Nitric Oxide xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Agmatine induced NO dependent rat mesenteric artery relaxation and its impairment in salt-sensitive hypertension ☆

Tushar V. Gadkari, Natalie Cortes, Kumpal Madrasi, Nikolaos M. Tsoukias, Mahesh S. Joshi *

Department of Biomedical Engineering, Florida International University, Miami, FL 33174, United States

ARTICLE INFO

Article history:
Received 17 June 2013
Revised 15 July 2013
Available online xxxx

Keywords:
Agmatine
Arginine decarboxylase
L-Arginine
Nitric oxide
α-2 Adreno receptor
Hypertension

ABSTRACT

L-Arginine and its decarboxylated product, agmatine are important mediators of NO production and vascular relaxation. However, the underlying mechanisms of their action are not understood. We have investigated the role of arginine and agmatine in resistance vessel relaxation of Sprague-Dawley (SD) and Dahl salt-sensitive hypertensive rats. Second or 3rd-order mesenteric arterioles were cannulated in an organ chamber, pressurized and equilibrated before perfusing intraluminally with agonists. The vessel diameters were measured after mounting on the stage of a microscope fitted with a video camera. The gene expression in Dahl rat vessel homogenates was ascertained by real-time PCR. L-Arginine initiated relaxations (EC_{50}, 5.8 ± 0.7 mM; $n = 9$) were inhibited by arginine decarboxylase (ADC) inhibitor, difluoromethylarginine (DFMA) (EC_{50}, 18.3 ± 1.3 mM; $n = 5$) suggesting that arginine-induced vessel relaxation was mediated by agmatine formation. Agmatine relaxed the SD rat vessels at significantly lower concentrations (EC_{50}, 138.7 ± 12.1 μM; $n = 22$), which was compromised by L-NAME (L-N^G-nitroarginine methyl ester, an eNOS inhibitor), RX821002 (α-2 AR antagonist) and pertussis toxin (G-protein inhibitor). The agmatine-mediated vessel relaxation from high salt Dahl rats was abolished as compared to that from normal salt rats (EC_{50}, 143.9 ± 23.4 μM; $n = 5$). The $\alpha\text{-}2_A$ AR, $\alpha\text{-}2_B$ AR and eNOS mRNA expression was downregulated in mesenteric arterioles of high-salt treated Dahl hypertensive rats. These findings demonstrate that agmatine facilitated the relaxation via activation of α-2 adrenergic G-protein coupled receptor and NO synthesis, and this pathway is compromised in salt-sensitive hypertension.

Introduction

Over the past 2 decades there has been a significant interest in understanding the role of NO in vascular relaxation. L-Arginine serves as a substrate for different nitric oxide synthases (NOS). The potential use of exogenous L-arginine as a therapeutic agent has been widely suggested in various physiological and pathophysiological processes such as wound healing [1–3], protein synthesis and muscle building [4], endocrine metabolism [5], erectile dysfunction [6], and a variety of cardiovascular functions [7–9]. Supplying arginine to the endothelial cells and vessels contributes to the enhanced NO synthesis and vessel relaxation despite its saturating cellular levels. Different mechanisms are hypothesized to explain this arginine paradox including endogenous NOS inhibitors and compartmentalization of intracellular arginine [10–13]. However, there still exists an ambiguity in understanding how exogenous L-arginine mediates NO-dependent relaxation [14]. The apparent benefits of L-arginine supplementation are difficult to reconcile with a purely substrate based mechanism for NO synthesis. An alternative proposed by us explains arginine's NOS substrate independent actions via the activation of α-2 adrenergic receptor (α-2 AR) as demonstrated in cultured endothelial cells [15].

