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Enhanced calcium entry via activation of NOX/PKC underlies increased vasoconstriction induced by methylglyoxal

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Abstract

Advanced glycation end-products (AGEs) play a pivotal role in macro- and micro-vascular diabetic complications. We investigated the mechanism by which methylglyoxal (an endogenous generator of AGEs) affects vascular contractility using the isolated artery technique. Contractile responses to vasoconstrictors phenylephrine (PE), angiotensin II (AngII), vasopressin (VP) and KCl were measured in the isolated rat aorta following one-our exposure to methylglyoxal (50-200 μM). The perfused rat kidney was employed to confirm the effect of methylglyoxal on microvessels. Methylglyoxal-induced changes in cytosolic calcium were measured in the smooth muscle layer of the aorta with the calcium-sensing fluorophore Fluo-4 AM. Methylglyoxal significantly increased maximal contraction of the rat aorta to PE, Ang II and VP. Similar results were seen in response to the depolarizing vasoconstrictor KCl in macro and micro vessels. The methylglyoxal-induced increases in aortic contraction mediated by the agonist and KCl were endothelium independent. Methylglyoxal-induced increases in KCl-dependent aortic contraction were abolished after the removal of extracellular calcium or in the presence of the calcium channel blocker nifedipine. Incubation with the antioxidant N-acetyl-L-cysteine (NAC), apocynin (a nonselective NADPH oxidase (NOX) inhibitor) or chelerythrine (a protein kinase C (PKC) inhibitor) prior to methylglyoxal pre-treatment reversed the methylglyoxal-induced increases

in the rat aortic contractility. In conclusion, the formation of AGEs increases vasoconstriction of both macro- and micro-vessels by increasing the voltage-activated calcium entry in vascular smooth muscles in a NOX and PKC dependent manner.

Keywords: methylglyoxal; diabetes mellitus; vascular contraction; rat; aorta.

1. Introduction:

Diabetes mellitus (DM) is a disease in which hyperglycemia develops in patients as a result of impaired insulin release and/or insulin resistance [1]. The World Health Organization registered a staggering 350 million cases of DM around the world in 2011 and the number is projected to increase to 440 million by the year 2030 [2].

Diabetic complications are becoming among the fastest growing causes of morbidity and mortality worldwide [1]. DM is associated with vascular complications and endothelial dysfunction, all of which directly affect vascular contractility [1]. High blood sugar levels seen in diabetic patients are believed to underlie the generation of AGEs. AGEs are formed in serum and tissues by non-enzymatic glycation, a reaction of sugars with amino groups of proteins and lipids [3]. Raised AGE levels are reported to correlate with diabetic complications, such

as nephropathy [4] and retinopathy [5]. AGEs could also be produced from sugar derivatives such as glyoxal and methylglyoxal [6].

Various studies have reported increased levels of glyoxal and methylglyoxal in plasma in diabetic patients [7] and in tissues in the streptozotocin-induced diabetic rat model [8]. Further studies demonstrated that methylglyoxal, when added to the drinking water, led to development of insulin resistance and hypertension in rats [9]. Levels of methylglyoxal also increased with ageing in spontaneously hypertensive rats [10].

However, only few studies investigated the effect of methylglyoxal on vascular reactivity. Pre-treatment of cultured rat aortic rings with methylglyoxal for 24 hours reduced endothelium-dependent relaxation due to reduction of expression and function of endothelial nitric oxide synthase [11]. Conversely, a short pre-treatment of rat vasculature with methylglyoxal either increased angiotensin II (Ang II) mediated contraction in the endothelial-dependent manner [12] or inhibited noradrenaline-mediated contraction in endothelium-independent fashion [13]. We recently reported that methylglyoxal reduced acetylcholine-mediated relaxation of the rat aorta precontracted with phenylephrine, but on the other hand, augmented phenylephrine-induced contraction [14]. Mechanisms of the methylglyoxal-induced increase in tension were not yet characterized. Therefore, this study aimed to investigate the effect of methylglyoxal on

contraction of isolated rat aorta in response to various agonists and elucidate the mechanistic basis of augmented vascular reactivity. The effects of methylglyoxal on microvascular reactivity in the perfused rat kidney preparation were also assessed *in vitro*. The outcome of this study provides a better understanding of the importance of AGEs in vascular diabetic complications.

