999.

[11] T. Matoba, H. Shimokawa, M. Nakashima, Y. Hirakawa, Y. Mukai, K. Hirano, H. Kanaide, and A. Takeshita, "Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice, "*J Clin Invest* vol. 106, pp. 1521-1530, 2000.

[12] T. Matoba, H. Shimokawa, H. Kubota, K. Morikawa, T. Fujiki, I. Kunihiro, Y. Mukai, Y. Hirakawa, and A. Takeshita, "Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries, "*Biochem Biophys Res Comm* vol. 290, pp. 909-913, 2002.

[13] K. Morikawa, H. Shimokawa, T .Matoba, H. Kubota, T. Akaike, MA. Talukder, M. Hatanaka, T. Fujiki, H. Maeda, S. Takahashi, and A. Takeshita, "Pivotal role of Cu,Zn-superoxide dismutase in endothelium-dependent hyperpolarization, " *J Clin Invest* vol. 112, pp. 1871-1879, 2003.

[14] H. Miura, JJ. Bosnjak, G. Ning. T. Saito, M. Miura, and DD. Gutterman, "Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles, " *Circ Res* vol. 92, pp.e31-e40, 2003.

[15] T. Yada, H. Shimokawa, O. Hiramatsu, T. Kajita, F. Shigeto, M. Goto, Y. Ogasawara, and F. Kajiya, "Hydrogen peroxide, an endogenous endothelium-derived hyperpolarizing factor, plays an important role in coronary autoregulation in vivo, "*Circulation* vol. 107, pp 1040-1045, 2003.

[16] T. Yada, H. Shimokawa, O. Hiramatsu Y. Haruna, Y. Morita, N. Kashihara, Y. Shinozaki, H. Mori, M. Goto, Y. Ogasawara, and F. Kajiya, "Cardioprotective role of endogenous hydrogen peroxide during ischemia-reperfusion injury in canine coronary microcirculation in vivo, " *Am J Physiol* vol. 291, pp H1138-46, 2006.

[17] CJ. Jones, DV. DeFily, JL. Patterson, and WM. Chilian, "Endothelium-dependent relaxation competes with alpha 1- and alpha 2-adrenergic constriction in the canine epicardial coronary microcirculation, "*Circulation* vol. 87, pp 1264-74, 1993.

[18] FS. Scornik, J. Codina, L. Birnbaumer, and L. Toro, "Modulation of coronary smooth muscle K_{Ca} channels by Gs alpha independent of phosphorylation by protein kinase A, "*Am J Physiol* vol. 265, pp H1460-1465, 1993.

[19] CM. Tan, S. Xenoyannis, and RD. Feldman, "Oxidant stress enhances adenylyl cyclase activation, "*Circ Res* vol. 277, pp 710-717, 1995.

[20] T. Yada, H. Shimokawa, O. Hiramatsu, Y. Shinozaki, H. Mori, M. Goto, Y. Ogasawara, and F. Kajiya F, "Important role of endogenous hydrogen peroxide in pacing-induced metabolic coronary vasodilation in dogs in vivo," *J Am Coll Cardiol* vol. 50, pp.1272-1278, 2007.

[21] E. Yamamoto, T. Yamashita, T. Tanaka, K. Kataoka, Y. Tokutomi, ZF. Lai, YF. Dong, S. Matsuba, H. Ogawa, and S. Kim-Mitsuyama, "Pravastatin enhances beneficial effects of olmesartan on vascular injury of salt-sensitive hypertensive rats, via pleiotropic effects, "*Arterioscler Thromb Vasc Biol*. vol. 27 pp.556-563, 2007

[22] T. Yada, O. Hiramatsu, A. Kimura, M. Goto, Y. Ogasawara, K. Tsujioka, S. Yamamori, K. Ohno, H. Hosaka, and F. Kajiya, "In vivo observation of subendocardial microvessels of the beating porcine heart using a needle-probe videomicroscope with a CCD camera," *Circ Res* vol. 72, pp.939-946, 1993

[23] FS. Scornik, J. Codina, L. Birnbaumer. and L. Toro, "Modulation of coronary smooth muscle K_{Ca} channels by Gs alpha independent of phosphorylation by protein kinase A," *Am J Physiol* vol. 265, pp.H1460- H1465, 1993.

[24] Y. Huang, and KH. Kwok, "Effects of putative K^+ channel blockers on beta-adrenoceptor-mediated vasorelaxation of rat mesenteric artery," *J Cardiovasc Pharmacol* vol. 29, pp.515-519, 1997.

[25] CM. Tan, S. Xenoyannis, and RD. Feldman, "Oxidant stress enhances adenylyl cyclase activation," *Circ Res* vol. 77, pp.710-717, 1995.

[26] K. Minami, Y. Hirata, A. Tokumura, Y. Nakaya, and K. Fukuzawa, "Protein kinase C-independent inhibition of the Ca^{2+}-activated K^+ channel by angiotensin II and endothelin-1,"*Biochem Pharmacol* vol. 49, pp 1051-1056, 1995.

[27] D. Diederich, D. Skopec, A. Diederich, and F. Dai, "Endothelial dysfunction in mesenteric resistance arteries of diabetic rats: Role of free radicals,"*Am J Physiol* vol. 266, pp H1153 – H1161, 1994.

[28] H. Brunner, JR. Cockcroft, J. Deanfield, et al, "Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension,"*J Hyper- tens* vol. 23, pp 233–246, 2005.

[29] N. Thengchaisri, R. Shipley, Y. Ren, J. Parker, and L. Kuo, "Exercise training restores coronary arteriolar dilation to NOS activation distal to coronary artery occlusion: role of hydrogen peroxide,"*Arterioscler Thromb Vasc Biol* vol. 27, pp 791-798, 2007.