ONLINE DATA SUPPLEMENT

SUPEROXIDE MODULATES MYOGENIC CONTRACTIONS OF MOUSE AFFERENT ARTERIOLES

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Methods

Animal preparation, dissection and mounting of afferent arterioles

The kidneys were sliced along the corticomedullary axis immediately after sacrifice, placed in 4ºC dissection solution and an afferent arteriole with glomerulus attached was microdissected using sharpened forceps under a stereomicroscope (model SZ40; Olympus Corp., Melville, NY) as described [1]. The afferent arterioles were identified in the cortex from the interlobular arterial tree. The arteriole with its glomerulus was transferred to a thermoregulated chamber on the stage of an inverted microscope (Olympus IX70, Olympus America, Inc., NY). Arterioles were perfused using a micromanipulator system (Vestavia Scientific, Vestavia Hills, AL) with concentric holding and perfusion pipettes made of custom glass tubes (Drummond Scientific Company, PA). The holding pipette had a tip aperture of approximately 24 μm. The arteriole was aspirated into this pipette. The inner perfusion pipette had a tip diameter of 6 μm. It was advanced into the lumen of the arteriole. The pressure at its tip was calibrated using a closed chamber connected to a DPM-1B pneumatic transducer calibrator (Bio-Tek Instruments, INC., Winooski, VT). Microdissection and cannulation were completed within 120 min, after which the bath was gradually warmed to 37°C and the arteriole stabilized for 20 minutes. The cannulated afferent arteriole was perfused with Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM, Sigma, St. Louis, MO) at 60 mmHg. The bath was perfused at 1 ml $\cdot$ min^{-1}. DMEM bubbled with 95% O_2 and 5% CO_2 and pH adjusted to 7.4 was used for dissection, bath and perfusion. The microperfused arteriole was displayed at ×400 magnification (Nomarski optics; Olympus Corp., Melville, NY) on a video monitor via a black and white camera (model NC 70; Dage-MTI, Inc., Michigan City, IN, USA) on an inverted microscope and recorded on VHS tape. Arterioles were selected according to the criteria of basal tone and a rapid constriction with KCl (100 mmol $\cdot$ l^{-1}) as described previously [2, 3].

Measurement of myogenic tone in afferent arterioles

The experiments were recorded by a video system, digitized, and monitored in real time. A full set of 20 mmHg pressure steps from zero to 135mmHg were undertaken in each arteriole in physiologic solution and in a perfusate without Ca^{++} and containing 5 x 10^{-3}M ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA, Sigma, St. Louis, MO) to abolish active tone. The active wall tension (AWT) was calculated as the difference between the tension measured during perfusion with these two solutions, as described (ref 1 of this section).

ROS Determination

ROS generation was assessed by fluorescence microscopy of perfused afferent arterioles with dihydroethidium (DHE) (Invitrogen, Carlsbad, CA). DHE is a cell-permeable ROS-sensitive fluorophore that is oxidized by $O_2^{\cdot -}$ to the highly fluorescent compound ethidium, which is trapped intracellularly and intercalated into DNA. This

method has been shown to also detect an oxidation product that differs from ethidium . Therefore additional studies were undertaken using another $O_2^{\cdot -}$ sensitive fluorophore, tempo-9-AC (Invitrogen, Carlsbad, CA) . [4]

Single-agent signal capture was achieved by cycling at 3 sec intervals between a 460- and 605-nm filter. Changes in $O_2^{\cdot -}$ were expressed as the ratio of ethidium:DHE fluorescence. The system used an Olympus IX70 fluorescence microscope equipped with dual photomultipliers (PMT, Photon Technology Int., Lawrenceville, NJ). Excitation was provided by a 75-W xenon arc lamp using a 380/460 nm wavelength combination isolated with a computer-controlled monochromator. Ethidium and DHE emit blue and red light, respectively, that were directed to a dual PMT assembly by a beam splitter that directed light to the two separate PMT using a 400-nm dichroic mirror and barrier filters centred at 460 and 605 nm, respectively. The ratio of ethidium:DHE was monitored in real time and recorded by software (Felix32; Photon Technology Int.).

