Chinese Journal of Physiology 53(1): 45-51, 2010
DOI: 10.4077/CJP.2010.AMH065

Reduction of Superior Mesenteric Hemodynamic Responsiveness to [Sar^1, Thr^8]-Angiotensin II and Bradykinin, But Not Sodium Nitroprusside, in the Presence of Homocysteine Infusion

Chia-Hung Yen[1], Yunn-Hwa Ma[2], Huang-Ping Yu[3], and Ying-Tung Lau[4]

[1]*Department of Life Science, National Pingtung University of Science and Technology, Pingtung*
[2]*Department of Physiology and Pharmacology, Chang Gung University, Taoyuan*
[3]*Department of Anesthesiology, Chang Gung Memorial Hospital, Taoyuan*
and
[4]*Department of Cosmetic Science, Chang Gung Institute of Technology*
Taoyuan, Taiwan, Republic of China

Abstract

Hyperhomocysteinemia (HHcy) has been shown to be an independent risk factor for cardiovascular diseases, superior mesenteric thrombosis and inflammatory bowel disease. Superior mesenteric artery (SMA) supplies the intestine and reduced SMA blood flow results in intestinal ischemia. Although *in vitro* studies have shown that endothelium-dependent vasorelaxation of SMA is reduced in the presence of homocysteine incubation, it is not confirmed with *in vivo* studies. In this work, we evaluated responsiveness of SMA to endothelium-dependent or -independent vasodilators and a vasoconstrictor in the absence and presence of acute HHcy *in vivo* to clarify effect of HHcy on superior mesenteric vascular function. Sodium nitroprusside (SNP), bradykinin (BK), and [Sar^1,Thr^8]angiotensin II ([Sar^1,Thr^8]-ANG II) were intravenously administrated in sequence in male Sprague-Dawley rats with or without $_{D,L}$-homocysteine infusion (6 mg/kg/min) through femoral vein. Agonists-induced changes in carotid artery blood pressure, superior mesenteric blood flow and vascular resistance were measured in the present study. We found that acute HHcy infusion had little effects on SNP-induced hemodynamic changes; however, BK-induced changes in blood pressure, blood flow and vascular resistance were significantly reduced in the presence of HHcy infusion. Additionally, HHcy also markedly decreased [Sar^1,Thr^8]-ANG II-induced superior mesenteric hemodynamic changes. These results demonstrated that responsiveness of SMA to vasoconstrictor, endothelium-dependent, but not endothelium-independent vasodilator, was inhibited in the presence of Hcy infusion. This HHcy-associated vascular hyporesponsiveness to vasoconstrictors and endothelium-dependent vasodilators may partially contribute to circulatory dysfunctions.

Key Words: hyperhomocysteine, superior mesentery, endothelium-dependent relaxation

Introduction

Elevated plasma level of homocysteine, hyperhomocysteinemia (HHcy), is associated with many pathophysiological conditions such as folate deficiency (10), methionine or homocysteine over-feeding (16, 46), enzyme (methylenetetrahydrofolate reductase, cystathionine beta-synthase, and methionine

Corresponding author: Ying-Tung Lau, Ph.D., Department of Cosmetic Science, Chang Gung Institute of Technology, 261 Wen Hwa 1 Rd., Kwei-Shan, Tao-Yuan 33333, Taiwan, Republic of China. E-mail: ytlau@gw.cgit.edu.tw
Received: November 14, 2008; Revised (Final Version): May 19, 2009; Accepted: June 9, 2009.
 ISSN : 0304-4920. http://www.cps.org.tw

synthase) defects (39), and chronic inflammatory diseases such as atherosclerosis (20), superior mesenteric artery occlusion/thrombosis (1, 14, 40) and inflammatory bowel disease (6). Although the mechanism of HHcy-affected vascular function is not clear, it is generally thought that vascular endothelium dysfunction is the major event in the pathological process of chronic inflammatory diseases (38). Evaluation of endothelial dysfunction (25), which is characterized by diminished endothelium-derived relaxation factors including endothelium-derived hyperpolarizing factor (EDHF), prostacyclin and nitric oxide (NO), is usually performed using endothelium-dependent vasodilators such as acetylcholine (ACh) or bradykinin (BK).

In this study, acute Hcy infusion was used in place of methionine (Met) infusion to induce HHcy. Met loading has been employed to induce HHcy *in vivo*; however, it also induces other effects. For example, acute Met loading results in a significant increase in plasma triglyceride (17) which influences hemodynamics (28, 34). Additionally, acute Met loading also increases plasma Met by almost thirty times (8), which may interfere with this effect of HHcy on hemodynamics and suggests side effects of Met (11, 23). Therefore, Hcy infusion avoids these potential complications in the investigation of the effects of HHcy on hemodynamics.

