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Impaired β -adrenoceptor-induced relaxation in small mesenteric arteries from DOCA-salt hypertensive rats is due to reduced K_{Ca} channel activity

Takayuki Matsumoto^{1,*}, Theodora Szasz², Rita C. Tostes³, and R. Clinton Webb²

¹Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan

²Department of Physiology, Georgia Health Sciences University, Augusta, GA 30912-3000, USA

³Department of Pharmacology, School of Medicine of Ribeirao Preto, University of Sao Paulo, 14049-900 Sao Paulo, SP, Brazil

Abstract

β -Adrenoceptor (β -AR)-mediated relaxation plays an important role in the regulation of vascular tone. β -AR-mediated vascular relaxation is reduced in various disease states and aging. We hypothesized that β -AR-mediated vasodilatation is impaired in DOCA-salt hypertension due to alterations in the cAMP pathway. β -AR-mediated relaxation was determined in small mesenteric arteries from DOCA-salt hypertensive and control uninephrectomized (Uni) rats. To exclude nitric oxide (NO) and cyclooxygenase (COX) pathways, relaxation responses were determined in the presence of L-NNA and indomethacin, NO synthase inhibitor and COX inhibitors, respectively. Isoprenaline (ISO)-induced relaxation was reduced in arteries from DOCA-salt compared to Uni rats. Protein kinase A (PKA) inhibitors (H89 or Rp-cAMPS) or adenylyl cyclase inhibitor (SQ22536) did not abolish the difference in ISO-induced relaxation between the groups. Forskolin (adenylyl cyclase activator)-induced relaxation was similar between the groups. The inhibition of IK_{Ca} / SK_{Ca} channels (TRAM-34 plus UCL1684) or BK_{Ca} channels (iberiotoxin) reduced ISO-induced relaxation only in Uni rats and abolished the relaxation differences between the groups. The expression of SK_{Ca} channel was decreased in DOCA-salt arteries. The expression of BK_{Ca} channel α subunit was increased whereas the expression of BK_{Ca} channel β subunit was decreased in DOCA-salt arteries. The expression of receptor for activated C kinase 1 (RACK1), which is a binding protein for BK_{Ca} channel and negatively modulates its activity, was increased in DOCA-salt arteries. These results suggest that the impairment of β -AR-mediated relaxation in DOCA-salt mesenteric arteries may be attributable to altered IK_{Ca} / SK_{Ca} and/or BK_{Ca} channels activities rather than cAMP/PKA pathway. Impaired β -AR-stimulated BK_{Ca} channel activity may be due to the imbalance between its subunit expressions and RACK1 upregulation.

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*Correspondence to: Takayuki Matsumoto, Ph.D., Hoshi University, Department of Physiology and Morphology, 2-4-41 Ebara, Shinagawa-ku, Tokyo, 142-8501, Japan, Phone 81-3-5498-5726, Fax.: 81-3-5498-5726, t-matsu@hoshi.ac.jp.

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Keywords

β -adrenoceptor; calcium-activated potassium channel; DOCA-salt; relaxation; mesenteric artery; RACK1

1. Introduction

Vascular tone is generated through a net balance between vasodilatation and vasoconstriction established via the effects of a number of neurotransmitters and hormones [viz. epinephrine, norepinephrine, angiotensin II, acetylcholine (ACh), nitric oxide (NO), etc.] [1]. Elevated arterial blood pressure in most types of hypertension is attributable to increased total peripheral resistance, which results, at least in part, from alterations in humoral and neurogenic components and in vascular endothelial and smooth muscle functions [2, 3]. Indeed, altered vascular tone, which is a characteristic feature of human and various experimental models of hypertension, has been associated not only with impaired endothelium-dependent vasodilatation and reduced endothelium-derived relaxing factors, including NO, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) signaling, but also with augmented vasoconstrictor signalings [4–9]. Growing evidence indicates that the activation of β -adrenoceptors (β -ARs) modulates the peripheral vascular tone, thereby controlling the distribution of blood flow to different tissues. In fact, gene-targeting techniques that selectively inactivate β -AR in mice have demonstrated that these animals exhibit decreased vasodilatation, increased blood pressure, as well as increased pressure responses induced by adrenaline infusion [10,11].