Agmatine [4-(aminobutyl)guanidine] is produced endogenously via decarboxylation of L-arginine by the endothelial arginine decarboxylase (ADC) [16,17] and it does not act as a substrate for NOS. A biological function for agmatine was suggested based on the observation that ADC activity transiently increased sevenfold during cerebral ischemia [18]. In addition, the importance of agmatine has been highlighted by its discovery as a novel neurotransmitter [19–21] demonstrating its potential to affect multiple biological targets. The presence of agmatine in serum [22] suggests a physiological role in the vasculature. Agmatine was shown to serve as a ligand for imidazoline and/or α-2 AR [23] and α-2 AR agonists mediate endothelium-dependent relaxation in mouse and rat aorta [24]. α-2 ARs (G-protein coupled receptors) play a pivotal role in the cardiovascular system and influence vascular tone at multiple

Abbreviations: ADC, arginine decarboxylase; DFMA, difluoromethylarginine; eNOS, endothelial nitric oxide synthases; L-NAME, L-NG-nitroarginine methyl ester; AR, adrenergic receptor; PTx, pertussis toxin.

* Corresponding author. Fax: +1 305 348 6954.
E-mail address: joshimahesh365@gmail.com (M.S. Joshi).

1089-8603/$ - see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.niox.2013.08.005

points. These receptors are targets for antihypertensive therapy and their stimulation produces long lasting drop in systemic blood pressure [25]. However, the signaling mechanisms participating in agmatine-initiated NO synthesis [15,26] and regulation of vascular tone is little understood, and the contribution of α-2 ARs is implicated in this process [15]. Compromised NO synthesis and endothelial dysfunction has been reported in the hypertensive vasculature including salt-sensitive hypertension [27,28]. However, the factors that are responsible for its impaired synthesis are varied and not clearly understood. Impaired α-2 AR function has been documented in several models of hypertension [29–31]. However, whether this impairment is a cause or an effect of hypertension remains to be elucidated.

Here we show that arginine-mediated arteriolar relaxations are due to agmatine produced by the actions of ADC and signaling via GPCR in rat microcirculation. Evidence is also presented documenting attenuated agmatine-mediated relaxation in Dahl salt-sensitive hypertensive rat mesenteric resistant arterioles, which correlates with reduced α-2 AR gene expression.

Materials and methods

Isolated mesenteric arteriole preparation

Resistance mesenteric arterioles of the 2nd or 3rd order (resting diameter ⩽150 μm) were isolated from male Sprague-Dawley (SD) and Dahl rats (250–300 g), cleaned of the surrounding tissue and cannulated at both ends on glass cannula. The organ chamber was maintained constant at 37 °C by superfusion with a modified Krebs–Ringer solution containing (mM): NaCl 145, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, HEPES 20, and Glucose 10.1, pH 7.4 [32,33]. Cannulated vessels were axially pre-stretched to remove any bents and to simulate physiological stretch conditions and were pressurized at 50 mmHg and allowed to equilibrate for 60 min before initiating the experiment. To establish a concentration that gave submaximal constriction, we constructed a dose–response curve to norepinephrine (NE). The vessels were preconstricted with continuous superfusion of NE and those that retained a constant pressure and a consistent constriction to NE (Fig. S1a and b), and fully responded to the acetylcholine (Fig. S3a) were included in the study. The presence of functional endothelium was assessed by the ability of acetylcholine (10 μM) to induce more than 90% relaxation. The vessel reactivity study was carried out by intraluminal perfusion with various agonist concentrations. This was achieved by an automated solenoid valve controlled pressure driven perfusion system. Diameter measurements were tracked in real time by mounting the perfusion chamber on the stage of an inverted microscope (Olympus, Center Valley, PA) fitted with a CCD camera (QImaging, Surrey, Canada). Post analysis was performed with IPLAB (BioVision Technologies, Exton, PA) and MATLAB (MathWorks, Natick, MA) software. Chemicals NE, L-NAME (L-N^G-nitroarginine methyl ester), RX821002, agmatine, L-arginine were obtained from Sigma–Aldrich Co. (St. Louis, MO) and pertussis toxin (PTx) were obtained from Tocris Bioscience (Ellisville, MO) and SPER-NO from Cayman Chemicals (Ann Arbor, MI).

Animal model

Male SD and Dahl salt-sensitive (SS/JrHsd) rats and their diet were purchased from Harlan Laboratories (Madison, WI). Sprague Dawley rats were maintained on standard pellet chow (2018 Teklad Global) rodent diet whereas the Dahl salt-sensitive rats were fed 0.49% NaCl diet (Harlan Cat. #TD 96208) or 4% NaCl diet (Harlan Cat. #TD 92034). The animals were housed in temperature and humidity controlled rooms with 12 h on/off light cycle at the animal care facility. All animal studies were performed following Institutional Animal Care and Use approved procedures.