Superior mesenteric artery (SMA) arises from the abdominal aorta which supplies the intestine from the duodenum through the transverse colon, and occlusion of SMA or reduced SMA blood flow results in intestinal ischemia. Although *in vitro* studies have demonstrated that superior mesenteric arteries treated with high concentrations of Hcy develop markedly impairment of acetylcholine-mediated endothelium-dependent vasorelaxation (35), it is not confirmed with *in vivo* studies. In fact, acute Hcy infusion has little effects in male rats on changes in mesenteric arteriolar diameter induced by ACh, a NO-dependent vasodilator or sodium nitroprusside, an endothelium-independent vasodilator (12). Therefore, the aim of this present study was to investigate whether acute hyperhomocysteinemia affects superior mesenteric responsiveness to endothelium-dependent vasodilator (*e.g.*, BK), endothelium-independent vasodilator (*e.g.*, SNP), and vasoconstrictor (*e.g.*, [Sar^1,Thr^8]-angiotensin II) *in vivo*. Here we report that hyporesponsiveness of superior mesenteric artery to vasoconstrictor and endothelium-dependent, but not endothelium-independent, vasodilator was observed in the presence of hyperhomocysteinemia.

Materials and Methods

Animals

Male Sprague Dawley (SD) rats were obtained from the National Laboratory Animal Center (Taipei, Taiwan, ROC) and were maintained in the Animal Center of Chang Gung University. Rats were housed in a 12:12-h light-dark cycle and were provided with standard rat chow (PMI Feeds, Inc., Lab Diet the Richmond standard #5010) and tap water freely. On the day of the surgery, 25 to 30-week old rats (~ 500 g) had been fasting for 16-18 hours. Experiments were performed according to the guideline of The Committees on Use and Care of Animals of Chang Gung University.

Preparation for Measurement of Blood Pressure and Blood Flow

Rats were anesthetized with thiobutabarbital (Inactin, 100 mg/kg, i.p.) and the body temperature was maintained at the range of 36-38°C with a servo-controlled homeothermic blanket control unit (Harvard, Edenbridge, KT, USA). Adequate depth of anesthesia was determined by withdrawal reflex to paw pinch. Tracheotomy was performed and PE-250 tubing was inserted to facilitate breathing. The right carotid artery was cannulated for measurement of blood pressure. Administration of fluids supplement (6% HAES-sertil and 0.9% saline, 2:3) or chemicals was conducted *via* a left femoral catheter with a syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA, USA) at the infusion rate of 180 µl/kg/min. Bladder was cannulated by a PE-250 tubing to ensure a patent urine flow. A midline abdominal incision was performed and the superior mesenteric artery, which is a branch of the descending aortic artery supplying the blood flow of intestine, was carefully isolated for a segment of 2-4 mm. A transtime ultrasound flow-meter (model T206; Transonic System, Ithaca, NY, USA) coupled to a flow probe (1 mm RB series, Transonic Systems) was used for direct determination of mesenteric blood flow. The probe was placed around the segment of superior mesenteric artery, which measured volume flow by calculating integrated transit times of ultrasonic beams to reflect the motion of liquid flowing through the vessel (30). Ultrasonic gel was used as an acoustic coupler between the flow probe and the vessel. Data of blood pressure and blood flow were recorded and analyzed by a Data Acquisition System (ML845, PowerLab 4/25, ADInstruments, Bella Bista, Australia) and the Chart™ software (ADInstruments). The mean of basal hemodynamic parameters, such as mean arterial pressure or mesenteric arterial blood flow, was obtained within 1-min period before the infusion of sodium nitroprusside (SNP, 5 µg/kg), bradykinin (BK, 5 µg/kg) or [Sar^1,Thr^8]-angiotensin II ([Sar^1,Thr^8]-ANG II, 10 ng/kg), and the selected

dosage of vasodilator or vasoconstrictor was the lowest dosage that caused significant hemodynamic changes based on our preliminary works. Mean of agonists-induced hemodynamic response was obtained within the period with maximal change after infusion. The averaging time of all measurement was 2 seconds.

Experimental Protocol

After the surgery, the infusion rate was reduced to 120 µl/kg/min, followed by equilibration for 30 min. Then initial baseline blood flow was taken 5 min prior to administration of SNP, BK or [Sar^1,Thr^8]-ANG II sequentially *via* femoral vein. The identical protocol was performed in another group with Hcy infusion (6 mg/kg/min) during the period of experiments.

Statistical Analysis

Data were expressed as means ± S.E.M, and statistical analysis was performed using unpaired *t* test (GraphPad Prism software version 4.0, GraphPad Software, Inc, Goleta, CA, USA). Significance was accepted at $P < 0.05$.

