J Pharmacol Sci **109**, 305 – 310 (2009)

Journal of Pharmacological Sciences

Full Paper

Methylglyoxal Inhibits Smooth Muscle Contraction in Isolated Blood Vessels

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Received November 8, 2008; Accepted December 25, 2008

Abstract. Methylglyoxal (MGO) is a metabolite of glucose. In addition to evidence that increased plasma MGO level is associated with diabetic vascular complications, recent studies demonstrated that MGO accumulated in vascular tissues of hypertensive animals. We hypothesized that MGO could directly affect vascular reactivity. To test the hypothesis, we examined effects of MGO on contraction of isolated blood vessels. Treatment of endothelium-denuded rat aorta with MGO (420 μM, 30 min) shifted the concentration–response curve for noradrenaline (NA: 1 nM – 1 μM) to the right. The inhibitory effect was concentration-dependent (MGO: 42 – 420 μM). Indomethacin (10 μM) and cimetidine (30 μM) could not prevent the inhibitory effect of MGO. However, a non-selective K^+-channel inhibitor, tetramethylammonium (10 mM), prevented it. Glibenclamide (3 μM), an ATP-sensitive K^+-channel inhibitor or apamin (1 μM), a small conductance Ca^{2+}-activated K^+-channel inhibitor was ineffective, but iberiotoxin (100 nM), a large conductance Ca^{2+}-activated K^+ (BK_{Ca})-channel inhibitor significantly prevented the effect of MGO. MGO (420 μM, 30 min) also inhibited the NA (1 nM – 1 μM)-induced contraction in mesenteric artery. The present results indicate that MGO has an inhibitory effect on contractility of isolated blood vessel, which is mediated via opening smooth muscle BK_{Ca} channel.

Keywords: glucose metabolite, vascular smooth muscle, contraction, potassium channel

Introduction

Methylglyoxal (MGO) is a metabolite of glucose. MGO is produced from dihydroxyacetone phosphate as a by-product during the formation of glyceraldehyde 3-phosphates in mammalian cells, including vascular smooth muscle cells (VSMCs) (1). MGO is further metabolized into advanced glycation end-products (AGEs) by non-enzymatic glycation of protein (2). It is also known that MGO is catalyzed to propanediol by aldose reductase (3).

It was reported that the plasma concentration of MGO is significantly increased in diabetic patients (4 – 6). Increased MGO-derived AGEs level in diabetic patients seems to correlate with diabetic complications such as diabetic nephropathy (7) and retinopathy (8). In addition, recent reports have demonstrated that accumulation of MGO increased in vascular tissues of spontaneous hypertensive rats (SHR) with aging (9). It was shown that increased aortic MGO level was associated with increased blood pressure in SHR. Furthermore, it was reported that administration of MGO by drinking water induced hypertension in Wistar-Kyoto rats (10). Although it is logical to hypothesize that MGO could directly affect vascular reactivity, there is no such report available. To test the hypothesis, we examined effects of MGO on contractility of isolated blood vessel and found that acute treatment of rat arteries with MGO inhibited smooth muscle contraction.

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Published online in J-STAGE on February 7, 2009 (in advance)
doi: 10.1254/jphs.08300FP

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 these rats was 4.8 mM at 5 weeks and 7.1 mM at 10 weeks. The thoracic aorta and superior mesenteric artery were isolated. After removal of fat and adventitia, the aorta was cut into strips (approximately 4-mm-wide, 8-mm-

long) and the mesenteric artery was cut into rings (1-mm in diameter) for the measurement of isometric tension. The endothelium was removed by rubbing the intimal surface with the flat face of a pair of forceps. Removal of the endothelium 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 (11, 12). The arterial preparations were repeatedly exposed to high-K^+ solution until the responses became stable (45 min). Noradrenaline (NA: 1 nM – 1 μM) was cumulatively applied and concentration–responses curves to NA were sequentially obtained in the same arterial preparation without (control) or with MGO (30 min). For each condition, we performed 2 sequential experiments (1st experiment: control and 2nd experiment: MGO alone or MGO + inhibitor) and confirmed that control sequential experiments performed after 30 min without MGO showed the same contraction profile as the preceding control.