Materials and methods:

2.1 Drugs and chemicals:

Vasopressin (20 units/mL) was manufactured by Swiss Parentrals PVT. Nifedipine, N-acetyl-L-cysteine (NAC) and chelerythrine were purchased from LKT Laboratories. Methylglyoxal, potassium chloride (KCl), angiotensin II (Ang II), phenylephrine and apocynin were purchased from Sigma–Aldrich, Dorset, UK. Fluo-4 AM was purchased from Thermo Fischer Scientific®, UK. Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was purchased from Hach company (Loveland, USA). Chelerythrine and apocynin were dissolved in dimethylsulfoxide (DMSO) and stored in aliquots at -20°C. All other drugs were dissolved in pure de-ionized water. Final DMSO concentration did not exceeded 0.1% and had no significant effect on aortic contraction (our preliminary data and previously published studies [14]).

2.2 Experimental animals:

Male Wistar rats (180-200 g) from the animal house facility at the King Abdul-Aziz University-Research Center were killed by decapitation. The descending aorta was carefully removed, placed in a cold Krebs–Henseleit buffer (KHB), cleaned of surrounding fat and connective tissue and then cut into ~3 mm rings in length.

2.3 Research design:

The research methodologies were in accordance with the Regulations of Research Bioethics on the Living Creatures of the National Committee of Biomedical Ethics, Kingdom of Saudi Arabia. The protocol was approved by the Research Ethics Committee, Faculty of Pharmacy, King Abdulaziz University (Reference No. 1438-110).

2.3.1 Macrovascular reactivity:

Isometric tension measurements from isolated thoracic aortae were performed using the organ bath technique as previously described [14]. Briefly, aortic rings were suspended in an organ bath filled with KHB containing (in mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5; $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 11; pH=7.4; at 37°C and bubbled with 95% O_2 / 5% CO_2. Vessels were kept under constant 1500 mg tension in the presence or absence of the AGE generating compound methylglyoxal. Aortic rings isolated from the same rat were incubated for 1 hour in

KHB (control) or in different concentrations of methylglyoxal (50, 100 or 200 μM). Cumulative concentration-response curve (CRC) to PE (10^{-8}-10^{-5} M), Ang II (10^{-9}-10^{-6} M), vasopressin (10^{-10}-10^{-7} M) or KCl (10-100 mM) were measured in the control and methylglyoxal-treated preparations. Agonists (PE, ANG II and vasopressin) were added in semi-logarithmic and KCl in 10 mM increments. To assess mechanisms underlying methylglyoxal-mediated effects, experiments were carried out in a nominally Ca^{2+}-free KHB (with 1 mM EGTA), and, separately, in the presence of 100 μM nifedipine, 1 mM NAC, 10 μM apocynin or 10 μM chelerythrine using KCl as a vasoconstrictor. All inhibitors were added 30 min prior to methylglyoxal pre-treatment. The responses were recorded by an isometric force transducer (MLT202) connected to a data acquisition system (Powerlab® 8/30) running Labchart Pro® 8.1.5 software (AD Instruments, Australia). Where stated, the endothelium was removed by gently rubbing the vessel lumen with a thin smooth pin and successful removal was verified as the lack of response to 1 μM acetylcholine.

2.3.2 Microvascular studies:

The effect of methylglyoxal on microvasculature was evaluated in an isolated perfused left kidney of the rat as described previously [15]. Briefly, after the animal was killed by decapitation and the left kidney was accessed via an incision in the abdomen. The left renal artery was exposed, cleaned of surrounding adipose

tissue and cannulated with an 18-gauge needle through an incision in the aorta. The cannula was stabilized with ligature placed around the renal artery. The kidney was flushed with 5 mL of heparinized saline solution (100 U/mL heparin), excised, transferred into a glass chamber and then perfused with KHB (37ºC, 95% +O_2, 5% CO_2) for 30 minutes before starting the experiment. A peristaltic pump (Masterflex®, Colepalmer, Jubail, Saudi Arabia) was used to maintain a flow rate of 3 mL/min and the effluent from the veins was allowed free drainage. Changes in perfusion pressure as the measure of microvascular resistance were measured using a pressure transducer connected to a Power Lab Data system (Gould P23ID, USA) and analyzed using Labchart Pro® 8.1.5 software (AD Instruments, Australia). An isolated kidney was perfused with or without methylglyoxal (100 µM) for 1 h followed by perfusion with KCl (10-100 mM).