Results

Effect of Hcy Infusion on Agonists-Induced Change in Mean Carotid Arterial Blood Pressure

Systemic administration of SNP or BK *via* femoral vein significantly reduced MAP by 15 ± 2 mmHg or 18 ±1 mmHg, respectively, and administration of [Sar^1,Thr^8]-ANG II markedly increased MAP by 22 ± 2 mmHg. However, in the presence of Hcy infusion, BK- or [Sar^1,Thr^8]-ANG II-, but not SNP-mediated MAP change was significantly inhibited. Results are summarized in Table 1.

SNP-Induced Mesenteric Hemodynamic Changes in the Absence and Presence of Hcy Infusion

Intravenous administration of SNP significantly increased MBF by 16 ± 1% and reduced VR by 28 ± 1% in the absence of Hcy infusion, respectively (open column, Fig. 1, A and B). These SNP-induced hemodynamic changes were not markedly altered in the presence of Hcy infusion (solid column, Fig. 1, A and B).

BK-Induced Mesenteric Hemodynamic Changes in the Absence and Presence of Hcy Infusion

In the absence of Hcy infusion, intravenous administration of BK significantly increased MBF and reduced VR by 20 ± 3% and 31 ± 3%, respectively

Table 1. Effects of Hcy infusion on sodium nitroprusside, bradykinin, and [Sar^1,Thr^8]-ANG II-induced mean arterial blood pressure changes

Agonists	Control	Hcy infusion
Sodium nitroprusside	-18.39 ± 0.63	-14.30 ± 2.78
Bradykinin	-14.93 ± 1.94	-7.09 ± 0.44*
[Sar^1,Thr^8]-ANG II	21.58 ± 2.01	12.33 ± 1.63*

Data are presented as means ± S.E.M. *$P < 0.05$ between control (n = 3) and Hcy-infused rats (n = 6).

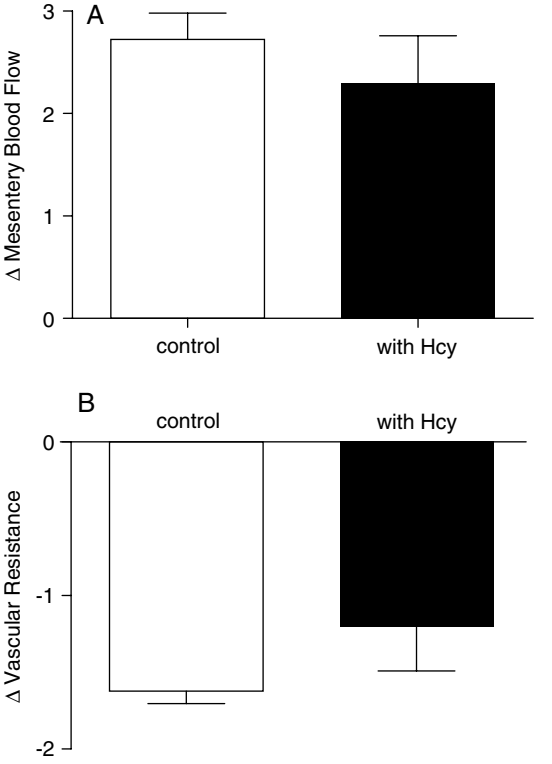

Fig. 1. Effects of Hcy infusion on sodium nitroprusside-induced changes in superior mesenteric blood flow (A) and vascular resistance (B). Data are as means ± S.E.M in control (n = 3) and Hcy-infused rats (n = 6).

(open column, Fig. 2, A and B.). However, in the presence of Hcy infusion, BK-mediated MBF increase and VR decrease were prominently reduced by 74 ± 13% and 61 ± 10%, respectively (solid column, Fig. 2, A and B).

[Sar^1,Thr^8]-ANG II-Induced Mesenteric Hemodynamic Changes in the Absence and Presence of Hcy Infusion