Statistical analyses

Results are expressed as a mean ± S.E.M. Statistical evaluation of the data 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. All pD_2 values were calculated as the $-\log_{10}EC_{50}$ (i.e., that concentration at which the half maximal effect occurred) by sigmoid curve fitting.

Chemicals

The chemicals used were as follows: NA, indomethacin, EDTA, cimetidine, tetramethylammonium (TMA), glibenclamide, apamin, MGO solution, iberiotoxin (Sigma, St. Louis, MO, USA) and ACh (Daiichi Pharmaceutical, Tokyo). Indomethacin, cimetidine, and glibenclamide were dissolved in DMSO (0.1%). Apamin was dissolved in acetic acid (0.05 mM). Other drugs were dissolved in distilled water.

Results

Effect of MGO on NA-induced contraction of endothelium-denuded thoracic aorta of rats

We first examined effects of pretreatment of rat aorta with MGO (420 μM, 30 min) on contraction induced by NA (1 nM – 1 μM). Treatment with MGO shifted the concentration–response curve for NA to the right (Fig. 1: A and B, open diamond). Maximum contraction induced by NA (1 μM; Fig. 1C, n = 47) or KCl (72 mM; Fig. 1D, n = 7) was not affected by the treatment with MGO (420 μM, 30 min). Figure 1B showed that MGO-mediated inhibition of NA-induced contraction was concentration-dependent (pD_2: 8.08 ± 0.05, n = 16 for the control; 7.81 ± 0.13, n = 4 for 42 μM MGO; 7.81 ± 0.15, n = 4 for 105 μM MGO; 7.70 ± 0.08, n = 4 for 210 μM MGO; 7.63 ± 0.11, n = 4 for 420 μM MGO; $P<0.05$, between the control and 210 μM MGO and $P<0.01$ between the control and 420 μM MGO). We observed that treatment with a higher concentration of MGO (4.2 mM) inhibited the NA (1 μM)-induced maximal contraction (n = 3, data not shown).

Effect of indomethacin or cimetidine on MGO-mediated inhibition of NA-induced contraction

To determine mechanisms responsible for the inhibitory effect of MGO, effects of indomethacin and cimetidine were examined. Treatment of rat aorta with a cyclooxygenase inhibitor, indomethacin (10 μM, 15 min), or a histamine H_2–receptor blocker, cimetidine (30 μM, 15 min), had no effects on MGO (420 μM, 30 min)-mediated inhibition of NA (1 nM – 1 μM)-induced contraction (In Fig. 2A, pD_2: 8.14 ± 0.05, n = 23 for the control; 7.91 ± 0.07, n = 11 for MGO; 7.89 ± 0.07, n = 12 for indomethacin + MGO and in Fig. 2B, pD_2: 8.11 ± 0.07, n = 9 for the control; 7.71 ± 0.11, n = 5 for MGO; 7.73 ± 0.11, n = 4 for cimetidine + MGO).

Effect of non-selective K^+-channel inhibitor on MGO-mediated inhibition of NA-induced contraction

To further explore the mechanisms, we used a non-selective K^+-channel inhibitor, TMA. Treatment with TMA (10 mM, 15 min) prevented the MGO (420 μM, 30 min)-mediated inhibition of NA (1 nM – 1 μM)-induced contraction (in Fig. 3, pD_2: 8.08 ± 0.04, n = 12 for the control; 7.81 ± 0.12, n = 5 for MGO; and 8.16 ± 0.07, n = 7 for TMA + MGO; $P<0.05$ between the control and MGO and $P<0.01$ between MGO and TMA + MGO). TMA alone had minimal effects on NA-induced contraction (n = 7, data not shown).