2.3.3 Intracellular calcium monitoring:

Intracellular calcium was monitored in the aortic smooth muscle layer using the fluorescence probe Fluo-4 AM as previously described with some modifications [16]. Isolated aortic rings (~ 3 mm length) were cut open longitudinally and the endothelium layer was gently rubbed off and incubated in 1.5 mL conical plastic tubes containing KHB without (control) or with methylglyoxal (100 µM) for 1 hour at 37ºC. Following the incubation, aortic preparations were carefully transferred to different conical plastic tubes with fresh KHB containing Fluo-4 AM

(0.2 μM) and incubated for 15 minutes at 37ºC. Pieces of aorta were transferred to conical plastic tubes containing dye-free KHB for 5 min at 37ºC. Aortic tissues with the smooth muscle layer facing upwards were placed in a 96-well black plate, secured in place with small clear plastic rings, and 200 μL KHB was added to each well. Calcium influx in aortic smooth muscles was stimulated by KCl (80 mM) and fluorescence intensity (λ_{ex}=480 nm, λ_{em}=506 nm) was measured every minute for 10 min using a SpectraMax® M3 monochromator plate reader (Molecular Devices, California, USA). Fluorescence was also measured in the presence of methylglyoxal and nifedipine (100 μM). Changes in intracellular calcium levels were expressed as Fluo-4 fluorescence intensity in arbitrary units.

2.4 Statistical analysis:

Values were expressed as mean ± standard error of the mean (SEM) with n representing the number of rats with 6-8 animals typically used in each experimental protocol. The difference between the groups was analyzed by the two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Statistical significance was determined as $p < 0.05$. Data were analyzed using Prism 5 software (Graphpad v5, CA, USA).

2. Results:

3.1 The effect of methylglyoxal on receptor-mediated contraction of the isolated rat aorta:

Figure 1A compares the effect of a one-hour incubation with methylglyoxal (50, 100 or 200 µM) on cumulative CRC to PE in isolated aortic rings with intact endothelium. Methylglyoxal enhanced PE-induced contraction at all concentrations, however, the effect was significantly different from the control at PE concentrations between 1×10^{-6} and 1×10^{-5} M after pre-treatment with 200 µM. 100 µM methylglyoxal also significantly increased contraction at 1×10^{-5} M of PE ($p<0.05$, Fig. 1A).

Notably, significant enhancement of contraction to PE (between 3×10^{-7} and 1×10^{-5} M) was also observed in endothelium-denuded aortic preparations pre-treated with 200 µM of methylglyoxal ($p<0.05$, Fig. 1B).

Qualitatively similar effects of methylglyoxal pre-treatment on contractions induced Ang II (Fig. 1C) and vasopressin (Fig. 1D) were also observed in endothelium-denuded rat aorta vessels. Although the responses in lower concentrations of methylglyoxal varied, pre-treatment with 200 µM caused significant potentiation of contraction to Ang II (at 1×10^{-6} M, $p<0.05$, Fig. 1C) and vasopressin (between 3×10^{-8} and 1×10^{-7} M, $p<0.05$, Fig. 1D) when compared to respective controls.

3.2 The effect of methylglyoxal on KCl-mediated responses in the rat aorta and in isolated perfused rat kidney:

To assess the role the voltage-activated calcium entry may play in methylglyoxal-mediated increases of agonist-induced contractions, cumulative CRCs to a depolarizing vasoconstrictor KCl were examined in endothelium-intact (Fig. 2A) and endothelium-denuded (Fig. 2B) aortic preparations, pre-treated with 50, 100 or 200 μM of methylglyoxal.

In endothelium-intact aortic rings, 50 μM methylglyoxal caused a marked increase in KCl-induced response that was significantly different at concentrations between 80 and 100 mM compared to the control ($p<0.05$). In higher concentrations of methylglyoxal, KCl-induced contraction tended to be reduced compared to that at 50 μM (Fig. 2A).

In endothelium-denuded vessels, contraction to KCl increased in all concentrations of methylglyoxal compared to the control with most significant differences observed between 30 and 100 mM KCl after pre-treatment with 100 μM methylglyoxal ($p<0.05$, Fig. 2B).

To assess if microvessels were affected by methylglyoxal in a similar fashion as conduit arteries, the isolated rat left kidney was perfused with 50 or 100 μM of methylglyoxal for 1 hour followed by perfusion with increasing

concentrations of KCl. Figure 2C shows that perfusion pressure was progressively increased after pre-treatment with methylglyoxal being significantly different at 80 and 100 mM KCl in 100 μM of methylglyoxal compared to the control kidney ($p<0.05$, Fig. 2C).

3.3 Role of extracellular calcium and voltage-activated calcium entry in methylglyoxal-mediated increases in KCl contraction:

To elucidate the role of extracellular calcium in methylglyoxal-mediated increase of KCl-induced contraction, CRCs to KCl were examined in endothelium-denuded aortic rings bathed in Ca^{2+}-free KHB. In this condition, KCl contractions were markedly reduced (Fig. 3A) compared to normal KHB (Fig. 2B) with no significant differences seen in any concentration of methylglyoxal when compared to the control group (Fig. 3A).