Intravenous administration of [Sar^1,Thr^8]-ANG

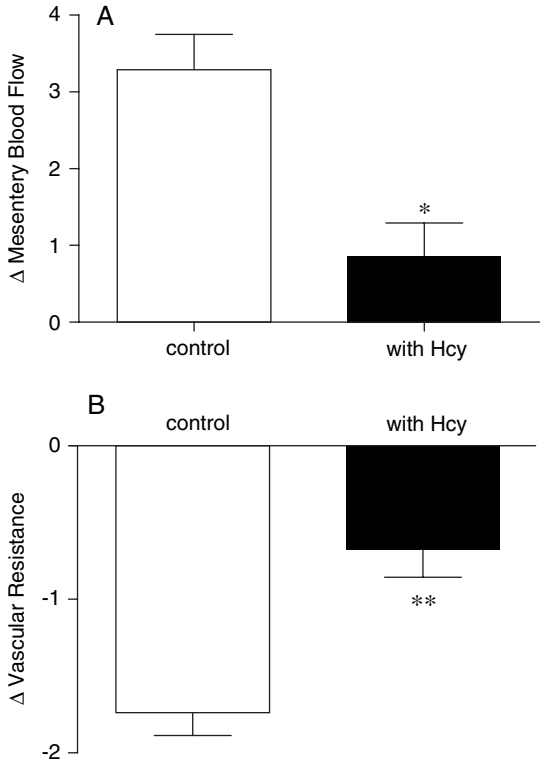

Fig. 2. Effects of Hcy infusion on bradykinin-induced changes in superior mesenteric blood flow (A) and vascular resistance (B). Data are as means ± S.E.M. **P* < 0.05 and ***P* < 0.01 between control (n = 3) and Hcy-infused rats (n = 6).

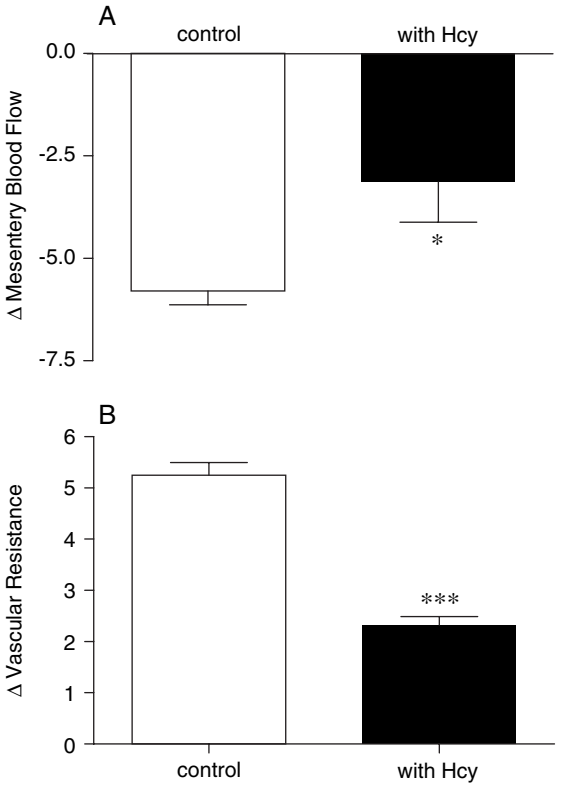

Fig. 3. Effects of Hcy infusion on [Sar[1],Thr[8]]ANG II -induced changes in superior mesenteric blood flow (A) and vascular resistance (B). Data are as means ± S.E.M. **P* < 0.05 and ****P* < 0.005 between control (n = 3) and Hcy-infused rats (n = 6).

II significantly reduced MBF and increased VR by 36 ± 2%, and 91 ± 4%, respectively (open column, Fig. 3, A and B) in the absence of Hcy infusion. Nevertheless, [Sar[1],Thr[8]]-ANG II-mediated MBF decrease and VR increase were markedly reduced by 46 ± 17% and 56 ± 3%, respectively in the presence of Hcy infusion (solid column, Fig. 3, A and B).

Discussion

In the present study, we demonstrated that hyperhomocysteinemia (HHcy) significantly reduced bradykinin (endothelium-dependent vasodilator), but not sodium nitroprusside (endothelium-independent vasodilator)-induced superior mesenteric hemodynamic changes (Fig. 2 and Fig. 1, respectively). Additionally, vasoconstrictor ([Sar[1],Thr[8]]-ANG II)-mediated hemodynamic changes were also inhibited in the presence of HHcy. This suggests that superior mesenteric artery shows hyporesponsiveness to vasoconstrictors and endothelium-dependent vasodilators even in the acute HHcy condition, and it may contribute to the vasculopathy of intestinal ischemia. Although we did not measure the plasma total homocysteine level in this study, the previous study has clearly shown that intravenous infusion of homocysteine at the dosage of 1.1 mg/kg/min for 5 min results in a steep rise of plasma total homocysteine level from 12.9 ± 1.0 μM to 101.5 ± 28.2 μM after 30 minutes, and to 53.3 ± 18.5 μM after 60 min (8), and this suggests that continuous intravenous administration of homocysteine at the dosage of 6 mg/kg/min in this study induces systemically or locally elevated homocysteine level.

In vitro studies have shown that high homocysteine concentration affects responsiveness of vascular smooth muscle cells to vasoconstrictors and that of endothelial cells to vasorelaxants (3, 41). In the present study, we further confirmed that *in vivo* acute homocysteine infusion did attenuate [Sar[1], Thr[8]]-ANG II-mediated vascular smooth muscle contraction and bradykinin-mediated endothelium-dependent relaxation. Our results and results from other reports clearly demonstrate that acute hyperhomocysteinemia indeed affect responsiveness of both vascular smooth muscle and endothelium to vasomotor agonists.