A number of different signaling pathways have been associated with β -AR-mediated vasodilatation in blood vessels. It is well recognized that the stimulation of β -ARs in blood vessels results in the activation of adenylyl cyclase (AC), which subsequently leads to the accumulation of intracellular cyclic adenosine 3',5' monophosphate (cAMP) and subsequent activation of cAMP-dependent protein kinase A (PKA) [12]. Accordingly, relaxation of vascular smooth muscle cells induced by the non-selective β -AR agonist isoprenaline (ISO) involves cAMP-dependent mechanisms since the β -AR subtypes are coupled to AC via Gs protein [13]. On the other hand, β -AR-induced relaxations are not exclusively triggered via cAMP-dependent mechanisms but also involve cAMP-independent mechanisms [13–15]. Moreover, stimulation of β -ARs in blood vessels leads to hyperpolarization of smooth muscle cells [16]. In smooth muscle and endothelium, K^+ channels are considered crucial effector proteins in the regulation of vascular tone and arterial blood pressure, and their activity is essential for the setting of the negative membrane potential (i.e., hyperpolarization) [8,17–20]. The vascular smooth muscle and endothelial cells express a variety of very diverse K^+ channels covering all K^+ channel gene subfamilies, such as ATP-sensitive K^+ channels (K_{ATP}), voltage-gated K^+ channels (K_V), and Ca^{2+} -activated K^+ channel (K_{Ca}) [8,17–20]. Previous reports suggested that β -AR-induced vascular hyperpolarization is linked to the activation of K_{ATP} channels [16,21]. Accordingly, vasorelaxant responses induced by activation of both β_1 - and β_2 -AR involve the opening of K_{ATP} channels in the rat perfused mesenteric bed [22]. Matsushita et al. [15] found that, in rat abdominal aortic smooth muscle, β_1 -/ β_2 -AR-mediated and cAMP-independent relaxant mechanisms are partly due to activation of the large-conductance K_{Ca} (BK_{Ca}) channel, whereas β_3 -AR-mediated relaxation is attributed to K_V channel.

Several lines of evidence suggest that the activation of β -ARs on endothelial cells leads to the production/release of endothelium-derived factors [23]. Over the last two decades, evidence has accumulated that β -AR activation contributes to vasodilatation via stimulation of endothelial NO biosynthesis [24–27]. At least in some vessels β -AR-mediated NO

production may greatly outweigh any direct vasorelaxant effect of β -AR located on vascular smooth muscle cells [26]. Furthermore, the production of prostacyclin by β -AR stimulation has been described in rabbit coronary endothelial cells [28]. Since complex interactions between different vasodilator pathways in vascular smooth muscle have been proposed for NO synthase (NOS) and cyclooxygenase (COX) [29,30], it may be difficult to understand the relationship among β -AR-mediated relaxation, cAMP/PKA pathway and K^+ channel activity in hypertension. Although impairment of β -AR-mediated vasodilatation has been described in both human and animal models of hypertension [12,31–35], no study has addressed the relaxation effects of β -AR-agonists in the absence of NOS- and COX-derived products in small mesenteric arteries obtained from deoxycorticosterone acetate (DOCA)-salt rat, a model of arterial hypertension [36–39].