Similar effects on KCl-induced contraction were seen in normal KHB in the presence of the L-type Ca^{2+} channel blocker nifedipine (Fig. 3B). Nifedipine significantly attenuated KCl-induced contraction between 70 and 100 mM following pre-treatment with 100 μM methylglyoxal ($p<0.05$, Fig. 3B).

To determine the effect on free cytosolic calcium, endothelium denuded aortic rings loaded with Fluo-4 (see Methods for details) were pre-incubated with methylglyoxal or methylglyoxal and nifedipine (all at 100 μM) and compared to

the control. All preparations were stimulated with 80 mM KCl (Fig. 3C). Methylglyoxal caused a significant increase in Fluo-4 fluorescence 2 min after stimulation with KCl. This effect was abolished by nifedipine (Fig. 3C).

3.4 The effect of NOX and PKC inhibitors on methylglyoxal-mediated increases in KCl-induced contraction:

To elucidate intracellular pathways which contribute to methylglyoxal-induced effects on KCl contraction, endothelium-denuded rat aortic rings were pre-treated with apocynin (a broad-spectrum NOX inhibitor, 10 µM), chelerythrine (a selective PKC inhibitor, 10 µM) or N-acetyl-L-cysteine (NAC) (an antioxidant, 1 mM) for 30 min prior a one-hour pre-treatment with 100 µM methylglyoxal followed by CRC to KCl (Fig. 4).

Apocynin, while having no effect alone, abolished the methylglyoxal-mediated increase in KCl contraction (Fig. 4A). Although chelerythine alone significantly attenuated KCl-induced contraction, it also completely blocked the effect of methylglyoxal to a similar degree as the inhibitor alone (Fig. 4B).

Likewise, the antioxidant NAC completely blocked the methylglyoxal-mediated increase in KCl contraction, although, when added alone, the antioxidant tends to enhance KCl responses albeit non-significantly (Fig. 4C).

4. Discussion:

Our study aimed at determining the mechanism by which AGEs may cause an increase in vasoconstriction responses, which is a key factor in diabetic vascular complications. We used methylglyoxal, one of reactive intracellular dicarbonyls, produced as a consequence of intracellular hyperglycaemia, which are thought to be a major source for formation of AGEs [17]. The main findings of this study show that methylglyoxal: i) increased agonist- and depolarization-induced contractions of large conduit arteries (the aorta) and microvessels (perfused kidney); ii) acted mainly in endothelium-independent fashion; iii) its effects were markedly dependent on depolarization-mediated voltage-activated calcium influx, and iv) its effects required activation of NOX and PKC.

Only two focused reports by Mukohda and colleagues directly evaluated the effect of methylglyoxal on agonist-induced contractions in large arteries. One study showed that exposure of the rat carotid artery methylglyoxal (420 μM) for 30 min increased Ang II-mediated contraction in the endothelium-dependent manner [12]. In another report, a 30 min pre-treatment with methylglyoxal inhibited noradrenaline-induced contraction in the endothelium-denuded aorta and superior mesenteric artery of the rat. The effect of methylglyoxal in the aorta was concentration independent (42-420 μM) with only 420 μM used in the mesenteric artery [13]. Above mentioned reports are in contrast to our findings where a

significant methylglyoxal-mediated potentiation of contraction of the rat aorta to both the α_1-adrenoceptor agonist phenylephrine and Ang II which were endothelium-independent for both agonists (Fig. 1B and 1C, respectively), as well as to vasopressin, an agonist that was not previously tested (Fig. 1D). Furthermore, phenylephrine-induced contraction tends to progressively increase with exposure to increasing concentrations of methylglyoxal (50-200 μM) in both endothelium-intact (Fig. 1A) and endothelium-denuded (Fig. 1B) vessels. The apparent disconcert between our findings and previous studies is likely to be due to the difference mainly in the duration of pre-treatment to methylglyoxal (1 hour pre-treatment used in this study vs 30 min in previous reports) and perhaps exposure to lower concentrations of methylglyoxal in our experiments.

Although tissue specificity cannot be entirely excluded, the ability of methylglyoxal to enhance contraction of endothelium-denuded aortic rings to three different agonists, phenylephrine, Ang II and vasopressin, strongly supports the presence of a common mechanism activated after a one-hour pretreatment on smooth muscle cells. Two principle mechanisms could be considered. One is via direct activation of $G\alpha_{q/11}$ coupled of α_1-adrenoceptors, angiotensin AT1 receptors [18] and vasopressin V1 receptors [19] on smooth muscles by respective agonists triggering inositol-1,4,5-trisphosphate (IP_3)-mediated intracellular calcium release. Another possibility could be an increased voltage-dependent calcium influx

triggered by membrane depolarization. The ability of agonists to depolarize smooth muscle cells via activation of non-selective cation channels, calcium-activated chloride channels or by activating voltage-activated calcium channels directly is well-recognized [20].