Results

As in a prior study [1], graded increases in perfusion pressure above 40 mmHg reduced luminal diameter progressively (Supplement Figure S1A) with a maximum response of 18.4 ± 5.3% (Figure S1B). There were linear increases in wall tension with pressure of vessels in a physiologic solution and in passive wall tension of vessels in a calcium-free solution containing EGTA (Figure S1C) and in active wall tension which was the difference between these two (Figure S1D).

Reference List

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(2) Lai EY, Martinka P, Fahling M, Mrowka R, Steege A, Gericke A, Sendeski M, Persson PB, Persson AE, Patzak A. Adenosine restores angiotensin II-induced contractions by receptor-independent enhancement of calcium sensitivity in renal arterioles. *Circ Res.* 2006;99:1117-1124.

(3) Patzak A, Lai EY, Mrowka R, Steege A, Persson PB, Persson AEG. AT1 receptors mediate angiotensin II-induced release of nitric oxide in afferent arterioles. *Kidney Int.* 2004;66:1949-1958.

(4) Fellner SK, Arendshorst WJ. Angiotensin II, reactive oxygen species, and Ca^{2+} signaling in afferent arterioles. *Am J Physiol Renal Physiol.* 2005;289:F1012-F1019.

Figures
Figure S1

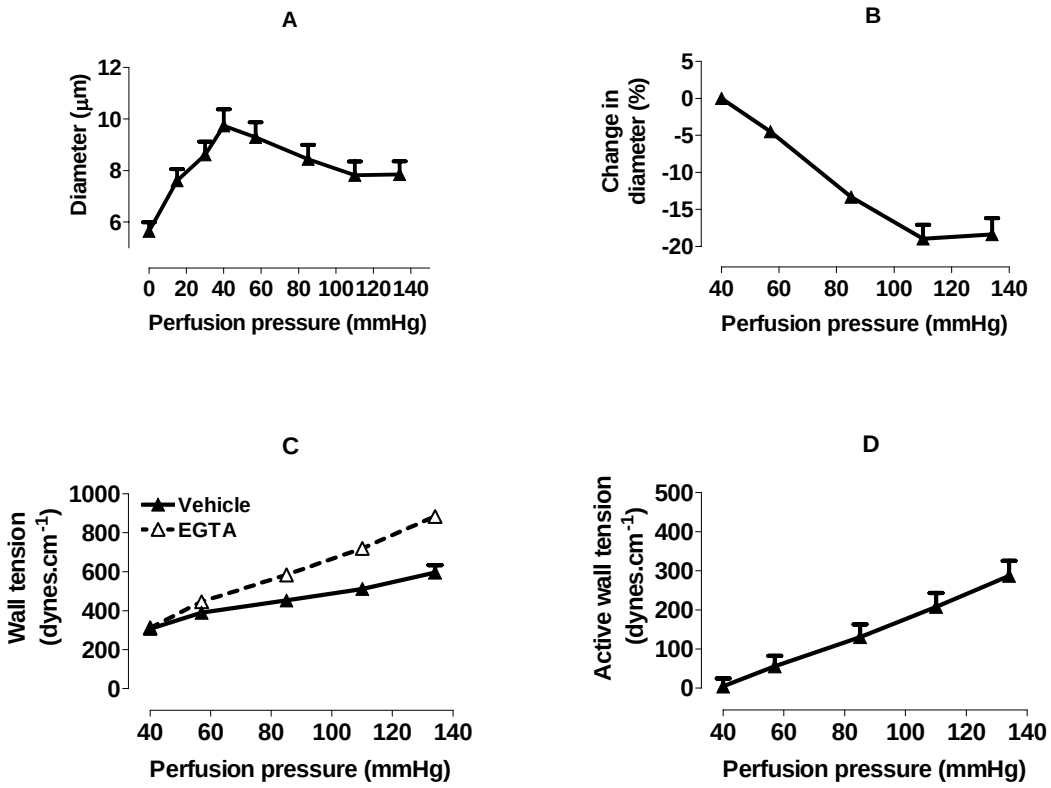

Figure Legends

Figure S1: Mean ± SEM values (n = 6) for responses of afferent arterioles to graded increases in perfusion pressure for diameter (A), change in diameter (B), wall tension with vehicle in physiology solution or with EGTA in calcium free solution (C) and active wall tension (D).