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Methylglyoxal Enhances Sodium Nitroprusside–Induced Relaxation in Rat Aorta

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Abstract. The concentration of methylglyoxal (MGO), a metabolite of glucose, increases in plasma of type II diabetic patients as well as in tissues of hypertensive rats. We have previously shown that MGO inhibited noradrenaline (NA)–induced smooth muscle contraction in rat aorta. However, the effect of MGO on relaxing responses in isolated blood vessel remains to be clarified. Thus, we examined if MGO affects acetylcholine (ACh)– or sodium nitroprusside (SNP)–induced vasodilation on NA (100 nM)–induced pre-contraction in rat thoracic aorta. Treatment of endothelium-intact aorta with MGO (420 μM, 30 min) did not change ACh (1 nM – 3 μM)–induced endothelium-dependent relaxation. In contrast, treatment of endothelium-denuded aorta with MGO shifted the concentration–response curve for SNP (0.1 – 300 nM) to the left. MGO increased reactive oxygen species (ROS) production in smooth muscle on analysis of protein carbonylation. Anti-oxidant agents such as tempol (10 μM), catalase (5000 U/mL), and nitric oxide synthase inhibitor, N^G-nitro-L-arginine methylester (100 μM) had no effect on MGO-induced enhancement of SNP-induced relaxation. However, iberiotoxin (100 nM), a large-conductance Ca^{2+}-activated K^+ (BK_{Ca})–channel inhibitor, significantly prevented the effect. The present study revealed that MGO enhanced SNP-induced relaxation in a ROS-independent manner via in part opening smooth muscle BK_{Ca} channels.

Keywords: glucose metabolite, vascular smooth muscle, relaxation, potassium channel, reactive oxygen species

Introduction

Methylglyoxal (MGO) is a reactive alpha-dicarbonyl compound. In the process of glycolysis, MGO is produced mainly from dihydroxyacetone phosphate as a by-product during the formation of glyceraldehyde 3-phosphates in all mammalian cells including vascular smooth muscle cells (1). MGO can also be formed from ketone body or threonine (2). MGO binds to and modifies arginine, lysine, and cysteine residues in proteins, which subsequently leads to the non-enzymatic formation of a variety of advanced glycation end-products (AGEs) (3). MGO also intracellularly reacts with nucleic acids (4). It is also known that MGO is catalyzed to propanediol by aldose reductase (5).

The plasma concentration of MGO is reported to be significantly increased in diabetic patients (6 – 8). Increased MGO-derived AGEs level in diabetic patients seems to correlate with diabetic complications, including diabetic retinopathy (9) and nephropathy (10). Furthermore, MGO might be related to neuropathy (11 – 13). In addition, recent reports have demonstrated that accumulation of MGO increased in aorta of spontaneous hypertensive rats (SHR) with aging and that increased MGO level in aorta was related to increased blood pressure in SHR (14). It was also demonstrated that administration of MGO by drinking water increased blood pressure in Wistar-Kyoto rats (15). Therefore it is highly likely that MGO could directly affect vascular reactivity. In fact, we have recently demonstrated that acute treatment of endothelium-denuded rat aorta and mesenteric artery with MGO inhibits contraction induced by noradrenaline (NA) (16). However, it remains to be clarified whether MGO could also affect vascular relaxing function. To explore this, we examined effects of MGO on both endothelium-dependent and -independent relaxations in

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rat isolated thoracic aorta.

Materials and Methods

Tissue preparation

Male Wistar rats (0.2 – 0.4 kg, 5 – 10-week-old) were anesthetized with urethane (1.5 g/kg, i.p.) and euthanized by exsanguination. The plasma glucose level of the rats was 4.8 mM at 5 weeks and 7.1 mM at 10 weeks in the non-fasted condition. The thoracic aorta was isolated. After removal of fat and adventitia, aorta was cut into strips (approximately 4-mm-wide, 8-mm-long) for the measurement of isometric tension (16, 17). Endothelium was removed by rubbing the intimal surface with the flat face of a pair of forceps, and it was confirmed by the lack of relaxation induced by acetylcholine (ACh, 1 μM). Animal care and treatment were conducted in conformity with institutional guidelines of The Kitasato University.

Measurement of isometric tension

The arterial preparations were placed in normal physiological salt solution, which contained 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23.8 mM $NaHCO_3$, 5.5 mM glucose, and 1 μM EDTA. The high-K^+ (72.7 mM) solution was prepared by replacing NaCl with equimolar KCl. These solutions were saturated with a 95% O_2 – 5% CO_2 mixture at 37°C and pH 7.4. Smooth muscle contractility was recorded isometrically with a force-displacement transducer (Nihon Kohden, Tokyo) as described previously (16, 17). The arterial preparations were equilibrated for 30 min under a resting tension of 1.0 g to allow development of a stable basal tone. The arterial preparations were repeatedly exposed to high-K^+ solution until the responses became stable (45 min). Concentration–responses curves were obtained by the cumulative application of ACh (1 nM – 3 μM) or sodium nitroprusside (SNP, 0.1 – 300 nM) to aorta pre-contracted with NA (100 nM). We performed 2 sequential experiments to achieve the concentration–response curves (1st experiment: control, 2nd experiment: MGO pretreatment). MGO was pretreated for 30 min before the NA-induced pre-contraction. We confirmed that control sequential experiments performed after 30 min without MGO showed the same contraction profile as the preceding control. NA (100 nM) caused a submaximal contraction in rat aorta, and there was no difference between the contraction in control and MGO-pretreated aorta [% relative to NA (1 μM)–induced maximal contraction: 93.79 ± 0.50% for the control and 91.29 ± 1.13% for MGO-pretreated aorta, n = 11)] (16).