The important role of the voltage-activated Ca^{2+} influx in methylglyoxal-mediated increases in vascular reactivity is supported by comparing CRCs to KCl in endothelium-intact and endothelium-denuded aortic rings before and after pre-treatment with methylglyoxal (Fig. 2A,B). The inhibitory effect of extracellular calcium removal and a complete block of the methylglyoxal-mediated increase in KCl-induced contraction and intracellular calcium concentration in rat aorta by the voltage-activated calcium channel blocker nifedipine (Fig. 3) are also consistent with this conclusion.

Notably, our findings differ from the observation made by Mukohda et al. (2009) who found no differences in KCl contraction in endothelium-denuded aortic preparations after a shorter 30 min pre-treatment with similar concentrations of methylglyoxal [13]. However, in cultured arteries exposed to low concentrations of methylglyoxal for 3 days KCl contraction was inhibited [21], supporting our conclusion that methylglyoxal effects are both time and concentration dependent.

Interestingly, KCl-induced contraction was reduced following pre-treatment with a higher 200 μM methylglyoxal in both endothelium-intact and endothelium-denuded preparations when compared with 50 and 100 μM (Fig 2, A and B). This reduction is unlikely due to activation of K^+ channels in smooth muscle cells which would be blocked by high KCl. Hence, the mechanism is different from activation of large-conductance Ca^{2+}-activated K^+ channels that attenuated noradrenaline-mediated contraction reported previously [15]. It is possible that high concentrations of methylglyoxal would inhibit nifedipine-sensitive voltage-activated calcium channels, as was demonstrated with phenylglyoxal in mouse cerebellum neurons [27], and, in this case, it may contribute to suppression of agonist-mediated contraction reported previously by Mukohda and colleagues.

The ability of methylglyoxal to potentiate KCl-induced vasoconstriction in isolated perfused kidney (Fig. 2C) in a similar manner as in large conduit vessels (Fig. 2A) could indicate the existence of common mechanisms in methylglyoxal-mediated effects that might be important in blood pressure control and renal complication in diabetes.

To elucidate downstream signaling pathways involved in methylglyoxal-mediated effects on KCl contraction the broad spectrum NOX inhibitor apocynin and selective inhibitor of PKC chelerythrine were used in endothelium-denuded aortic rings (Fig. 4,A and B). Both inhibitors completely blocked the

methylglyoxal-mediated increases in KCl contraction. Correspondingly, reduction of oxidative stress with the antioxidant NAC also inhibited the effect of methylglyoxal (Fig. 4C). The link between increased oxidative stress, hyperglycaemia and AGEs has been well recognized [22]. Previous studies showed that NOX inhibitors, including apocynin, prevented the endothelium-dependent increase in Ang II responses during short-term exposure to methylglyoxal [12], while NAC inhibited decreases in noradrenaline-induced contraction associated with increased NOX activity induced by a 3-day exposure to methylglyoxal in cultured endothelium-denuded mesenteric arteries [21]. Our data demonstrate that a one-hour exposure to methylglyoxal increases nifedipine-sensitive voltage-activated calcium entry in aortic smooth muscle cells via activation of NOX and PKC. Although detailed mechanisms including specific NOX and PKC isoforms involved will require a separate study, calcium-sensitive isoforms of PKC are likely to be involved in the absence of agonists. Indeed, NOX mediated activation of the Ca^{2+}-sensitive PKCα led to increased L-type calcium channel activity in vascular smooth muscle cells [23].

In conclusion, we have described a novel effect of methylglyoxal on vascular reactivity demonstrating that methylglyoxal enhances vascular contractility in both conduit and resistance vessels in response to a range of vasoconstricting agents. This effect resided within vascular smooth muscle cells and is likely to be

mediated by an increase in the nifedipine-sensitive voltage-activated Ca^{2+} influx mediated by increased NOX and PKC activity.

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References

[1] A. Chawla, R. Chawla, S. Jaggi, Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum?, Indian J Endocrinol Metab, 20 (2016) 546-551.

[2] M. Donald, J. Dower, R. Ware, B. Mukandi, S. Parekh, C. Bain, Living with diabetes: rationale, study design and baseline characteristics for an Australian prospective cohort study, BMC Public Health, 12 (2012) 8.

[3] L. Wu, Is methylglyoxal a causative factor for hypertension development?, Can J Physiol Pharmacol, 84 (2006) 129-139.