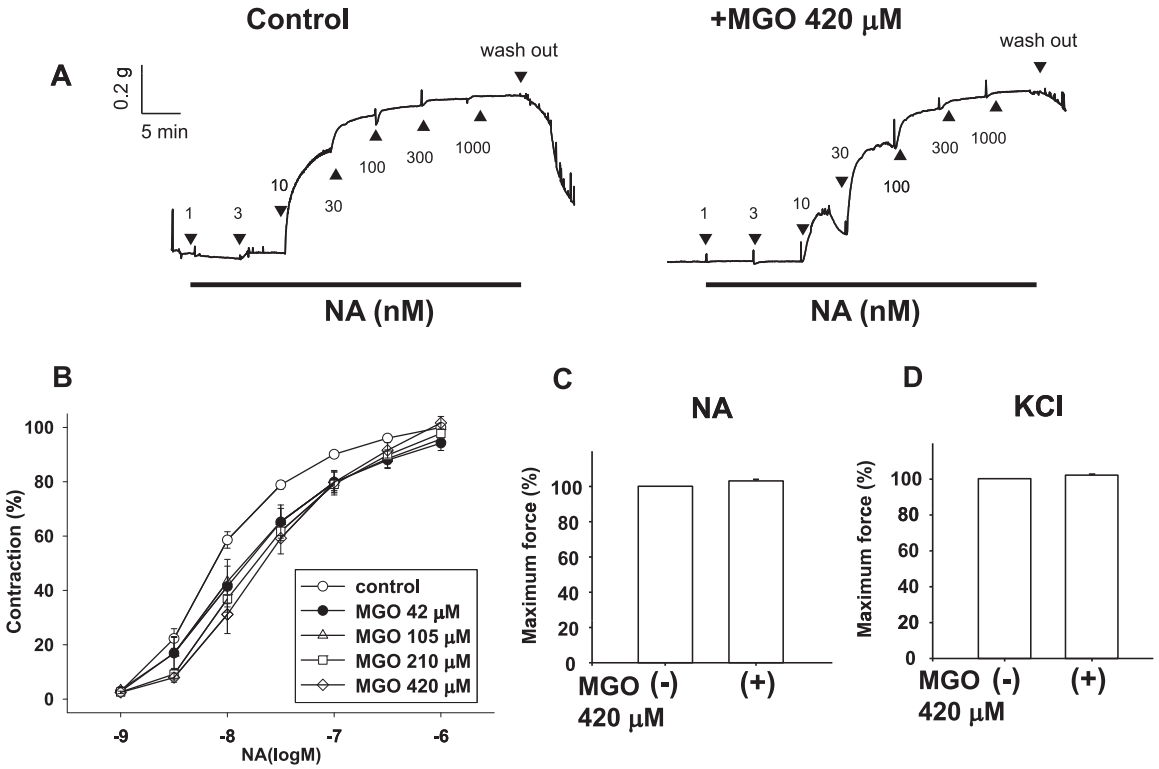

Fig. 1. Effect of pretreatment with methylglyoxal (MGO) on noradrenaline (NA)-induced concentration-dependent contraction. A: NA (1 nM – 1 μM) was cumulatively applied to endothelium-denuded rat aorta in the absence [MGO (–): control] or presence of MGO (420 μM, 30 min). B: Concentration-dependent effect of MGO (0 – 420 μM) on NA-induced contraction is shown. Results were each expressed as a mean ± S.E.M. 100% represents NA (1 μM)-induced maximal contraction in control artery. Control: open circle, n = 16; 42 μM MGO: closed circle, n = 4; 105 μM MGO: open triangle, n = 4; 210 μM MGO: open square, n = 4; 420 μM MGO: open diamond, n = 4. C, D: Effect of pretreatment with MGO (420 μM, 30 min) on NA (1 μM, n = 47) (C)- or KCl (72 mM, n = 7) (D)-induced maximum contraction. Data were expressed relative to MGO (–).