We used 420 μM MGO for the following reasons (16): 1) It was reported that plasma MGO concentration in poorly controlled human diabetic patients is about 400 μM (6). 2) Although others demonstrated that it was much less (7, 8), local MGO concentration in tissues is suggested to be much higher than plasma level (18).

Determination of protein carbonylation by Western blotting

Production of reactive oxygen species (ROS) was examined by detecting protein carbonylation using Western blotting. Carbonylated proteins are indicative of direct oxidation of side chains of amino acid residues. After derivatized with 2,4-dinitrophenylhydrazine (DNPH), carbonylated proteins were detected using anti-dinitrophenol (DNP) antibody. The aorta was cut into rings for the measurement of protein carbonylation. After removal of the endothelium, the arterial preparations were stabilized for 30 min in normal physiological salt solution saturated with a 95% O_2 – 5% CO_2 mixture at 37°C and pH 7.4 without resting tension. After the treatment with MGO (420 μM) for 30 min, protein lysates were obtained by homogenizing aorta with Triton-based lysis buffer (1% Triton X-100, 20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM β-glycerol phosphate, 1 mM NA_3VO_4, 1 μg/ml leupeptin, and 0.1% protease inhibitor mixture; Nacalai Tesque, Kyoto). Protein concentration in lysates was determined by the bicinchoninic acid method (Pierce, Rockford, IL, USA). Equal amounts of proteins (10 – 15 μg) were separated by SDS-PAGE (7.5%) and transferred to a nitrocellulose membrane (Pall Corporation, Ann Arbor, MI, USA) (19, 20). After treatment with 20% methanol in 80% Tris-buffered saline (10 mM Tris-HCl, pH 7.4, 140 mM NaCl) for 5 min, the membrane was incubated with 2 N HC1 containing 10 mM DNPH (SHIMA Laboratories, Tokyo) for 5 min at room temperature. After the reaction, the membrane was washed with 2 N HCl for 15 min and treated with 50% methanol for 35 min (21, 22). Then the membrane was blocked with 2% skim milk for 60 min, incubated with anti-DNP rabbit antibody (1:500 – 1000 dilution, SHIMA Laboratories) at 4°C overnight, and the membrane-bound antibodies were visualized using horseradish peroxidase–conjugated secondary antibodies (1:10000 dilution, 1 h) and the EZ-ECL system (Biological Industries, Kibbutz Beit Haemek, Israel). Equal loading of proteins was ensured by measuring α-actin (DAKO, Glostrup, Denmark) expression.

Statistical analyses

Results are expressed as the mean ± S.E.M. Statistical evaluation of the date was performed by the paired or unpaired Student's *t*-test for comparisons between two groups and by ANOVA followed by Bonferroni's test for comparisons in more than three groups. Results were considered significant when the *P*-value was less than 0.05.

Chemicals

The chemicals used were as follows: apamin, 4-aminopyridine (4-AP), EDTA, glibenclamide, iberiotoxin (IbTx), MGO solution, NA, pyrrolidine dithiocarbamate (PDTC), SNP, 4-hydroxy-TEMPO (tempol), tetramethylammonium (Sigma, St. Louis, MO, USA); catalase (Calbiochem, San Diego, CA, USA); 8-bromo-cyclic GMP (8-Br-cGMP) (Enzo, Plymouth Meeting, PA, USA); N^G-nitro-L-arginine methylester (L-NAME) (Dojindo, Kumamoto); and ACh (Daiichi Pharmaceutical, Tokyo). All drugs were dissolved in distilled water.

Results

Effect of MGO on ACh-induced relaxation of endothelium-intact thoracic aorta of rats

We first examined effects of pretreatment of endothelium-intact rat aorta with MGO (420 μM, 30 min) on relaxation induced by ACh (1 nM – 3 μM) on NA (100 nM)–induced pre-contraction. MGO had no effect on ACh-induced relaxation (Fig. 1, n = 8, maximal relaxation: 58.40 ± 6.94% for the control and 67.39 ± 6.75% for MGO).