[4] A.A. Mostafa, E.W. Randell, S.C. Vasdev, V.D. Gill, Y. Han, V. Gadag, A.A. Raouf, H. El Said, Plasma protein advanced glycation end products, carboxymethyl cysteine, and carboxyethyl cysteine, are elevated and related to nephropathy in patients with diabetes, Mol Cell Biochem, 302 (2007) 35-42.

[5] D.S. Fosmark, P.A. Torjesen, B.K. Kilhovd, T.J. Berg, L. Sandvik, K.F. Hanssen, C.D. Agardh, E. Agardh, 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, 55 (2006) 232-236.

[6] N. Seidler, Basic Biology of GAPDH, GAPDH: Biological Properties and Diversity, Springer, 2013, pp. 1-36.

[7] A. Lapolla, F. Piarulli, G. Sartore, A. Ceriello, E. Ragazzi, R. Reitano, L. Baccarin, B. Laverda, D. Fedele, Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease, Diabetes Care, 30 (2007) 670-676.

[8] Q. Wei, X. Ren, Y. Jiang, H. Jin, N. Liu, J. Li, Advanced glycation end products accelerate rat vascular calcification through RAGE/oxidative stress, BMC Cardiovasc Disord, 13 (2013) 13.

[9] Q. Guo, T. Mori, Y. Jiang, C. Hu, Y. Osaki, Y. Yoneki, Y. Sun, T. Hosoya, A. Kawamata, S. Ogawa, M. Nakayama, T. Miyata, S. Ito, Methylglyoxal contributes to the development of insulin resistance and salt sensitivity in Sprague-Dawley rats, J Hypertens, 27 (2009) 1664-1671.

[10] Y. Liu, M. Yu, L. Zhang, Q. Cao, Y. Song, Y. Liu, J. Gong, Soluble receptor for advanced glycation end products mitigates vascular dysfunction in spontaneously hypertensive rats, Mol Cell Biochem, 419 (2016) 165-176.

[11] S. Turkseven, E. Ertuna, G. Yetik-Anacak, M. Yasa, Methylglyoxal causes endothelial dysfunction: the role of endothelial nitric oxide synthase and AMP-activated protein kinase α, J Basic Clin Physiol Pharmacol, 25 (2014) 109-115.

[12] M. Mukohda, H. Yamawaki, M. Okada, Y. Hara, Methylglyoxal augments angiotensin II-induced contraction in rat isolated carotid artery, J Pharmacol Sci, 114 (2010) 390-398.

[13] M. Mukohda, H. Yamawaki, H. Nomura, M. Okada, Y. Hara, Methylglyoxal inhibits smooth muscle contraction in isolated blood vessels, J Pharmacol Sci, 109 (2009) 305-310.

[14] H.M. El-Bassossy, A.A. Elberry, S.A. Ghareib, Geraniol improves the impaired vascular reactivity in diabetes and metabolic syndrome through calcium channel blocking effect, J Diabetes Complications, 30 (2016) 1008-1016.

[15] H.M. El-Bassossy, R. El-Fawal, A. Fahmy, M.L. Watson, Arginase inhibition alleviates hypertension in the metabolic syndrome, Br J Pharmacol, 169 (2013) 693-703.

[16] H.M. El-Bassossy, A. Fahmy, D. Badawy, Cinnamaldehyde protects from the hypertension associated with diabetes, Food Chem Toxicol, 49 (2011) 3007-3012.

[17] S. Marques, T. Trevisan, C. Maia, A. Breuer, R.W. Owen, Improved Methods for the Rapid Formation and Prevention of Advanced Glycation End Products (AGEs) In Vitro by Coupling to the Hypoxanthine/Xanthine Oxidase Assay System, Biomedicines, 6 (2018).

[18] L.G. Navar, E.W. Inscho, S.A. Majid, J.D. Imig, L.M. Harrison-Bernard, K.D. Mitchell, Paracrine regulation of the renal microcirculation, Physiol Rev, 76 (1996) 425-536.

[19] K.K. Henderson, K.L. Byron, Vasopressin-induced vasoconstriction: two concentration-dependent signaling pathways, J Appl Physiol (1985), 102 (2007) 1402-1409.

[20] J. Mironneau, N. Macrez-Leprêtre, Modulation of Ca2+ channels by alpha 1A- and alpha 2A-adrenoceptors in vascular myocytes: involvement of different transduction pathways, Cell Signal, 7 (1995) 471-479.

[21] M. Mukohda, T. Morita, M. Okada, Y. Hara, H. Yamawaki, Long-term methylglyoxal treatment impairs smooth muscle contractility in organ-cultured rat mesenteric artery, Pharmacol Res, 65 (2012) 91-99.