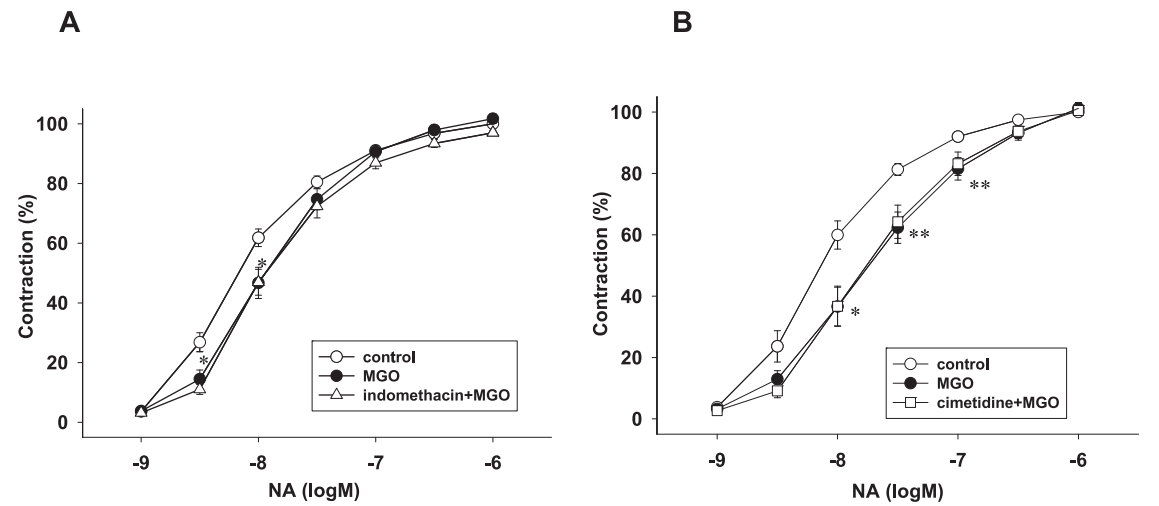

Fig. 2. Concentration–contraction relationships for NA in endothelium-denuded rat aorta in the absence (control: open circle; n = 23 for A, n = 9 for B) or presence of MGO (420 μM, 30 min) pretreated without (MGO: closed circle; n = 11 for A, n = 5 for B) or with indomethacin (10 μM, 15 min, open triangle, n = 12) (A) or cimetidine (30 μM, 15 min, open square, n = 4) (B). Results were each expressed as a mean ± S.E.M. 100% represents NA (1 μM)-induced maximal contraction in control artery. *P<0.05, **P<0.01, control *vs* MGO.

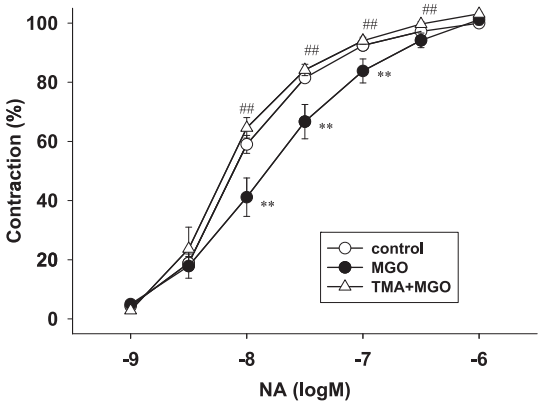

Fig. 3. Effect of a non-selective K^+-channel inhibitor on MGO-mediated inhibition of NA-induced contraction. Results were each expressed as a mean ± S.E.M. 100% represents NA (1 μM)-induced maximal contraction in control artery. Control: open circle, n = 12; MGO: closed circle, n = 5; TMA + MGO: open triangle, n = 7. **P<0.01, control *vs* MGO. $^{\#\#}P$<0.01, MGO *vs* TMA + MGO.