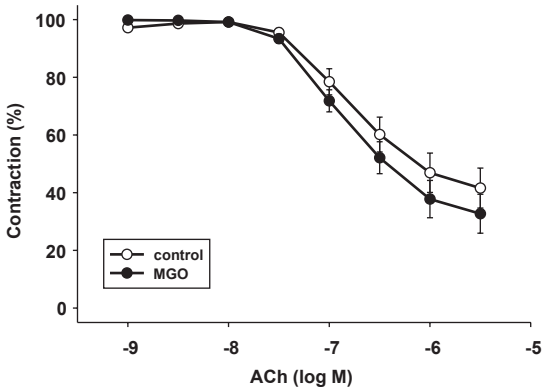

Fig. 1. Effect of pretreatment of endothelium-intact rat aorta with methylglyoxal (MGO; 420 μM, 30 min) on relaxation induced by acetylcholine (ACh, 1 nM – 3 μM) on noradrenaline (NA, 100 nM)–induced pre-contraction. ACh was cumulatively added after the contraction induced by NA had reached a steady state. Results were expressed as the mean ± S.E.M. 100% represents NA-induced pre-contraction. Control: open circle, n = 8, MGO: closed circle, n = 8.

Effect of MGO on SNP-induced relaxation of thoracic aorta of rats

Next, we examined whether MGO could affect SNP-induced relaxation in aorta. Treatment of endothelium-denuded aorta with MGO (420 μM, 30 min) shifted the concentration–response curve for SNP to the left (Fig. 2A, n = 28, pD_2: 8.31 ± 0.26 for the control and 8.66 ± 0.35 for MGO, $P < 0.01$; maximal relaxation: 99.99 ± 0.07% for the control and 100.02 ± 0.03% for MGO). Treatment of endothelium-intact aorta with MGO also shifted the concentration–response curve for SNP to the left (Fig. 2B, n = 6, pD_2: 8.26 ± 0.24 for the control and 8.60 ± 0.30 for MGO, $P < 0.05$; maximal relaxation: 99.91 ± 0.09% for the control and 101.01 ± 0.68% for MGO). Next, we examined whether MGO could affect cGMP analogue (8-Br-cGMP)–induced relaxation in endothelium-denuded aorta. Treatment with MGO (420 μM, 30 min) shifted the concentration–response curve for

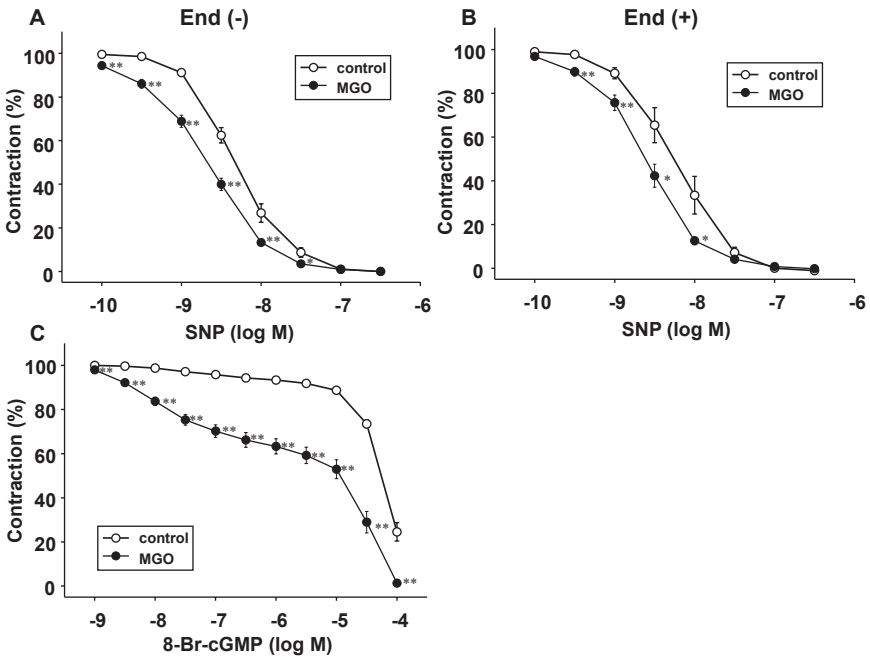

Fig. 2. Effect of pretreatment of endothelium-denuded [A, End (−); control: open circle, n = 28] or -intact [B, End (+); control: open circle, n = 10] rat aorta with MGO (420 μM, 30 min; MGO: closed circle, n = 28 for A and n = 10 for B) on relaxation induced by sodium nitroprusside (SNP, 0.1 – 300 nM). C) Effect of pretreatment of endothelium-denuded rat aorta with MGO (420 μM, 30 min) on relaxation induced by 8-bromo-cyclic GMP (8-Br-cGMP; 1 nM – 100 μM, n = 12; control: open circle and MGO: closed circle) on NA (100 nM)–induced pre-contraction was also examined. SNP or 8-Br-cGMP was cumulatively added after the contraction induced by NA had reached a steady state. Results were expressed as the mean ± S.E.M. 100% represents NA-induced pre-contraction. *$P < 0.05$, **$P < 0.01$: control vs. MGO.