Therefore, in the present study, we designed experiments to investigate the mechanisms underlying the hypertension-associated impairment of β -AR-mediated dilatation in the rat small mesenteric artery. We hypothesized that β -AR-mediated vasodilatation is impaired in DOCA-salt hypertension due to alterations in cAMP pathway and/or K_{Ca} channel activity. Since complex interactions between different vasodilator pathways in vascular smooth muscle have been proposed for COX and NOS, we performed all functional experiments in the presence of indomethacin and N^G -Nitro-L-arginine (L-NNA), to inhibit COX and NOS, respectively. Under these conditions, we determined whether cAMP/PKA pathway or K^+ channels might be responsible for the impairment of β -AR-mediated relaxation in arterial hypertension. We also determined whether mesenteric arteries from control and hypertensive rats exhibit differential expression of the BK_{Ca} channel subunits.

2. Materials and methods

2.1. Reagents

ACh, DOCA, phenylephrine (PE), indomethacin, UCL1684, 1-[(2-chlorophenyl)diphenylmethyl]-1*H*-pyrazole (TRAM-34), forskolin (FSK), isoprenaline hydrochloride (ISO), Rp-adenosine 3',5'-cyclic monophosphorothioate triethylammonium salt hydrate (Rp-cAMPS), atenolol, glybenclamide and antibody against β -actin were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). SQ22536, H89 dihydrochloride, formoterol hemifumarate, CL316243 disodium salt and iberiotoxin (IbTX) were obtained from Tocris Bioscience (Ellisville, MO, USA). 9,11-dideoxy-9 α ,11 α -methanoeperoxy prostaglandin $F_{2\alpha}$ (U46619) was from Cayman Chemical (Ann Arbor, MI, USA). N^G -Nitro-L-arginine (L-NNA) was obtained from Calbiochem (San Diego, CA, USA). Drugs were dissolved in sterile HPLC grade water or dimethyl sulfoxide. Indomethacin was dissolved first in a small amount of 10^{-1} M Na_2CO_3 solution and then made up to the final volume with HPLC grade water. The antibody against BK_{Ca} channel $\beta 1$ subunit was obtained from Abcam (Cambridge, MA, USA). The antibodies against SK3 and BK_{Ca} channel α subunit were obtained from Alomone Labs (Jerusalem, Israel). The antibody against receptor for activated C kinase 1 (RACK1) was obtained from BD Biosciences (San Jose, CA, USA). Horseradish peroxidase-linked secondary anti-mouse and anti-rabbit antibodies were obtained from GE Healthcare (Piscataway, NJ, USA).

2.2. Animals and experimental design

Male Wistar rats (8 weeks-old, 250–340 g; Harlan Laboratories, Indianapolis, IN, USA) were used in this study. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals and approved by the Georgia Health Sciences University Committee on the Use of Animals in Research and Education. The animals were housed two or three per cage on a 12-h light/dark cycle and fed a standard chow diet with water *ad libitum*.

DOCA-salt hypertension was induced in rats as previously reported [36–39]. Briefly, all animals were uninephrectomized under anesthesia and were given no further treatment (control Uni group) or received 1% NaCl plus 0.2% KCl in the drinking water and DOCA silastic pellets (0.2 g/kg), which were implanted subcutaneously in the scapular region (DOCA-salt group). The duration of treatment was 4 to 5 weeks.

One week before euthanasia, systolic blood pressure was measured by the tail-cuff method using a CODA system (Kent Scientific Corporation). At the end of 4 to 5 weeks of treatment, second-order mesenteric artery was isolated and submitted to experimental procedures.