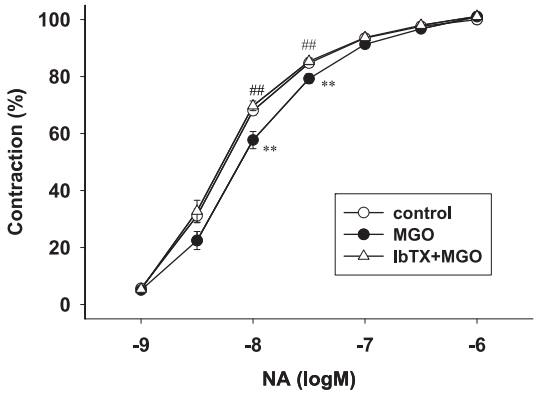

Fig. 5. Concentration–contraction relationships for NA in endothelium-denuded rat aorta in the absence (control: open circle, n = 26) or presence of MGO (420 μM, 30 min) pretreated without (MGO: closed circle, n = 14) or with iberiotoxin (100 nM IbTX, 15 min, open triangle, n = 12). Results were each expressed as a mean ± S.E.M. 100% represents NA (1 μM)-induced maximal contraction in control artery. **P<0.01, control *vs* MGO; $^{\#\#}P$<0.01, MGO *vs* IbTX + MGO.

Effect of K^+-channel inhibitors on MGO-mediated inhibition of NA-induced contraction

We next analyzed which type of K^+ channel is responsible for the inhibitory effect of MGO. Treatment with an ATP-sensitive K^+ (K_{ATP})-channel inhibitor, glibenclamide (3 μM, 15 min), or a small conductance Ca^{2+}-activated K^+ (SK)-channel inhibitor, apamin (1 μM, 15 min), did not prevent the inhibitory effect of MGO (in Fig. 4A, pD_2: 8.11 ± 0.03, n = 8 for the control; 7.89 ± 0.08, n = 4 for MGO; 7.75 ± 0.06, n = 4 for gliben + MGO and in Fig. 4B, pD_2: 8.05 ± 0.05, n = 11 for the control; 7.68 ± 0.10, n = 6 for MGO; 7.74 ± 0.09, n = 5 for apamin + MGO).

In contrast, a large conductance Ca^{2+}-activated K^+ (BK_{Ca})-channel inhibitor, iberiotoxin (100 nM, 15 min), significantly prevented the inhibitory effect of MGO (in Fig. 5, pD_2: 8.23 ± 0.03, n = 26 for the control; 8.07 ± 0.05, n = 14 for MGO; and 8.25 ± 0.04, n = 12 for iberiotoxin + MGO; P<0.01 between the control and MGO and P<0.01 between MGO and iberiotoxin + MGO). Iberiotoxin alone had no effects on NA-induced contraction (n = 6, data not shown).