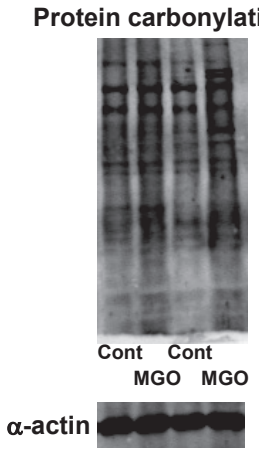

Fig. 3. Effect of MGO on protein carbonylation. Representative immunoblots showing total protein carbonylation in endothelium-denuded rat aorta treated without (control, n = 6) or with MGO (420 μM, 30 min, n = 6) are shown. Protein carbonylation was analyzed by Western blotting using anti-dinitrophenol (DNP) antibody. Broad staining in response to anti-DNP antibody represents carbonylated proteins. Equal loading of proteins was ensured by measuring α-actin expression.

8-Br-cGMP to the left (Fig. 2C, n = 12, pD_2: 4.22 ± 0.25 for the control and 5.50 ± 0.89 for MGO, $P < 0.01$; maximal relaxation: 75.43 ± 4.19% for the control and 98.65 ± 0.73% for MGO, $P < 0.01$).

Effect of MGO on protein carbonylation

To determine mechanisms responsible for the enhanced effect of MGO on SNP-induced relaxation, protein carbonylation, which represents increased ROS production, was detected by Western blotting. In the protein samples obtained from the endothelium-denuded aorta treated with MGO (420 μM, 30 min), broad staining in response to anti-DNP antibody was observed compared with the untreated control samples (Fig. 3, n = 6), indicating that MGO produced ROS in smooth muscle of rat aorta.

Effect of anti-oxidant drugs on MGO-mediated enhancement of SNP-induced relaxation

To examine whether MGO-produced ROS contributes to the enhancement of SNP-induced relaxation, we used anti-oxidant drugs. Firstly, treatment of endothelium-denuded aorta with tempol (10 μM, 15 min), a superoxide scavenger, had no effect on the enhanced effect of MGO (Fig. 4A, n = 7 for the control, n = 4 for MGO, and n = 4 for tempol + MGO). Catalase (5000 U/mL, 15 min), which metabolites H_2O_2 to H_2O, was also ineffective on MGO (Fig. 4B, n = 7 for the control, n = 4 for MGO, and n = 4 for catalase + MGO). There are reports showing that this concentration of tempol (10 μM) completely prevented obesity-induced superoxide production in rat mesenteric artery (23) and that catalase (less than 5000 U/mL) significantly reduced xanthine oxidase– (24) or endothelium-derived (25) H_2O_2 production in rat aorta. A nitric oxide (NO) synthase inhibitor, L-NAME (100 μM, 15 min) had also no effect (Fig. 4C, n = 8 for the control, n = 4 for MGO, and n = 4 for L-NAME + MGO), suggesting that ROS is not responsible for the MGO-mediated enhancement of SNP-induced relaxation. We

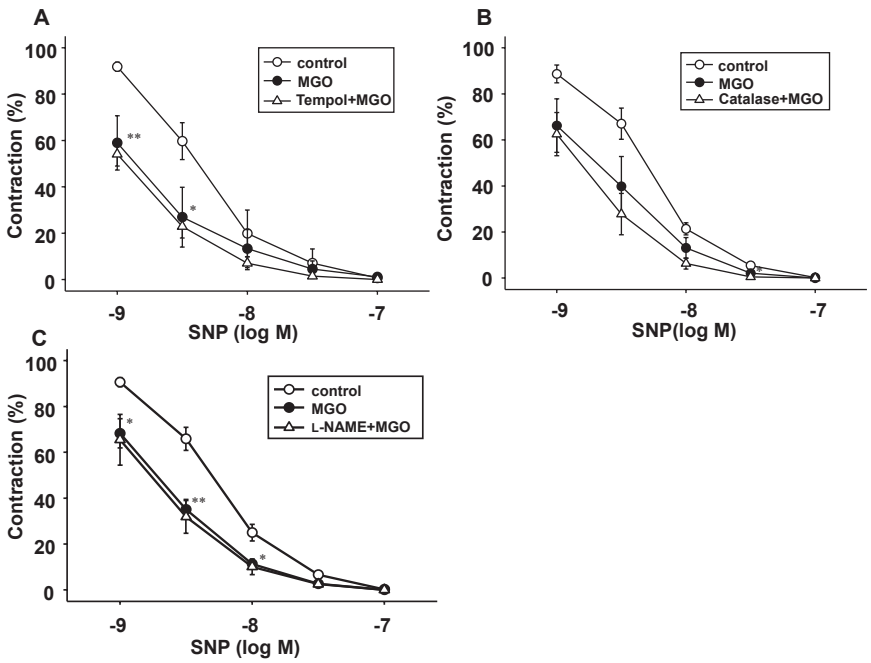

Fig. 4. Concentration–contraction relationships for SNP in endothelium-denuded rat aorta in the absence (control: open circle, n = 7) or presence of MGO (420 μM, 30 min) pretreated without (MGO: closed circle, n = 4) or with tempol (10 μM, 15 min, open triangle, n = 4) (A), catalase (5000 U/mL, 15 min, open triangle, n = 4) (B), or N^G-nitro-L-arginine methylester (L-NAME; 100 μM, 15 min, open triangle, n = 4) (C). Results were each expressed as the mean ± S.E.M. 100% represents NA (100 nM)–induced pre-contraction. *$P < 0.05$, **$P < 0.01$: control vs. MGO.