2.3. Vascular functional study

Arterial isometric force was recorded as previously described [37]. After euthanasia, the mesentery was rapidly isolated and placed in an ice-cold physiologic salt solution (PSS) containing: 130.0 mmol/L NaCl, 4.7 mmol/L KCl, 14.9 mmol/L NaHCO₃, 5.5 mmol/L glucose, 1.18 mmol/L KH₂PO₄, 1.17 mmol/L MgSO₄·7H₂O, 1.6 mmol/L CaCl₂·2H₂O, and 0.026 mmol/L ethylenediaminetetraacetic acid. Second-order branches of mesenteric artery were carefully cleaned of fat and connective tissue under a stereomicroscope. The mesenteric arteries were then cut into 2 mm rings (internal diameter $\approx$ 150–250 μ m) (several rings were obtained from each animal) and mounted on an isometric Mulvany-Halpern myograph (Danish MyoTech; Aarhus, Denmark) filled with 5 mL PSS and continuously gassed with 95% O₂- 5% CO₂ at 37 C and recorded by a PowerLab 8/SP data acquisition system (ADInstruments). The rings were stretched until an optimal resting tension, which was established from preliminary length-active tension curves, of 2.0 mN [37,40], and then allowed to equilibrate for at least 45 min. Arterial integrity was assessed by contracting the segments with a depolarizing concentration of 120 mM potassium chloride (KCl) and subsequently, with PE (10^{-5} M) followed by relaxation with ACh (10^{-5} M).

After being washed, for all relaxation studies, the rings were equilibrated for 30 min in the combined presence of 10^{-4} M L-NNA (NOS inhibitor) and 10^{-5} M indomethacin (COX inhibitor) before the administration of the thromboxane analog (U46619, 3×10^{-7} M). Cumulative concentration-response curves to ISO (10^{-9} – 10^{-5} or 10^{-4} M), a classical nonselective β -AR agonist [41], formoterol (10^{-9} – 10^{-5} M), a selective β_2 -AR agonist [42] and CL316243 (10^{-9} – 10^{-4} M), a selective β_3 -AR agonist [41,43], were performed. To study the role of β_1 -AR in ISO-mediated relaxations under NOS/COX inhibition, we performed concentration-response curves to ISO in the presence of 10^{-5} M atenolol (selective β_1 -AR antagonist [42,44]). To study the role of cAMP/PKA pathway in ISO-mediated relaxations under NOS/COX inhibition, ISO responses were determined in the presence of 10^{-4} M SQ22536 (AC inhibitor [45]), 10^{-7} or 10^{-6} M H89 (PKA inhibitor [27,46]), or 5×10^{-5} M Rp-cAMPS (PKA inhibitor [44]). To study the role of K_{Ca} channels or K_{ATP} channels in ISO-mediated relaxations under NOS/COX inhibition, responses to ISO were determined in the presence of 10^{-5} M TRAM-34 [inhibitor of intermediate conductance K_{Ca} (IK_{Ca}) [47–49]] plus 10^{-7} M UCL1684 [inhibitor of small conductance K_{Ca} (SK_{Ca}) [49, 50]], 10^{-7} M IbTX (inhibitor of BK_{Ca} [47,51]), or 10^{-6} M glibenclamide (inhibitor of K_{ATP} [16]). These concentrations of inhibitors were chosen based on previous studies.

2.4. Western blotting for SK3, BK_{Ca} subunits and RACK1

Mesenteric arteries from DOCA-salt and Uni groups were rapidly isolated, cleaned from fat, dissected, and frozen in liquid N₂. Proteins [50 μ g (for SK3), 40 μ g (for α subunit of BK_{Ca} and RACK1) or 20 μ g (for β_1 subunit of BK_{Ca})] extracted from the mesenteric bed were separated by electrophoresis on a 10% polyacrylamide gel and transferred to a nitrocellulose

membrane. Nonspecific binding sites were blocked with 5% skim milk in Tris-buffered saline solution with Tween (0.1%) for 1 h at room temperature. Membranes were then incubated with antibodies overnight at 4 C. Antibodies were as follows: anti-SK3 (1:500; Alomone Labs), anti-BK_{Ca} channel α subunit (1:500; Alomone Labs), anti-BK_{Ca} channel β 1subunit (1:1000; Abcam), anti-RACK1 (1:1000; BD Biosciences), and β -actin (1:15,000; Sigma). After incubation with secondary antibodies, signals were revealed with chemiluminescence, visualized by autoradiography, and quantified densitometrically. Results were normalized by β -actin expression and expressed as a percentage of control. The β -actin expression was similar between DOCA-salt and Uni groups in mesenteric arteries (data not shown).