Although both ACh and BK induce endothelium-dependent vasorelaxation, the major mediator is different. Nitric oxide (NO) plays a major role and EDHF only a minor role in ACh-mediated vasorelaxa-

tion (21); however, NO and EDHF equally contribute to the BK-mediated vasorelaxation (27). Furthermore, superior mesenteric vascular bed is more sensitive to BK than to ACh in rats (7, 42). Additionally, [Sar^1, Thr^8]-ANG II, which is an analogue of ANG II which exerts agonistic pressor activity in humans (5, 18), was used in the present study. Based on the previous results, it is concluded that cellular mechanisms of vascular smooth muscle contraction induced by ANG II are endothelium-independent (22). Although NO reduced ANG II-induced vasoconstriction, the antagonism between ANG II and NO was not due to ANG II-induced NO production (32). All of the chemicals-induced hemodynamic changes including blood pressure, blood flow and vascular resistance in the present study were returned to the baseline condition prior to administration of chemicals.

Evidence has accumulated on variable responses to different endothelium-dependent vasodilators in the same vasculture in the absence and presence of HHcy. In pancreatic vascular bed of male Wistar rat, Hcy infusion (0.413 to 41.3 mg/kg/min) completely abolishes ACh-mediated vasodilatation, but it has no effect on adenosine-mediated vasodilatation (37). Similarly, in young hypertensive rats, different levels of ACh- and BK-mediated vasorelaxation of mesenteric vascular bed were observed (45). Additionally, mesenteric vascular bed is more sensitive to BK than to ACh (7, 42). In the present study, acute HHcy only affected endothelium-dependent vasodilatation (BK), but not endothelium-independent (SNP) vascular response to NO in superior mesentery *in vivo*, and this finding is supported by *in vitro* studies showing that BK-induced vasorelaxation is reduced by acute HHcy (35). However, the effect of HHcy on superior mesentery is different from that observed on mesenteric arteriole (diameter ranging between 18 to 39 μm) (12), and this suggests that vessels with different diameters would have different sensitivities to HHcy.

In order to investigate whether the effect of HHcy on BK-induced hemodynamic changes is NO-dependent, we also attempted to conduct the study in the presence of N^w-Nitro-L-arginine methyl ester (L-NAME), a constitutive nitric oxide synthase (NOS) inhibitor. We employed the intravenous bolus administration of L-NAME (10 mg/kg) and found that mesenteric arterial blood flow was dramatically reduced from around 16-18 mL/min to 3-4 mL/min, and its effect lasted for more than one hour. This result is consistent with those of other studies using NO synthase inhibitors including L-NMMA, N^w-Nitro-L-arginine, L-NAME, which induce increases in systemic arterial blood pressure (2, 33, 36), total peripheral resistance (2, 33), and decrease in blood flow of stomach, small intestine, colon, pancreas, spleen kidney, mesentery (33) and forearm blood flow (15). Since response to endothelium-dependent vasodilator has shown a significant dependence on resting blood flow (4), *in vivo* administration of constitutive NOS inhibitor-induced low baseline level of blood flow could complicate the effect of HHcy on BK-mediated blood flow. These results suggest that chemically or mechanically removing endothelium *in vivo* would induce severe inflammatory responses and would affect systemic hemodynamics; therefore, using wire myograph system to compare intact rings with denuded rings would be an alternative method to demonstrate whether the effect of homocysteine on the vasculature is endothelium-dependent or not in further studies.

In addition to inducing NO production, BK also induces the release of vasodilatory substances such as prostacyclin and endothelium-derived hyperpolarizing factor (8, 13, 27). Increasing evidence has also demonstrated an intimate interaction between Hcy and prostacyclin or endothelium-derived hyperpolarizing factor (19, 24). Further investigation of the role of non-NO mediators in the negative effects of HHcy on BK-mediated mesenteric hemodynamics should provide additional insights on the nature of HHcy impairment on endothelium.

Vascular hyporesponsiveness to vasoconstrictors or vasodilators associated with circulatory failure is generally observed in lipopolysaccharide-treated animals (26) as well as in HHcy-treated rats in this study. Additionally, increased homocysteine levels observed in patients with cardiovascular diseases or inflammatory bowel disease (IBD) may play a role in the pathogenesis of atherosclerosis (20) or ulcerative colitis (9, 29). Further evidence indicates that IBD patients have increased risks of early atherosclerosis than healthy controls (31). Therefore, our findings that acute HHcy-mediated hyporesponsiveness of superior mesenteric artery, which is originated in the abdominal aorta and supplies the small intestine and the ascending colon, to ANG II analog and BK is consistent with dysfunction caused by defective perfusion.