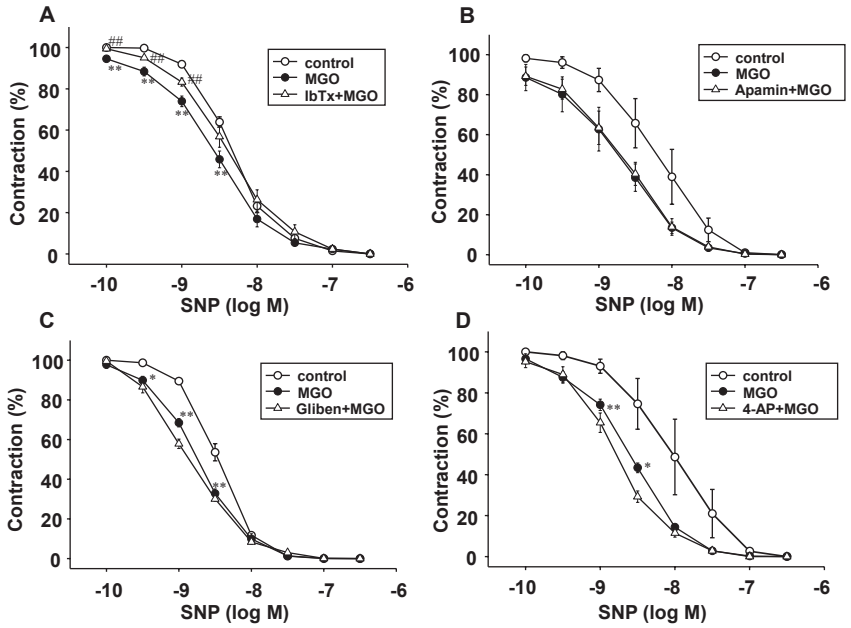

Fig. 5. Concentration–contraction relationships for SNP in endothelium-denuded rat aorta in the absence (control: open circle, n = 7 for A, n = 5 for B, n = 4 for C, and n = 4 for D) or presence of MGO pretreated without (MGO: closed circle, n = 7 for A, n = 5 for B, n = 4 for C, and n = 4 for D) or with iberiotoxin (IbTx; 100 nM, 15 min, open triangle, n = 7) (A), apamin (1 μM, 15 min, open triangle, n = 5) (B), glibenclamide (gliben; 3 μM, 15 min, open triangle, n = 4) (C), or 4-aminopyridine (4-AP; 1 mM, 15 min, open triangle, n = 4) (D). Results were expressed as the mean ± S.E.M. 100% represents NA (100 nM)–induced pre-contraction. *$P < 0.05$, **$P < 0.01$: control vs. MGO; $^{\#\#}P < 0.01$: MGO vs. IbTx + MGO.

also observed that PDTC (10 μM, 15 min), a widely used anti-oxidant to eliminate ROS in the activation of NF-κB pathways (26, 27), was also ineffective on MGO (n = 3, data not shown).

Effect of K^+-channel blockers on MGO-mediated enhancement of SNP-induced relaxation

We have previously demonstrated that MGO inhibits NA-induced contractility in rat aorta, which is mediated via opening smooth muscle large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels (16). To examine the possible involvement of K^+ channels, we used selective K^+-channel inhibitors. IbTx (100 nM, 15 min), a BK_{Ca}-channel inhibitor partially prevented the enhanced effect of MGO on SNP-induced relaxation (Fig. 5A, n = 7, pD_2: 8.34 ± 0.29 for the control, 8.54 ± 0.35 for MGO, and 8.38 ± 0.37 for IbTx + MGO). On the other hand, apamin (1 μM, 15 min), a small-conductance Ca^{2+}-activated K^+–channel inhibitor; glibenclamide (3 μM, 15 min), an ATP-sensitive K^+–channel inhibitor; or 4-AP (1 mM, 15 min), a voltage-gated potassium channel inhibitor, had no effect (in Fig. 5B, n = 5, pD_2: 8.21 ± 0.28 for the control, 8.76 ± 0.40 for MGO, and 8.66 ± 0.38 for apamin + MGO; in Fig. 5C, n = 4, pD_2: 8.47 ± 0.23 for the control, 8.74 ± 0.33 for MGO, and 8.92 ± 0.41 for glibenclamide + MGO; in Fig. 5D, n = 4, pD_2: 8.03 ± 0.26 for the control, 8.58 ± 0.35 for MGO, and 8.78 ± 0.35 for 4-AP + MGO). In contrast, a non-selective K^+-channel inhibitor, tetramethylammonium reversed the effects of MGO (pD_2: 8.33 ± 0.26 for the control, 8.55 ± 0.30 for MGO, and 8.33 ± 0.36 for TMA + MGO, n = 6, data not shown).