2.5. Data analysis and statistics

Experimental values were calculated relative to the maximal changes from the contraction produced by U46619 in each segment. Concentration-response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 5.0; GraphPad Software Inc., San Diego, CA, USA). Data are expressed as means $\pm$ SEM. Statistical analysis of the concentration-response curves was performed by using 2-way analysis of variance (ANOVA) for comparison between the groups. When compared to groups, data were analyzed using ANOVA with *post-hoc* Bonferroni test or Student's *t*-test. Western blot data were analyzed by 1-sample *t* test. The values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Body weight and systolic blood pressure of the rats

At 3 or 4 weeks after the surgery, the body weight of the DOCA-salt rats was lower than that of control Uni rats (347.0 ± 11.9 g, $n=24$ vs. 421.3 ± 6.4 g, $n=28$, $P < 0.001$; respectively). DOCA-salt rats displayed higher systolic blood pressure in comparison with Uni rats (181 ± 5 mmHg, $n=24$ vs. 130 ± 2 mmHg, $n=28$, $P < 0.001$; respectively).

3.2. β -AR agonist-induced relaxation was reduced in small mesenteric arteries from DOCA-salt rats (under NOS and COX inhibitions)

Since NO- and COX metabolites-mediated signaling adds further complexity to the regulation of vascular tone, β -AR-mediated relaxation was determined under simultaneous inhibition of NO production and prostaglandins (by 10^{-4} M L-NNA and 10^{-5} M indomethacin, respectively). Under these conditions, ISO-induced relaxation was greatly impaired in the DOCA-salt group compared to the Uni group (Fig. 1A).

To investigate which β -ARs are responsible for the impaired response in mesenteric arteries from DOCA-salt rats, we examined the relaxant effects of selective β -AR agonists [β_2 -AR selective agonist (Fig. 1B) and β_3 -AR selective agonist (Fig. 1C)], and of ISO in the presence of a selective β_1 -AR antagonist (Fig. 1D), in the presence of NOS/COX inhibitors. The β_2 -AR selective agonist formoterol produced vasodilation, which was reduced in the DOCA-salt group vs. Uni group (Fig. 1B). The β_3 -AR selective agonist CL316243 did not cause vasodilation in mesenteric arteries from either the DOCA-salt or Uni groups (Fig. 1C). The ISO-induced relaxation was largely blocked by the β_1 -AR antagonist atenolol in both groups (Fig. 1D). It should be noted that in the presence of atenolol, ISO-induced relaxation was still reduced in the DOCA-salt vs. the Uni group (Fig. 1D).

3.3. cAMP/PKA pathway does not contribute to the impaired ISO-induced relaxation in DOCA-salt mesenteric artery

Several reports suggested that β -AR-induced vasodilatation was mediated by cAMP-dependent and -independent pathways [12,13]. To investigate a differential contribution of the cAMP/PKA pathway in mesenteric arteries from DOCA-salt rats, we evaluated the effect of cAMP/PKA pathway inhibitors on ISO-induced relaxation in the presence of NOS/COX inhibitors (Fig. 2). Pretreatment of mesenteric arteries with an AC inhibitor [SQ22536 (10^{-4} M)] slightly, but not significantly, reduced ISO-induced relaxations in both groups (Fig. 2A). Rp-cAMPS (5×10^{-5} M), which is a PKA inhibitor, did not significantly change ISO-induced relaxation in arteries from DOCA-salt or Uni rats (Fig. 2B). H89, which is another PKA inhibitor, did not affect (at 10^{-7} M or slightly reduced (at 10^{-6} M) ISO relaxation in arteries from both groups (Fig. 2C). It should be noted that these inhibitors did not abolish the difference in ISO-induced relaxation between the groups (Figs. 2A, 2B, and 2C). Moreover, the relaxation induced by forskolin, an AC activator, was similar between mesenteric arteries from the DOCA-salt and Uni groups (Fig. 2D). These results suggested that the cAMP/PKA pathway does not contribute to the impaired ISO-induced relaxation in mesenteric arteries from DOCA-salt rats.

