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Constriction velocities of renal afferent and efferent arterioles of mice are not related to SMB expression

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Background. Constriction of renal arterioles contributes significantly to the control of perfusion and glomerular filtration. Afferent but not efferent arterioles express smooth muscle myosin heavy chain B (SMB) (with a 5′-insert of seven amino acids). The aim of the present study was to investigate (*1*) the constriction characteristics of afferent and efferent arterioles under physiologic load and (*2*) whether expression of SMB may causally contribute to these constriction characteristics.

Methods. We compared constriction parameters [constriction amplitude, maximal rate of constriction velocity ("dc/dt_{max}"), and time to half-maximal constriction ($t_{1/2}$) of in vitro perfused renal afferent and efferent arterioles of wild-type (smb(+/+)] and homozygous SMB knockout [smb(−/−)] mice upon stimulation with angiotensin II (Ang II) (10^{-8} mol/L) and potassium chloride (KCl) (100 mmol/L). SMB expression was investigated by double-labeling immunofluorescence.

Results. Contraction amplitude and dc/dt_{max} of mouse afferent arterioles upon Ang II stimulation were significantly greater compared to efferent arterioles. However, constriction amplitudes, dc/dt_{max}, and $t_{1/2}$ of afferent as well as efferent arterioles upon Ang II stimulation were similar in smb(+/+) and smb(−/−) mice. Constriction amplitudes upon KCl stimulation of afferent arterioles were similar in both smb(+/+) and smb(−/−) mice. Furthermore, KCl-induced dc/dt_{max} and $t_{1/2}$ of afferent arterioles were similar in both smb(+/+) and smb(−/−) mice. SMB expression could be detected in afferent but not efferent arterioles in smb(+/+) mice. No SMB expression in either arteriole could be observed in smb(−/−) mice.

Conclusion. Our results suggest that the presence of different alternatively 5′-spliced smooth muscle-myosin heavy chain (SM-MHC) isoforms does not dominate the different contractile features of physiologically loaded renal afferent or efferent arterioles.

Key words: afferent arteriole, efferent arteriole, smooth muscle, myosin heavy chains, juxtaglomerular apparatus.

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Type II myosin is the molecular motor which causes smooth muscle cell (SMC) contraction by cyclic interaction with actin and hydrolysis of adenosine triphosphate (ATP). The native myosin II consists of two heavy chains (myosin heavy chain, MHC) with around 200 kD each, which revealed a 140 nm alpha-helical "rod" domain and a pear-shaped N-terminal "head" domain with around 20 nm length. Two pairs light chains (myosin light chain, MLC) with 17 kD (essential MLC) and 20 kD (regulatory MLC) are noncovalently associated with each MHC close to the head domain [1, 2].

Three different MHC genes are expressed in SMCs, namely, SM-MHC as well as MHC which are also expressed in nonmuscle (NM) cells (NM-MHCA and NM-MHCB [3–5]). SM-MHC is exclusively expressed in SMCs and located on the human chromosome 16p13.13 [6]. Various MHC isoenzymes have distinct functions in SMCs. They form different contractile systems which are recruited during phasic contraction (the SM-MHC) or during tonic contraction upon sustained activation (the NM-MHC isoenzymes) [7, 8].

The SM-MHC gene transcript is alternatively spliced at both the 3′-(carboxy-terminal myosin tail) and at the 5′-end (amino-terminal head domain). Inclusion of a 39 nucleotide exon which encodes nine amino acids at the most 3′-terminus generates the translation of a SM-MHC isoenzyme with 1443 amino acids and around 200 kD (SM2) with a shortened tail region. Exclusion of this exon causes the translation of an elongated tail with 1477 amino acids and around 204 kD (SM1), containing a nonhelical tailpiece domain with a phosphorylatable serine residue [9–11].

5′-splicing of SM-MHC is accomplished by excision of a highly conserved exon with 21 nucleotide (seven amino acids) causing a shortened flexible surface loop (loop 1) close to the ATP-binding 25K/50K junction [12–14]. SM-MHC without the 5′-insert were designated as "SMA" isoforms (SM1A and SM2A), those with 5′-insert as "SMB" forms (SM1B and SM2B).