Discussion

In the present study, we examined the effect of MGO, a metabolite of glucose, on both endothelium-dependent and -independent relaxations in isolated blood vessel. Treatment of rat aorta with MGO (420 μM, 30 min) had no effect on the ACh-induced endothelium-dependent relaxation, but significantly increased the SNP-induced endothelium-independent relaxation. The effect of MGO is independent of ROS production. We have finally determined that MGO-induced enhancement of SNP-induced relaxation is partly due to opening smooth muscle BK_{Ca} channels. We have previously shown that MGO (420 μM, 30 min) inhibited the NA-induced smooth muscle contraction in rat aorta and mesenteric artery (16). These results collectively indicate that acute treatment with MGO results in the vasorelaxing effects in isolated blood vessel. To the best of our knowledge, the results are the first demonstration and we clarified the novel effect of MGO on relaxation of isolated blood vessel.

We performed the current study based on the hypothesis that MGO may induce hypertension by directly impairing relaxation of rat aorta, since it was reported that accumulation of MGO increased in vascular tissues of SHR and that increased aortic MGO level was associated with increased blood pressure (14). In present study, MGO rather increased the SNP-induced relaxation. There are several explanations for this discrepancy: 1) Changes that MGO produced in vascular reactivity could be a compensatory mechanism in the initial phase of exposition. 2) Hypertension and diabetes are chronically developed diseases. In the present study, however,

we determined the acute effects of MGO on arterial relaxation. Therefore, we need further studies to explore chronic effects of MGO on vascular reactivity.

We found that MGO was effective on the relaxation induced by SNP, but not the relaxation induced by ACh in aorta. The results seem controversial, since it is reasonable to assume in endothelium-intact aorta that MGO could affect not only vascular endothelium but also smooth muscle. It was previously demonstrated in vascular endothelial cells that MGO increased cyclooxygenase-2–derived product (19) or ROS (28, 29), which is one of endothelium-derived contracting factors (EDCFs). Conversely, it might be possible that MGO could decrease endothelium-derived relaxation factor (EDRF) including NO, since MGO induced endothelium cell injury/death (19, 30). Therefore why MGO had no effect on relaxation induced by ACh might be explained by the idea that the results are the combined effects of MGO-mediated enhancement of relaxation in smooth muscle and increased EDCFs or decreased EDRF in endothelium. The results that MGO also augmented the SNP-induced relaxation in endothelium-intact aorta support this concept.

We showed that treatment of endothelium-denuded rat aorta with MGO increased carbonylated proteins, which indicates increased ROS production. It was reported that H_2O_2 could elicit relaxation of endothelium-denuded blood vessels such as rat aorta, bovine coronary arteries, and human placental arteries and veins, presumably due to the effect of cGMP (31 – 34). In the present study, however, treatment of endothelium-denuded rat aorta with catalase, which metabolizes H_2O_2 to H_2O, had no effect on the MGO-mediated enhancement of relaxation induced by SNP. Thus it is suggested that MGO-induced H_2O_2 is not responsible for the effects of MGO. In addition, the present study demonstrated that neither O_2^- nor NO was responsible for the MGO-mediated enhancement of the relaxation.

In our study, the mechanisms of MGO-induced activation of BK_{Ca} channel remain to be clarified. There are reports showing that cAMP or cGMP activates BK_{Ca} channels via protein kinase A– or protein kinase G–dependent phosphorylation in smooth muscle of airway or coronary artery (35 – 42). To support this, recent studies indicated that the activity of BK_{Ca} channels are regulated by phosphorylation states of serine or tyrosine residues (43, 44). It was also reported that 12,14-dichlorodehydroabietic acid (diCl-DHAA) enhanced the activity of BK_{Ca} α-subunit via increasing sensitivity to both Ca^{2+} and membrane potential (45). It was also demonstrated that estradiol activated BK_{Ca} channels via binding to the regulatory β-subunit of the BK_{Ca} channel (46). Thus it is possible to assume that MGO activates BK_{Ca} channel via 1) second messengers–dependent phosphorylation, 2) changing the sensitivity of the channel to Ca^{2+} or membrane potential, or 3) direct binding to a particular subunit of the channel. In the present study, MGO significantly augmented the 8-Br-cGMP–induced relaxation, suggesting that MGO might enhance the sensitivity of smooth muscle to cGMP-mediated relaxing mechanisms rather than increasing the cGMP levels. Further biochemical studies might help to clarify the mechanisms through which MGO activates smooth muscle BK_{Ca} channels.

In summary, we for the first time demonstrated that MGO has an enhancing effect on SNP-induced relaxation of rat aortic smooth muscle. The effect was in part mediated via opening smooth muscle BK_{Ca} channels. Since we only clarified the acute effects of MGO on relaxation, further studies are necessary to explore the chronic effects of MGO in both blood vessels and at the whole animal level.