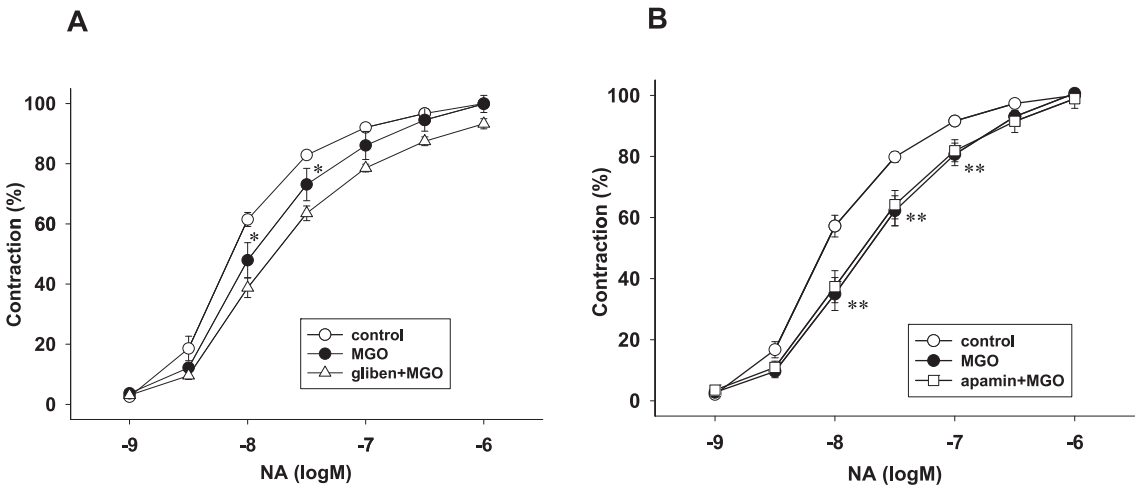

Fig. 4. Concentration–contraction relationships for NA in endothelium-denuded rat aorta in the absence (control: open circle; n = 8 for A, n = 11 for B) or presence of MGO (420 μM, 30 min) pretreated without (MGO: closed circle; n = 4 for A, n = 6 for B) or with glibenclamide (3 μM, 15 min, open triangle, n = 4) (A) or apamin (1 μM, 15 min, open square, n = 5) (B). Results were each expressed as a mean ± S.E.M. 100% represents NA (1 μM)-induced maximal contraction in control artery. *P<0.05, **P<0.01, control *vs* MGO.

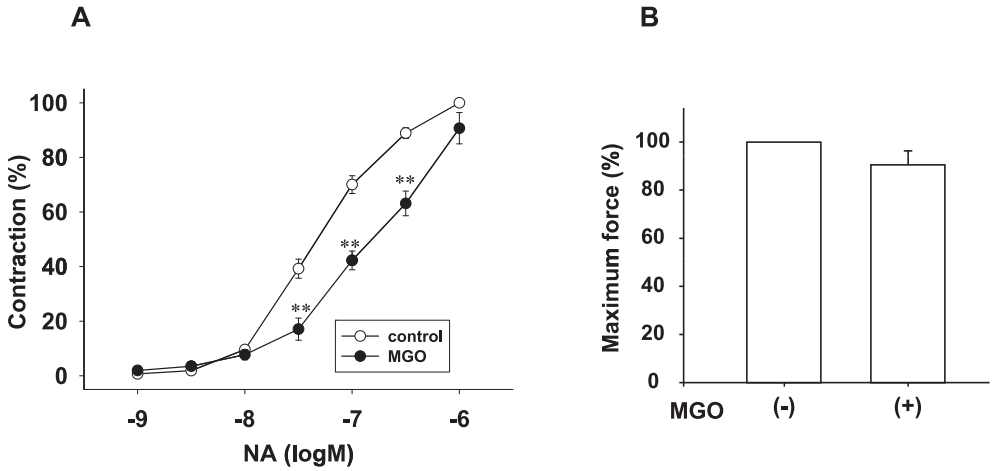

Fig. 6. Effect of pretreatment with MGO on NA-induced contraction in mesenteric artery of rats. A: Concentration–contraction relationships for NA (1 nM – 1 μM) in endothelium-denuded rat mesenteric artery in the absence (control, open circle) or presence of MGO (420 μM, 30 min, closed circle). Results were each expressed as a mean ± S.E.M. of 5 experiments. 100% represents NA (1 μM)-induced maximal contraction in control artery. **P<0.01, control *vs* MGO. B: Effect of pretreatment with MGO on NA (1 μM)-induced maximum contraction. Data were each expressed relative to MGO (–) (n = 5).