3.4. Contribution of K_{Ca} channels to ISO-mediated relaxation

In view of the published evidence that activation of K_{Ca} channels plays an important role in the regulation of β -AR-mediated relaxation and EDHF-mediated relaxation [13,14,18], we examined the contribution of such channels to ISO-mediated relaxation, in the presence of NOS/COX inhibition (Fig. 3). To examine a role for the IK_{Ca} / SK_{Ca} channels, which are important for EDHF actions [49–51], rings were incubated with TRAM-34 (10^{-5} M) and UCL1684 (10^{-7} M), inhibitors of IK_{Ca} and SK_{Ca} , respectively. ISO-induced relaxation was markedly reduced by these treatments in the Uni group, but not in the DOCA-salt group (Fig. 3A). Iberitoxin (10^{-7} M), an inhibitor of BK_{Ca} channel markedly inhibited ISO-induced relaxation in arteries from Uni rats, but not in DOCA-salt arteries (Fig. 3B). Pretreatment of mesenteric arteries with a K_{ATP} channel inhibitor [glibenclamide (10^{-6} M)] slightly increased ISO-induced relaxations in both groups (Fig. 3C). It should be noted that treatments with K_{Ca} inhibitors (Figs. 3A and 3B), but not K_{ATP} inhibitor (Fig. 3C), abolished the relaxation differences between the groups, suggesting that IK_{Ca} / SK_{Ca} channels and BK_{Ca} channels, but not K_{ATP} channel, greatly contribute to the defective of ISO-mediated relaxation in DOCA-salt mesenteric artery.

3.5. Expression of SK_{Ca} , BK_{Ca} channel subunits and RACK1 in small mesenteric arteries

We next examined the expressions of SK_{Ca} and BK_{Ca} channel subunits in small mesenteric arteries by Western blotting analysis (Fig. 4). Protein expression levels of SK_{Ca} were significantly reduced in mesenteric arteries of DOCA-salt rats compared with Uni rats (Fig. 4A). BK_{Ca} channels are composed of pore-forming α and regulatory $\beta 1$ subunits [52–54]. The expression of BK_{Ca} channel α subunit was increased in the DOCA-salt group (vs. the Uni group) (Fig. 4B), whereas the expression of BK_{Ca} channel $\beta 1$ subunit was reduced in the DOCA-salt group (vs. the Uni group) (Fig. 4C).

RACK1 is a seven-bladed-beta-propeller protein with seven internal Trp-Asp 40 (WD40) repeats, and participates in cell signaling by functioning as a scaffold protein [55]. RACK1 can bind BK_{Ca} channel and is thought to impair its function [56]. Therefore, we next investigated the expression of RACK1 protein in mesenteric arteries from DOCA-salt and Uni rats (Fig. 5). Interestingly, the protein expression of RACK1 was significantly increased in mesenteric arteries from DOCA-salt rats vs. arteries from Uni rats (Fig. 5).

4. Discussion

Uncertainty still surrounds the role of vascular β -ARs, and their associated signaling, on vascular function. However, consistent evidence provided by a number of studies shows an impaired vasodilation in response to β -ARs activation in several experimental models of hypertension [12,31–35]. The principal findings from the present study, performed on small mesenteric arteries isolated from DOCA-salt hypertensive and their normotensive (Uni) controls, under NOS and COX inhibition, are: 1) β -AR agonists-induced vascular relaxations are reduced in the DOCA-salt group; 2) the AC/cAMP/PKA pathway does not contribute to the impaired ISO-induced vascular relaxation; 3) the inhibition of IK_{Ca}/SK_{Ca} channels or BK_{Ca} channels suppresses ISO-induced relaxation in arteries from controls, but not in arteries from the DOCA-salt group and abolishes the differences in β -AR-mediated relaxation between the Uni and DOCA-salt groups; and 4) the inhibition of K_{ATP} channels does not abolish the differences in ISO-induced relaxation between the Uni and DOCA-salt groups. Moreover, a down-regulation of SK_{Ca} channels, an imbalanced expression of BK_{Ca} subunits, (viz. increased α subunit and decreased $\beta 1$ subunit), as well as an upregulation of RACK1 protein expression are present in mesenteric arteries from the DOCA-salt group.