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References

1 Wu L. Is methylglyoxal a causative factor for hypertension development? Can J Physiol Pharmacol. 2006;84:129–139.
2 Turk Z. Glycotoxines, carbonyl stress and relevance to diabetes and its complications. Physiol Res. In press 2009.
3 Yim HS, Kang SO, Hah YC, Chock PB, Yim MB. Free radicals generated during the glycation reaction of amino acids by methylglyoxal. A model study of protein-cross-linked free radicals. J Biol Chem. 1995;270:28228–28233.
4 Thornalley PJ. Protecting the genome: defence against nucleotide glycation and emerging role of glyoxalase I overexpression in multidrug resistance in cancer chemotherapy. Biochem Soc Trans. 2003;31:1372–1377.
5 Vander Jagt DL, Hunsaker LA. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. Chem Biol Interact. 2003;143–144:341–351.
6 Lapolla A, Flamini R, Dalla Vedova A, Senesi A, Reitano R, Fedele D, et al. Glyoxal and methylglyoxal levels in diabetic patients: quantitative determination by a new GC/MS method. Clin Chem Lab Med. 2003;41:1166–1173.
7 McLellan AC, Thornalley PJ, Benn J, Sonksen PH. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. Clin Sci (Lond). 1994;87:21–29.
8 Thornalley PJ, Hooper NI, Jennings PE, Florkowski CM, Jones AF, Lunec J, et al. The human red blood cell glyoxalase system in diabetes mellitus. Diabetes Res Clin Pract. 1989;7:115–120.
9 Fosmark DS, Torjesen PA, Kilhovd BK, Berg TJ, Sandvik L, Hanssen KF, et al. Increased serum levels of the specific advanced glycation end product methylglyoxal-derived hydroimidazolone are associated with retinopathy in patients with type 2 diabetes