Chronic and acute HHcy seems to have distinct effects on ROS and NO production. Long-term Hcy treatment limits nitric oxide availability (44) and increased ROS production in cultured human umbilical vein endothelial cells (43, 47); however, short-term (30 min) Hcy incubation reduced ROS production and increased NO production (43). Increasing evidence suggests that the interaction between NO and ROS is an important factor for the regulation of vascular tone. Therefore, short-term Hcy-mediated elevated NO production may partly be due to the vascular hyporesponsiveness.

In conclusion, acute HHcy *in vivo* reduced superior mesenteric artery responsiveness to vasocon-

strictor ([Sar^1,Thr^8]-ANG II), endothelium-dependent vasodilator (BK), but not endothelium-independent vasodilator (SNP). These results provide the *in vivo* evidence to suggest that short-term elevated homocysteine concentration could result in vascular hyporesponsiveness to vasoconstrictors and endothelium-dependent vasodilators, and this partially contributes to the circulatory failure and intestinal ischemia.

Acknowledgments

Our work was supported by grants from the National Science Council (NSC 95-2320-B-182-030-MY3), Chang Gung Memorial Hospital (CMRPD140152) and Chang Gung Molecular Medicine Research Center (EMRPD150261) to Y.T. Lau and NSC95-2320-B-020-001 to C.H. Yen.