There have been some controversial studies about the role of endothelium-derived factors, such as NO and COX metabolites on β -AR-induced vascular relaxation. For instance, endothelium denudation inhibited β -AR-mediated relaxation in rat aorta [25], pulmonary artery [57], mesenteric arteries [58] and dog coronary artery [59], suggesting that endothelial NO and cyclic GMP system are involved. Indeed, NOS inhibition attenuated β -AR-induced relaxation in rat aorta [60], pulmonary artery [57], and mesenteric arteries [58]. However, there are others studies showing that NOS inhibition or endothelium denudation failed to suppress β -AR-mediated relaxation [61]. Moreover, age-related differences in β -AR-mediated relaxation were associated with changes in COX activity [62]. Therefore, in the present study, to exclude the effects of NOS and COX signaling, we compared β -AR-mediated relaxation in small mesenteric arteries from DOCA-salt and Uni rats in the presence of L-NNA and indomethacin.

At present, at least three populations of β -AR subtypes have been described to be involved in the β -adrenergic responses in blood vessels [43,63]. In various vessels, stimulation of β_1 -, β_2 -, and β_3 -ARs leads to vasodilation. Briones et al. [41] have provided a very comprehensive study of β -AR relaxation in the small mesenteric arteries, using a range of agonists and antagonists and direct visualization of the receptors. β -ARs are mainly, but not exclusively located to the smooth muscle layers, and the agonist/antagonist profile suggests that vasodilation is due to the activation of β_1 -ARs. In the present study, we found that 1) non-selective β -AR agonist ISO-induced relaxation was smaller in DOCA-salt rats than in control rats; 2) selective β_2 -AR agonist-induced relaxation was also smaller in DOCA-salt rats than in control rats; 3) selective β_3 -AR agonist failed to cause vasodilation in both groups; 4) ISO-induced relaxation was largely blocked by the selective β_1 -AR antagonist atenolol; and 5) atenolol-resistant ISO-induced relaxation was still smaller in arteries from DOCA-salt rats vs. control rats. These results suggest that the activation of β_1 - and β_2 -, but not β_3 -AR induces vasodilation in small mesenteric arteries under NOS/COX inhibition, and β_1 - and/or β_2 -AR-activated signaling is impaired in small mesenteric arteries from DOCA-salt rats.

Generally, the activation of β -ARs leads to Gs/AC/cAMP/PKA activation [12]. On the other hand, cAMP-independent pathways are also involved in β -AR-induced relaxation in various vessels [13–15]. To address the association between the cAMP pathway and β -AR-induced relaxation, we used a pharmacological approach. In the present study, we found that the difference of ISO-induced relaxation between the DOCA-salt and Uni groups was not

abolished by treatment with AC inhibitor (Fig. 2A) or PKA inhibitors (Figs. 2B and 2C). These results suggest that cAMP-independent signaling may be responsible for the impaired ISO-induced vascular relaxation in DOCA-salt rats. Further support for this hypothesis is provided by the observations that the AC activator FSK produced similar relaxation in small mesenteric arteries from the DOCA-salt and Uni groups.

