Electronic supplementary material

Methods

Isometric tension measurements Healthy adult male Wistar rats or adult Wistar rats with human *GLO-I* overexpression (GLO-I TG) (300–450 g) were killed by CO_2 inhalation. The viscera were exposed in order to remove the superior mesenteric vascular arcade, which was then pinned on to a dissecting dish containing cold KRB: 118 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 25 mmol/l $NaHCO_3$, 1.1 mmol/l $MgSO_4$, 2.5 mmol/l $CaCl_2$ and 5.0 mmol/l glucose. A second-order branch of the superior mesenteric artery was cleaned of fat and connective tissue, and a segment of approximately 8.0 mm in length removed and divided into four equal segments. These segments were then mounted in a myograph organ bath (610M; Danish Myotechnology by J. P. Trading, Aarhus, Denmark) with two steel 40 μm wires inserted through the lumen of the segments. After mounting the tissues, the organ bath solution was changed to fresh Krebs maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2 (pH 7.4). Prior to the normalisation procedure, the arteries were rested for 15 min without tension. The internal diameter of each vessel was normalised as described by Halpern and Mulvany [1]. This was performed by stretching the vessel to a diameter that yielded a wall tension equivalent to 90% of that given by a transmural pressure of 100 mmHg (this diameter was 326±37 μm for wild-type rats and 336±25 μm for *GLO-I* transgenic rats). The isometric tension generated by the vessels was recorded using Powerlab 4/25 (ADInstruments, London, UK) connected to the Myo-Interface. Endothelial viability was assessed by application of 10 μmol/l acetylcholine during contraction with 10 μmol/l phenylephrine. Viable endothelium resulted in 94±6% relaxation. NO-mediated effects were investigated using acetylcholine (0.01–10.00 μmol/l) and SNP (0.01–10.00 μmol/l) during contraction with 65 mmol/l K^+, which blocks endothelium-derived hyperpolarising factors. NO-mediated vasorelaxation was measured after incubation with or without methylglyoxal after pre-treatment with or without the antioxidant NAC (Sigma Aldrich, St Louis, MO, USA) or the superoxide dismutase mimetics EUK-134 and MnIIITMP (Invitrogen, San Diego, CA, USA). During some experiments arteries were exposed to additional high glucose (25 and 35 mmol/l; Sigma Aldrich) or mannitol (35 mmol/l; Sigma Aldrich). During the experiments, 10 μmol/l indometacin was used to block synthesis of prostaglandins. Iso-osmotic buffer containing 65 mmol/l K^+ was prepared by mixing appropriate volumes of KRB with a KRB in which all NaCl was replaced by KCl.

NO measurement Deoxygenated 50 mmol/l phosphate buffer (pH 7.4) was prepared under a N_2 atmosphere. Deoxygenated water was spiked with •NO gas for about 1 min and 2 µl of the •NO spiked water was added to 20 ml 50 mmol/l phosphate buffer (pH 7.4) in a thermostat-controlled test tube (37°C). During measurements the test tube was kept under a N_2 atmosphere. The •NO concentration was monitored with an Iso-NO meter (World Precision Instruments, Sarasota, FL, USA), which was coupled to a MacLab interface (ML020 MacLab/8; ADInstruments) and recorded with 'Chart' software (Kipp, Delft, the Netherlands). The decrease in •NO concentration was followed in time in the presence or absence of the test compound in solution. The stock solution of the test compound was incubated at 37°C for 10 min before use. During this procedure, the solution in the test vessel was mixed using a magnetic stirrer. The natural logarithm of the •NO concentration was plotted against time. To determine the rate constant, the •NO concentration was expressed in arbitrary units, since the reaction showed (pseudo) first-order reaction kinetics.

Reference

[1] Halpern W, Mulvany MJ (1977) Tension responses to small length changes of vascular smooth muscle cells [proceedings]. J Physiol 265:21P–23P