mellitus. Metabolism. 2006;55:232–236.
10 Mostafa AA, Randell EW, Vasdev SC, Gill VD, Han Y, Gadag V, et al. Plasma protein advanced glycation end products, carboxymethyl cysteine, and carboxyethyl cysteine, are elevated and related to nephropathy in patients with diabetes. Mol Cell Biochem. 2007;302:35–42.
11 Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, et al. Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. Am J Pathol. 1998;153:1149–1155.
12 Oya T, Hattori N, Mizuno Y, Miyata S, Maeda S, Osawa T, et al. Methylglyoxal modification of protein. Chemical and immunochemical characterization of methylglyoxal-arginine adducts. J Biol Chem. 1999;274:18492–18502.
13 Nakadate Y, Uchida K, Shikata K, Yoshimura S, Azuma M, Hirata T, et al. The formation of argpyrimidine, a methylglyoxal-arginine adduct, in the nucleus of neural cells. Biochem Biophys Res Commun. 2009;378:209–212.
14 Wang X, Desai K, Chang T, Wu L. Vascular methylglyoxal metabolism and the development of hypertension. J Hypertens. 2005;23:1565–1573.
15 Vasdev S, Ford CA, Longerich L, Parai S, Gadag V, Wadhawan S. Aldehyde induced hypertension in rats: prevention by N-acetyl cysteine. Artery. 1998;23:10–36.
16 Mukohda M, Yamawaki H, Nomura H, Okada M, Hara Y. Methylglyoxal inhibits smooth muscle contraction in isolated blood vessels. J Pharmacol Sci. 2009;109:305–310.
17 Nomura H, Yamawaki H, Mukohda M, Okada M, Hara Y. Mechanisms underlying pioglitazone-mediated relaxation in isolated blood vessel. J Pharmacol Sci. 2008;108:258–265.
18 Randell EW, Vasdev S, Gill V. Measurement of methylglyoxal in rat tissues by electrospray ionization mass spectrometry and liquid chromatography. J Pharmacol Toxicol Methods. 2005;51:153–157.
19 Yamawaki H, Saito K, Okada M, Hara Y. Methylglyoxal mediates vascular inflammation via JNK and p38 in human endothelial cells. Am J Physiol Cell Physiol. 2008;295:C1510–C1517.
20 Yamawaki H, Hara N, Okada M, Hara Y. Visfatin causes endothelium-dependent relaxation in isolated blood vessels. Biochem Biophys Res Commun. 2009;383:503–508.
21 Nakamura A, Goto S. Analysis of protein carbonyls with 2,4-dinitrophenyl hydrazine and its antibodies by immunoblot in two-dimensional gel electrophoresis. J Biochem. 1996;119:768–774.
22 Sato T, Seyama K, Sato Y, Mori H, Souma S, Akiyoshi T, et al. Senescence marker protein-30 protects mice lungs from oxidative stress, aging, and smoking. Am J Respir Crit Care Med. 2006;174:530–537.
23 Bouvet C, Belin de Chantemele E, Guihot AL, Vessieres E, Bocquet A, et al. Flow-induced remodeling in resistance arteries from obese zucker rats is associated with endothelial dysfunction. Hypertension. 2007;50:248–254.
24 Auch-Schwelk W, Katusic ZS, Vanhoutte PM. Contractions to oxygen-derived free radicals are augmented in aorta of the spontaneously hypertensive rat. Hypertension. 1989;13:859–864.
25 Hink J, Thom SR, Simonsen U, Rubin I, Jansen E. Vascular reactivity and endothelial NOS activity in rat thoracic aorta during and after hyperbaric oxygen exposure. Am J Physiol Heart Circ Physiol. 2006;291:H1988–H1998.
26 Brennan P, O'Neill LA. 2-mercaptoethanol restores the ability of nuclear factor kappa B (NF kappa B) to bind DNA in nuclear extracts from interleukin 1-treated cells incubated with pyrollidine dithiocarbamate (PDTC). Evidence for oxidation of glutathione in the mechanism of inhibition of NF kappa B by PDTC. Biochem J. 1996;320:975–981.
27 Fernandes RS, McGowan AJ, Cotter TG. Mutant H-ras overexpression inhibits drug and U.V. induced apoptosis. Anticancer Res. 1996;16:1691–1705.
28 Schalkwijk CG, Vermeer MA, Stehouwer CD, te Koppele J, Princen HM, van Hinsbergh VW. Effect of methylglyoxal on the physico-chemical and biological properties of low-density lipoprotein. Biochim Biophys Acta. 1998;1394:187–198.
29 Hsieh CL, Huang CN, Lin YC, Peng RY. Molecular action mechanism against apoptosis by aqueous extract from guava budding leaves elucidated with human umbilical vein endothelial cell (HUVEC) model. J Agric Food Chem. 2007;55:8523–8533.
30 Baden T, Yamawaki H, Saito K, Mukohda M, Okada M, Hara Y. Telmisartan inhibits methylglyoxal-mediated cell death in human vascular endothelium. Biochem Biophys Res Commun. 2008;373:253–257.
31 Thomas G, Ramwell P. Induction of vascular relaxation by hydroperoxides. Biochem Biophys Res Commun. 1986;139:102–108.
32 Omar HA, Figueroa R, Tejani N, Wolin MS. Properties of a lactate-induced relaxation in human placental arteries and veins. Am J Obstet Gynecol. 1993;169:912–918.
33 Mohazzab HK, Kaminski PM, Fayngersh RP, Wolin MS. Oxygen-elicited responses in calf coronary arteries: role of H_2O_2 production via NADH-derived superoxide. Am J Physiol. 1996;270:H1044–H1053.
34 Hayabuchi Y, Nakaya Y, Matsuoka S, Kuroda Y. Hydrogen peroxide-induced vascular relaxation in porcine coronary arteries is mediated by Ca^{2+}-activated K^+ channels. Heart Vessels. 1998;13:9–17.
35 Kume H, Takai A, Tokuno H, Tomita T. Regulation of Ca^{2+}-dependent K^+-channel activity in tracheal myocytes by phosphorylation. Nature. 1989;341:152–154.
36 Minami K, Fukuzawa K, Nakaya Y, Zeng XR, Inoue I. Mechanism of activation of the Ca(2+)-activated K^+ channel by cyclic AMP in cultured porcine coronary artery smooth muscle cells. Life Sci. 1993;53:1129–1135.
37 Robertson BE, Schubert R, Hescheler J, Nelson MT. cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. Am J Physiol. 1993;265:C299–C303.
38 Scornik FS, Codina J, Birnbaumer L, Toro L. Modulation of coronary smooth muscle KCa channels by Gs alpha independent of phosphorylation by protein kinase A. Am J Physiol. 1993;265:H1460–H1465.
39 Taniguchi J, Furukawa KI, Shigekawa M. Maxi K^+ channels are stimulated by cyclic guanosine monophosphate-dependent protein kinase in canine coronary artery smooth muscle cells. Pflugers Arch. 1993;423:167–172.
40 Torphy TJ. Beta-adrenoceptors, cAMP and airway smooth muscle relaxation: challenges to the dogma. Trends Pharmacol Sci. 1994;15:370–374.
41 Standen NB, Quayle JM. K^+ channel modulation in arterial smooth muscle. Acta Physiol Scand. 1998;164:549–557.
42 Schubert R, Nelson MT. Protein kinases: tuners of the BK_{Ca} channel in smooth muscle. Trends Pharmacol Sci. 2001;22:505–512.
43 Yokoshiki H, Seki T, Sunagawa M, Sperelakis N. Inhibition of

Ca^{2+}-activated K^+ channels by tyrosine phosphatase inhibitors in rat mesenteric artery. Can J Physiol Pharmacol. 2000;78:745–750.

44 Zhou XB, Arntz C, Kamm S, Motejlek K, Sausbier U, Wang GX, et al. A molecular switch for specific stimulation of the BKCa channel by cGMP and cAMP kinase. J Biol Chem. 2001;276:43239–43245.

45 Sakamoto K, Nonomura T, Ohya S, Muraki K, Ohwada T, Imaizumi Y. Molecular mechanisms for large conductance Ca^{2+}-activated K^+ channel activation by a novel opener, 12,14-dichloro-dehydroabietic acid. J Pharmacol Exp Ther. 2006;316:144–153.

46 Valverde MA, Rojas P, Amigo J, Cosmelli D, Orio P, Bahamonde MI, et al. Acute activation of Maxi-K channels (hSlo) by estradiol binding to the beta subunit. Science. 1999;285:1929–1931.