After acclimatization for 1 week, 6-weeks old Dahl salt-sensitive rats were separated into 2 diet groups; normal salt (NS), fed 0.49% NaCl diet and high salt (HS), fed 4% NaCl diet for 5 weeks. Systolic blood pressure was measured weekly by the tail-cuff method [34] and HS rats consistently demonstrated sustained hypertension (BP > 200 mmHg) while NS rats remained normotensive (BP < 160 mmHg) (Fig. S2). The rats were euthanized by CO_2 inhalation and vascular reactivity assessed on isolated, cannulated and pressurized mesenteric arterioles as described above.

Determination of plasma nitrite

Blood derived from rats was centrifuged immediately at 5000*g* for 5 min and plasma collected. The nitrite analysis was carried out using iodine/iodide in glacial acetic acid supplemented with 1% v/v antifoam SE-15 (Sigma–Aldrich) using an ozone based chemiluminescence analyzer (Sievers, model 280i) as described [35].

Real time-polymerase chain reaction (RT-PCR)

RT-PCR was carried out on mesenteric tissue from Dahl rats [36], cleaned of fat and stabilized with RNA*later* (Qiagen, Valencia, CA). The tissue was homogenized (∼30 mg) with a sonicator in RLT buffer (Qiagen), the lysate centrifuged (10,000*g*) and total RNA purified with reagents from RNeasy® Fibrous Tissue Mini Kit (Qiagen). A first strand cDNA synthesis was performed using purified mRNA by Superscript III RT (Invitrogen, Grand Island, NY) in a thermocycler (MJ Research). The new cDNA strand was purified with QIAquick® PCR Purification Kit (Qiagen). Pure cDNA (∼10 ng) was reacted with Power SYBR Green PCR Master Mix reagent (Applied Biosystems, Mountain View, CA) in RNase-free water in a StepOne RT-PCR system (Applied Biosystems). The relative expression of $\alpha\text{-}2_A$, $\alpha\text{-}2_B$ AR and eNOS was determined using β-actin as a housekeeping gene. The primers used were: $\alpha\text{-}2_A$; TTT GCA CGT CGT CCA TAG TG (forward) and CAG TGA CAA TGA TGG CCT TG (reverse). $\alpha\text{-}2_B$; AAA CAC TGC CAG CAT CTC CT (forward) and CTG GCA ACT CCC ACA TTC TT (reverse). eNOS; CAA CGCTAC CAC GAG GAC ATT (forward) and CTC CTG CAA AGA AAA GCT CTG G (reverse). β-actin; TCC TAG CAC CAT GAA GAT C (forward) and AAA CGC AGCTCA GTA ACA G (reverse). Standard curves (initial amount of cDNA versus Ct values) were tested for each set of primers, demonstrating that for the similar range of total cDNA amplification the efficiency of target genes and housekeeping gene (β-actin) were equal. 'No reverse transcription control' was used where the PCR reaction was run in the absence of reverse transcriptase. Expression of the gene of interest was divided by the housekeeping gene and expressed as fold-change compared with the corresponding normal-salt rat group.

Data analysis

Relaxations were expressed as percentage of NE (2 μM) induced contraction. The vasodilation was studied by obtaining the maximal response and EC_{50} values were then calculated by fitting the concentration–response relationship to a logistic function. Amplified transcripts from RT-PCR were quantified using the comparative threshold cycle method ($2^{-\Delta\Delta Ct}$) with β-actin as a normalizer and the corresponding sample from the normal salt fed rat mesentery as internal control.

All data were expressed as mean ± SEM with n representing independent rat experiments. Statistical significance was tested using a paired t-test with $P < 0.05$ considered significant.

Please cite this article in press as: T.V. Gadkari et al., Agmatine induced NO dependent rat mesenteric artery relaxation and its impairment in salt-sensitive hypertension, Nitric Oxide (2013), http://dx.doi.org/10.1016/j.niox.2013.08.005