[22] H. Vlassara, J. Uribarri, Advanced glycation end products (AGE) and diabetes: cause, effect, or both?, Curr Diab Rep, 14 (2014) 453.

[23] G.C. Amberg, S. Earley, S.A. Glapa, Local regulation of arterial L-type calcium channels by reactive oxygen species, Circ Res, 107 (2010) 1002-1010.

Figure Legends:

Figure 1: Effect of methylglyoxal (MG) on agonist-induced contraction of the rat aorta in the presence and absence of endothelium. (A) and (B) show the effect of phenylephrine (PE) in endothelium-intact (A) and endothelium-denuded vessels (B), (C) and (D) show the effect of angiotensin II (Ang II) and vasopressin (VP), respectively, in endothelium-denuded preparations following one-hour pre-incubation with different concentrations of methylglyoxal as indicated. Values are shown as mean ± SEM, $^*p < 0.05$, compared to control, n= 7.

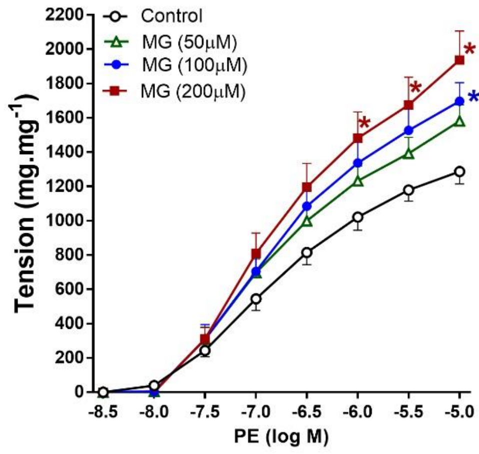
Figure 2: Effect of methylglyoxal on KCl-mediated responses in endothelium-intact and endothelium-denuded rat aortic rings (A, B) and in the isolated perfused rat kidney (C). (A) and (B) compares KCl responses in endothelium-intact and endothelium-denuded aortic preparations, respectively, after one-hour exposure to different concentrations of methylglyoxal (MG) as indicated by the symbols. Note difference in scale in (A) and (B). (C) shows the changes in perfusion pressure to KCl following one-hour pre-treatment of the isolated rat kidney with 50 and 100 μM of methylglyoxal. Values are expressed as mean ± SEM, $^*p < 0.05$, compared to control, n = 6-8.

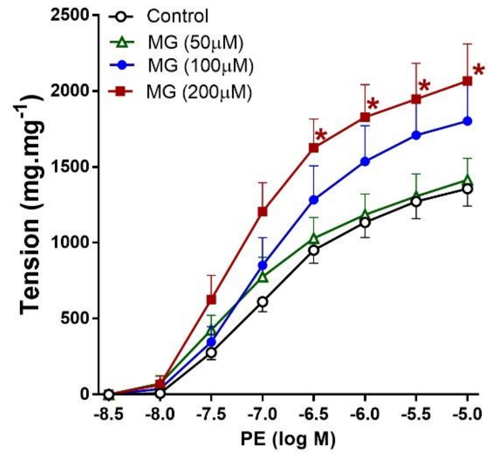
Figure 3: The role of extracellular calcium entry in methylglyoxal-mediated enhancement of KCl-induced contraction in the endothelium denuded rat aorta. Concentration-response curves to KCl recorded in the presence of different concentrations of methylglyoxal (MG) (50,100 or 200 μM) in Ca^{2+}-free KHB (+1 mM EGTA) (A) and in the presence of nifedipine (100 μM) (B). Time-dependent changes in Fluo-4 fluorescence intensity (expressed in arbitrary units (a.u.)) in rat aortic smooth muscles stimulated with 80 mM KCl (added at 0 min) under control condition and after pre-treatment with 100 μM MG or MG + nifedipine (both at 100 μM) (C). Values are expressed as mean ± SEM, *$p < 0.05$, compared to control, n = 7.