Effect of MGO on NA-induced contraction of mesenteric artery of rats

We finally examined the effect of pretreatment of endothelium-denuded mesenteric artery with MGO on contraction induced by NA (1 nM – 1 μM). Treatment with MGO (420 μM, 30 min) shifted the concentration-response curve for NA to the right (in Fig. 6A, n = 5, pD_2: 7.30 ± 0.05 for the control and 6.77 ± 0.08 for MGO, P<0.01). The maximum contraction induced by NA (1 μM) was not affected by the treatment with MGO (Fig. 6B, n = 5).

Discussion

The major findings of the present study are that acute treatment of endothelium-denuded rat aorta with MGO decreased the sensitivity of contraction to NA. The inhibitory effect of MGO is independent of prostacyclin or histamine production. We have finally determined that the effect of MGO is mediated via opening smooth muscle BK_{Ca} channel. It was also confirmed that acute treatment of rat mesenteric artery with MGO inhibited the contractile sensitivity to NA, suggesting that the effect of MGO is not specific to muscular type vessels. Here, we for the first time clarified the effects of MGO on contractility of isolated blood vessels.

We found that MGO is effective on the contraction induced by NA but not high K^+. Contrastingly, previous studies showed that NS1619, a widely used benzimidazolone BK_{Ca}-channel opener, inhibited high-K^+ induced contraction in rat aorta (13) and basilar artery (14). However, the inhibitory effect might be derived from a nonspecific effect since it was shown that NA1619 inhibited L-type Ca^{2+} channel (13 – 15). Therefore, it is suggested that MGO might be a more specific BK_{Ca}-channel opener than NS 1619. It was also found that MGO (420 μM) has the unique property of being able to change the sensitivity of contraction to NA without inhibiting the maximal force. Although we could not state the exact reason for this, a higher concentration of MGO (4.2 mM) inhibited the maximal force induced by NA.

In the present study, mechanisms of BK_{Ca}-channel activation by MGO remain unclear. It was previously reported that estradiol activated the BK_{Ca} channel from bovine aortic smooth muscle cells (16). It was shown that the activation was mediated by estradiol binding to the regulatory β-subunit of BK_{Ca} channel. Alternatively, it is well known that cAMP or cGMP activates BK_{Ca} channels via PKA- or PKG-dependent phosphorylation (17 – 20). In addition, there are reports demonstrating that isoprenaline activates BK_{Ca} channel in coronary arterial smooth muscle (21, 22). It was shown that β-adrenaline receptor–mediated activation of Gsα stimulated the α-subunit of the BK_{Ca} channel. Thus it is possible to assume that MGO might directly activate BK_{Ca} channel by physically binding to or indirectly activate BK_{Ca} channel via second messengers–dependent mechanisms. Further biochemical studies might help to clarify the mechanisms through which MGO activates smooth muscle BK_{Ca} channel.

We showed that MGO inhibited the contractility of vascular smooth muscle in a concentration-dependent manner (42 – 420 μM, Fig. 1B). There is a report

showing that the plasma MGO concentrations in patients with poorly controlled diabetes are about 400 μM (4), whereas other studies reported that it was much less (5, 6). It is suggested that local MGO concentration in tissues is much higher than the plasma level (23). Based on the above reports as well as the concentration-dependent inhibitory effects of MGO, it seems likely that our results are pathophysiologically relevant. Since we only clarified the acute effects of MGO, further studies are necessary to explore the chronic effects of MGO on vascular reactivity in both blood vessels and whole animal levels.

In summary, we demonstrated for the first time that MGO has inhibitory effects on NA-induced contraction of rat aorta and mesenteric artery. The inhibitory effect was mediated via opening smooth muscle BK_{Ca} channel. Further studies might contribute to provide mechanistic insights into roles of MGO in the development of diabetes-associated vascular complications.

Acknowledgements

This study was supported by a Grant-in-Aid for scientific research (#19790195) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; Kitasato University Research Grant for Young Researchers; and Research Grants from the Takeda Science Foundation and the Uehara Memorial Foundation (to Dr. H. Yamawaki).

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