References

1. Alexander, T., Rajnish, R., Balakrishnan, R. and Shallam, J.F. Hyperhomocysteinemia presenting as superior mesenteric artery thrombosis. *Indian J. Gastroenterol.* 24: 78-79, 2005.
2. Charkoudian, N., Joyner, M.J., Barnes, S.A., Johnson, C.P., Eisenach, J.H., Dietz, N.M. and Wallin, B.G. Relationship between muscle sympathetic nerve activity and systemic hemodynamics during nitric oxide synthase inhibition in humans. *Am. J. Physiol*. 291: H1378-H1383, 2006.
3. Chen, C., Conklin, B.S., Ren, Z. and Zhong, D.S. Homocysteine decreases endothelium-dependent vasorelaxation in porcine arteries. *J. Surg. Res*. 102: 22-30, 2002.
4. Chowienczyk, P.J., Cockcroft, J.R. and Ritter, J.M. Blood flow responses to intra-arterial acetylcholine in man: effects of basal flow and conduit vessel length. *Clin. Sci*. 87: 45-51, 1994.
5. Cody Jr., R.J., Tarazi, R.C., Fouad, F.M. and Bravo, E.L. Hemodynamics of a new angiotensin antagonist, [Sar^1, Thr^8]AII, in hypertensive man. *Circulation* 61: 338-344, 1980.
6. Danese, S., Sgambato, A., Papa, A., Scaldaferri, F., Pola, R., Sans, M., Lovecchio, M., Gasbarrini, G., Cittadini, A. and Gasbarrini, A. Homocysteine triggers mucosal microvascular activation in inflammatory bowel disease. *Am. J. Gastroenterol.* 100: 886-895, 2005.
7. de P Rodrigues, S.F., dos Santos, R.A., Silva-Antonialli, M.M., Scavone, C., Nigro, D., Carvalho, M.H., de Cassia Tostes, R. and Fortes, Z.B. Differential effect of losartan in female and male spontaneously hypertensive rats. *Life Sci.* 78: 2280-2285, 2006.
8. De Vriese, A.S., Blom, H.J., Heil, S.G., Mortier, S., Kluijtmans, L.A., van de Voorde, J. and Lameire, N.H. Endothelium-derived hyperpolarizing factor-mediated renal vasodilatory response is impaired during acute and chronic hyperhomocysteinemia. *Circulation* 109: 2331-2336, 2004.
9. Drzewoski, J., Gasiorowska, A., Malecka-Panas, E., Bald, E. and Czupryniak, L. Plasma total homocysteine in the active stage of ulcerative colitis. *J. Gastroenterol. Hepatol*. 21: 739-743, 2006.
10. Eikelboom, J.W., Lonn, E., Genest, J., Hankey, G. and Yusuf, S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann. Intern. Med.* 131: 363-375, 1999.
11. Erdmann, K., Grosser, N. and Schroder, H. L-methionine reduces oxidant stress in endothelial cells: role of heme oxygenase-1, ferritin, and nitric oxide. *AAPS J*. 7: E195-E200, 2005.
12. Fu, W.Y., Dudman, N.P., Perry, M.A. and Wang, X.L. Homocysteine attenuates hemodynamic responses to nitric oxide *in vivo*. *Atherosclerosis* 161: 169-176, 2002.
13. Gosink, E.C. and Forsberg, E.J. Effects of ATP and bradykinin on endothelial cell Ca^{2+} homeostasis and formation of cGMP and prostacyclin. *Am. J. Physiol*. 265: C1620-C1629, 1993.
14. Gradman, W.S., Daniel, J., Miller, B. and Haji-Aghaii, M. Homocysteine-associated acute mesenteric artery occlusion treated with thrombectomy and bowel resection. *Ann. Vasc. Surg.* 15: 247-250, 2001.
15. Green, D.J., Bilsborough, W., Naylor, L.H., Reed, C., Wright, J., O'Driscoll, G. and Walsh, J.H. Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *J. Physiol*. 562: 617-628, 2005.
16. Hanratty, C.G., McGrath, L.T., McAuley, D.F., Young, I.S. and Johnston, G.D. The effects of oral methionine and homocysteine on endothelial function. *Heart* 85: 326-330, 2001.
17. Hart, S.R., Mangoni, A.A., Swift, C.G. and Jackson, S.H. Lack of significant effects of methionine loading on endothelial function in elderly volunteers. *Heart Lung Circ*. 15: 358-361, 2006.
18. Hata, T., Ogihara, T., Nakamaru, M., Gotoh, S., Masuo, K., Saeki, S., Kumagai, A. and Kumahara, Y. Effect of three angiotensin II antagonists, [Sar^1,Thr^8]-, [Sar^1,Ile^8]- and [Sar^1,Ala^8]angiotensin II on blood pressure and endocrine factors in normal subjects. *Eur. J. Clin. Pharmacol*. 23: 7-10, 1982.
19. Heil, S.G., De Vriese, A.S., Kluijtmans, L.A., Mortier, S., Den Heijer, M. and Blom, H.J. The role of hyperhomocysteinemia in nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF)-mediated vasodilatation. *Cell. Mol. Biol*. 50: 911-916, 2004.
20. Herrmann, W. Significance of hyperhomocysteinemia. *Clin. Lab.* 52: 367-374, 2006.
21. Honda, H., Moroe, H., Fujii, H., Arai, K., Notoya, Y. and Kogo, H. Short term hypercholesterolemia alters N^G-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation by acetylcholine in rabbit renal artery. *Jpn. J. Pharmacol*. 85: 203-206, 2001.
22. Kanaide, H., Ichiki, T., Nishimura, J. and Hirano, K. Cellular mechanism of vasoconstriction induced by angiotensin II: it remains to be determined. *Circ. Res*. 93: 1015-1017, 2003.
23. Kanani, P.M., Sinkey, C.A., Browning, R.L., Allaman, M., Knapp, H.R. and Haynes, W.G. Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocyst(e)inemia in humans. *Circulation* 100: 1161-1168, 1999.
24. Lee, H.Y., Chae, I.H., Kim, H.S., Park, Y.B., Choi, Y.S., Lee, Y.W., Park, S.J. and Cha, Y.J. Differential effects of homocysteine on porcine endothelial and vascular smooth muscle cells. *J. Cardiovasc. Pharmacol*. 39: 643-651, 2002.
25. Lentz, S.R. Mechanisms of homocysteine-induced atherothrombosis. *J. Thromb. Haemost*. 3: 1646-1654, 2005.
26. Lin, S.L., Lee, Y.M., Chang, H.Y., Cheng, Y.W. and Yen, M.H. Effects of naltrexone on lipopolysaccharide-induced sepsis in rats. *J. Biomed. Sci*. 12: 431-440, 2005.
27. Luksha, L., Nisell, H. and Kublickiene, K. The mechanism of EDHF-mediated responses in subcutaneous small arteries from healthy pregnant women. *Am. J. Physiol*. 286: R1102-R1109, 2004.
28. Mancuso, D.J., Han, X., Jenkins, C.M., Lehman, J.J., Sambandam, N., Sims, H.F., Yang, J., Yan, W., Yang, K., Green, K., Abendschein, D.R., Saffitz, J.E. and Gross, R.W. Dramatic accumulation of triglycerides and precipitation of cardiac hemodynamic dysfunction during brief caloric restriction in transgenic myocardium expressing human calcium-independent phospholipase $A_{2\gamma}$. *J. Biol. Chem*. 282: 9216-9227, 2007.
29. Morgenstern, I., Raijmakers, M.T., Peters, W.H., Hoensch, H. and Kirch, W. Homocysteine, cysteine, and glutathione in human colonic mucosa: elevated levels of homocysteine in patients with inflammatory bowel disease. *Dig. Dis. Sci*. 48: 2083-2090, 2003.
30. Pan, H.L., Deal, D.D., Xu, Z. and Chen, S.R. Differential resoponses of regional sympathetic activity and blood flow to visceral