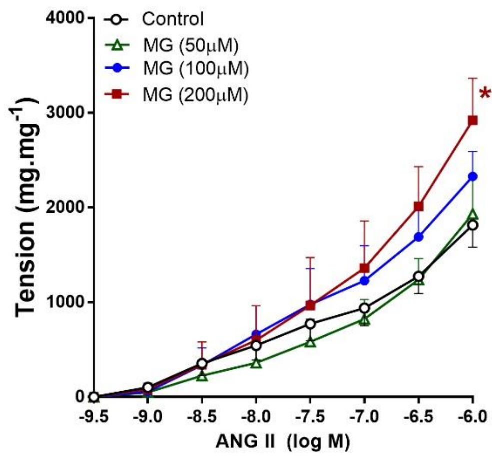
Figure 4: Effects of apocynin, chelerythrine and N-acetyl-L-cysteine (NAC) on methylglyoxal-induced increases in KCl contraction in the endothelium-denuded rat aorta. Comparison of concentration-response curves to KCl recorded in the presence of 10 μM apocynin (Apo) (A), 10 μM chelerythrine (Chel) (B) and 1 mM NAC (C) in the absence and following the pre-treatment with 100 μM methylglyoxal (MG) (blue tringles). Control (in the absence of the inhibitors or MG) and the effect of MG alone (black circles) are shown for comparison. Values

are expressed as mean ± SEM, n=8. * and # indicate $p < 0.05$ compared to the control (*) and the MG-treated (#) preparations.

FIGURE 1







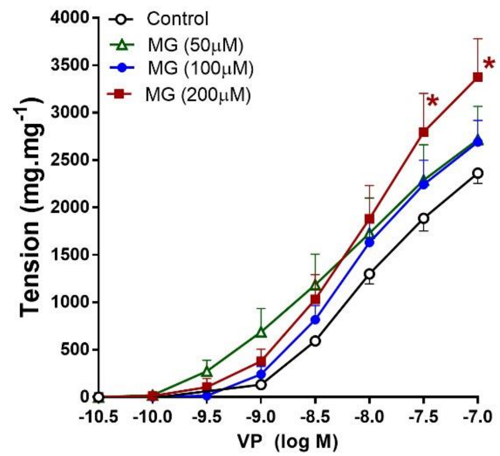


FIGURE 2

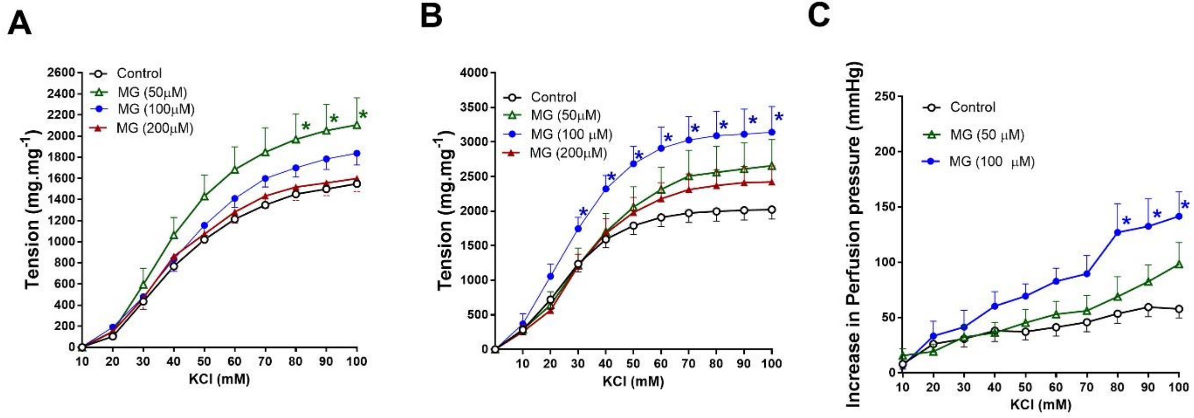
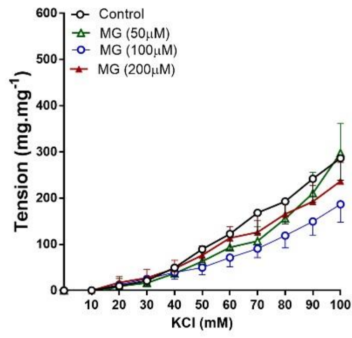
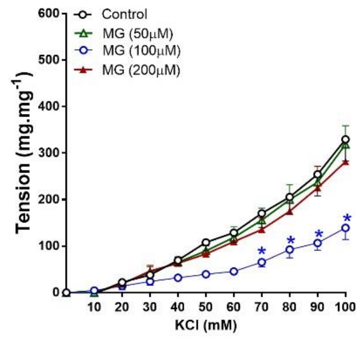


FIGURE 3





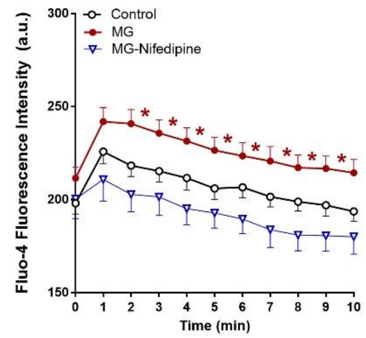


FIGURE 4