Alternative 3′-splicing in the carboxy terminus of SM-MHC isoforms seems not to be associated with motor properties of myosin [15] or shortening velocity of smooth muscle preparations [16]. However, alternatively 5′-spliced myosin isoenzymes revealed different kinetic and conferred distinct contractile features to muscle preparations. Due to the proximity to the ATP binding pocket, the length of loop 1 may regulate the rate of adenosine diphosphate (ADP) release from the catalytic domain and, therefore cross-bridge detachment [17], thus determining V_{max} [18] and the duration of the duty cycle [19]. In fact, SMB (with 5′-insertion and elongated loop 1) revealed a reduced duration of the duty cycle, increased shortening velocity as well as increased actin-activated ATPase activity and velocity of actin filament sliding in the in vitro motility assay [13, 20, 21]. In a recently generated knockout mouse model with targeted ablation of exon 5b [22], the SMA isoenzymes were the sole expressed SM-MHC isoenzymes which conferred decreased contraction kinetics to smooth muscle preparations. In the same animal model, addition of magnesium ADP (MgADP) revealed a stronger effect on the force-generating states of SMA [15], supporting the hypothesis that a shorter loop 1 releases ADP slower and increases the duration of the duty cycle. Please note, however, that results of these studies reflect the kinetic features of myosin in vitro or under unloaded rather than under physiologically loaded conditions.

Renal afferent and efferent arterioles express different alternatively 5′- and 3′-spliced MHC isoforms. Afferent arterioles expressed SM1, SM2, SMA, and SMB, while efferent arterioles expressed mainly SM1 and SMA [23, 24]. In a recent study [24], SMB expression in afferent arterioles was associated with enhanced constriction velocity of afferent arterioles upon angiotensin II (Ang II) or norepinephrine stimulation. However, it is not clear, whether the presence of SMB or the different microstructures and responsiveness to Ang II and norepinephrine [25, 26] of renal afferent and efferent arterioles are mainly responsible for the different constriction velocities. To answer this question, we examined Ang II responsiveness of efferent arterioles and the Ang II and KCl responsiveness of afferent arterioles of wild-type [smb(+/+)] and homozygous SMB knockout [smb(−/−)] mice. While there was a significant difference of Ang II–induced constriction amplitude and velocity between renal afferent and efferent arterioles, we did not observe any significant difference of constriction parameters of afferent and efferent arterioles between smb(+/+) and smb(−/−) mice.

METHODS

Animal model

We used a homozygous SMB knockout mouse model (smb−/−) which has been recently introduced [22]. The homozygous SMB knockout and the corresponding wild-type mice (smb+/+) were generated by breeding heterozygous SMB knockout mice. Animals were fed with standard mouse chow and allowed free access to tap water. All animal procedures adhered to the guidelines for care and handling of animals established by the United States Department of Health and Public Services and published by the National Institutes of Health.

Constriction studies of renal afferent and efferent arterioles

Physiologic salt solution (PSS) was used with the following composition: NaCl 115, $NaHCO_3$ 25, K_2HPO_4 2.5, $CaCl_2$ 1.3, $MgSO_4$ 1.2, and glucose 5.5 (mmol/L). The KCl solution consisted of 100 mmol/L KCl, whereby 95 mmol/L NaCl were substituted by KCl. Dulbecco's modified Eagle's medium (DMEM) (1000 mL) was supplied with 100 mg streptomycin, and 100000 U penicillin. The bicarbonate buffered solution was equilibrated with 5% CO_2 and 95% O_2. The pH was adjusted to 7.4 after addition of bovine serum albumin (BSA). The oxygen partial pressure was below 200 mm Hg in the perfusion and bath solution during the experiment. The pH was stable. The concentration of BSA in DMEM, which was used for dissection, and in PSS (bath solution) was 0.1%. The perfusate of the arterioles consisted of PSS with a BSA concentration of 1%. BSA was obtained from SERVA Electrophoresis (Heidelberg Germany). Ang II, DMEM, streptomycin, and penicillin were from Sigma-Aldrich (München, Germany).

Male mice (body mass was 21 to 33 g and age was between 12 and 15 weeks) were used in this study. After killing the animal, the kidneys were immediately removed and sliced along the corticomedullary axis. The arterioles were prepared at 4°C in DMEM, which was enriched with 0.1% albumin. The dissection procedure of the afferent and efferent arterioles was the same as described before [27]. The macula densa, the adjacent parts of the tubule, and the glomerulus were preserved. In case of the dissection of an afferent arteriole the efferent arteriole was removed by cutting close to the glomerulus. The same was done with the afferent arteriole when using the efferent arteriole for the experiment. This assured standard conditions in regard to the total vascular resistance of preparation, and consequently the flow. The preparation was transferred into a thermoregulated chamber (volume 1.5 mL) (VETEC, Rostock, Germany) on a stage of an inverted microscope (Axiovert 100) (Carl Zeiss, Oberkochen, Germany). The perfusion system allowed movement and adjustment of concentric, holding and perfusion pipettes (Luigs & Neumann, Ratingen, Germany). The pipettes were made by customizing glass tubes (Drummond Scientific Company, Broomall, PA, USA). The holding pipette into which the free end of the arteriole was aspirated had an aperture of roughly 26 μm at the tip and a constriction of about 20 μm. The inner