afferent stimulation. *Am. J. Physiol*. 280: R1781-R1789, 2001.
31. Papa, A., Danese, S., Urgesi, R., Grillo, A., Guglielmo, S., Roberto, I., Bonizzi, M., Guidi, L., De Vitis, I., Santoliquido, A., Fedeli, G., Gasbarrini, G. and Gasbarrini, A. Early atherosclerosis in patients with inflammatory bowel disease. *Eur. Rev. Med. Pharmacol. Sci*. 10: 7-11, 2006.
32. Parekh, N., Dobrowolski, L., Zou, A.P. and Steinhausen, M. Nitric oxide modulates angiotensin II- and norepinephrine-dependent vasoconstriction in rat kidney. *Am. J. Physiol*. 270: R630-R635, 1996.
33. Pizcueta, P., Pique, J.M., Fernandez, M., Bosch, J., Rodes, J., Whittle, B.J. and Moncada, S. Modulation of the hyperdynamic circulation of cirrhotic rats by nitric oxide inhibition. *Gastroenterology* 103: 1909-1915, 1992.
34. Polak, K., Schmetterer, L., Luksch, A., Gruber, S., Polska, E., Peternell, V., Bayerle-Eder, M., Wolzt, M., Krebs, M. and Roden, M. Free fatty acids/triglycerides increase ocular and subcutaneous blood flow. *Am. J. Physiol*. 280: R56-R61, 2001.
35. Pruefer, D., Scalia, R. and Lefer, A.M. Homocysteine provokes leukocyte-endothelium interaction by downregulation of nitric oxide. *Gen. Pharmacol.* 33: 487-498, 1999.
36. Pullamsetti, S.S., Maring, D., Ghofrani, H.A., Mayer, K., Weissmann, N., Rosengarten, B., Lehner, M., Schudt, C., Boer, R., Grimminger, F., Seeger, W. and Schermuly, R.T. Effect of nitric oxide synthase (NOS) inhibition on macro- and microcirculation in a model of rat endotoxic shock. *Thromb. Haemost*. 95: 720-727, 2006.
37. Quere, I., Hillaire-Buys, D., Brunschwig, C., Chapal, J., Janbon, C., Blayac, J.P., Petit, P. and Loubatieres-Mariani, M.M. Effects of homocysteine on acetylcholine- and adenosine-induced vasodilatation of pancreatic vascular bed in rats. *Br. J. Pharmacol*. 122: 351-357, 1997.
38. Ross, R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362: 801-809, 1993.
39. Selhub, J. Homocysteine metabolism. *Annu. Rev. Nutr*. 19: 217-246, 1999.
40. Stern, J.M., Saver, J.L., Boldy, R.M. and DeGregorio, F. Homocysteine associated hypercoagulability and disseminated thrombosis—a case report. *Angiology* 49: 765-769, 1998.
41. Tasatargil, A., Sadan, G. and Karasu, E. Homocysteine-induced changes in vascular reactivity of guinea-pig pulmonary arteries: role of the oxidative stress and poly (ADP-ribose) polymerase activation. *Pulm. Pharmacol. Ther*. 20: 265-272, 2007.
42. Thomas, G.R., Thiemermann, C., Walder, C. and Vane, J.R. The effects of endothelium-dependent vasodilators on cardiac output and their distribution in the anaesthetized rat: a comparison with sodium nitroprusside. *Br. J. Pharmacol*. 95: 986-992, 1988.
43. Tsen, C.M., Hsieh, C.C., Yen, C.H. and Lau, Y.T. Homocysteine altered ROS generation and NO accumulation in endothelial cells. *Chinese J. Physiol*. 46: 129-136, 2003.
44. van Guldener, C. and Stehouwer, C.D. Hyperhomocysteinemia, vascular pathology, and endothelial dysfunction. *Semin. Thromb. Hemost.* 26; 281-289, 2000.
45. Wirth, K.J., Linz, W., Wiemer, G. and Scholkens, B.A. Differences in acetylcholine- and bradykinin-induced vasorelaxation of the mesenteric vascular bed in spontaneously hypertensive rats of different ages. *N.-S. Arch. Pharmacol*. 354: 38-43, 1996.
46. Yen, C.H. and Lau, Y.T. Vascular responses in male and female hypertensive rats with hyperhomocysteinemia. *Hypertension* 40: 322-328, 2002.
47. Zhang, B.Q., Hu, S.J., Qiu, L.H., Zhu, J.H., Xie, X.J., Sun, J., Zhu, Z.H., Xia, Q. and Bian, K. Effects of Astragalus membranaceus and its main components on the acute phase endothelial dysfunction induced by homocysteine. *Vascul. Pharmacol*. 46: 278-285, 2007.