There is an emerging body of evidence suggesting that K^+ channels play an important role in the control vascular tone and the β -AR-mediated response [8,14,17–20]. The three subtypes of K_{Ca} channels [i.e., SK_{Ca} , IK_{Ca} , and BK_{Ca} channels] are present in the vascular wall [18] and in healthy arteries, SK_{Ca}/IK_{Ca} channels are preferentially located in endothelial cells, while BK_{Ca} channels are preferentially expressed in vascular smooth muscle cells [18]. Moreover, the activation of SK_{Ca}/IK_{Ca} channels on endothelial cells plays an important role as a source of EDHF signaling [8,18,49–51]. Indeed, β -AR-induced hyperpolarization in vessels has been linked to the activation of K_{ATP} channels [16], and vasorelaxant response to both β_1 - and β_2 -AR activation involves the opening of K_{ATP} channels in the rat perfused mesenteric bed [22]. Matsushita et al. [15] found that in the rat abdominal aortic smooth muscle β_1 -/ β_2 -AR-mediated and cAMP-independent relaxant mechanisms were partly due to the BK_{Ca} channel. Therefore, we have examined the relative contributions of K_{ATP} , IK_{Ca}/SK_{Ca} , and BK_{Ca} to ISO-mediated responses in DOCA-salt small mesenteric arteries, under conditions of preserved EDHF signaling (i.e., under NOS/COX inhibition). In the present study, the combined treatment with IK_{Ca}/SK_{Ca} inhibitors (Fig. 3A) and treatment with BK_{Ca} channel inhibitor (Fig. 3B) abolished the differences in ISO-induced relaxation between Uni and DOCA-salt groups. In contrast, the treatment with a K_{ATP} channel inhibitor did not abolish such differences (Fig. 3C). These results strongly suggest that defective IK_{Ca}/SK_{Ca} and/or BK_{Ca} channel activity is responsible for the impaired ISO-induced relaxation in the small mesenteric arteries from DOCA-salt rats. In agreement with our functional data, a differential expression of SK_{Ca} and BK_{Ca} channels was observed between the groups. Small mesenteric arteries from DOCA-salt rats, when compared with arteries from Uni rats, exhibited decreased levels of SK_{Ca} channel.

BK_{Ca} channels are expressed largely in vascular smooth muscle, where they function as negative-feedback regulators of vascular tone [18–20]. BK_{Ca} channels are composed of pore-forming α and regulatory $\beta 1$ subunits [18–20,52–54]. Upregulation of BK_{Ca} channel α subunit expression and function occurs in vascular smooth muscle from aldosterone-salt hypertensive rats and SHR [64,65]. In the present study, the imbalanced expression of BK_{Ca} channel subunits, such as increased α subunit (in agreement with a previous report [66]) and decreased $\beta 1$ subunit were observed in small mesenteric arteries from DOCA-salt hypertensive rats. The activity of the BK_{Ca} channel is regulated not only by expression of its subunits, but also by various messengers including calcium, heterotrimeric GTP binding proteins, protein phosphatase, and protein kinases [20,67–69]. Moreover, RACK1, which is a 7-sided propeller protein with 7WD40 repeats that participates in cell signaling by functioning as a scaffold protein [55], binds to BK_{Ca} channels in rat basilar and pulmonary artery smooth muscle cells, a process that is thought to impair BK_{Ca} channel function [56]. In the present study, the expression of RACK1 protein was increased in mesenteric arteries from DOCA-salt hypertensive rats. Although the mechanisms by which RACK1 interacts with the G protein-coupled receptor β -AR and BK_{Ca} channel subunits remain unclear at present, the reduction of β -AR-stimulated BK_{Ca} activation in DOCA-salt mesenteric arteries might be attributable to the imbalanced expression of the BK_{Ca} channels subunits and the increased RACK1 protein expression. Further studies will be required to determine the molecular mechanisms of BK_{Ca} channel activation induced by β -AR stimulation in small mesenteric arteries.

5. Conclusions

In summary, the present results suggest that a defective β -AR-mediated relaxation in small mesenteric arteries is seen in DOCA-salt hypertensive rats, under NOS and COX inhibition. Such impaired responses may be attributable to impairment in K_{Ca} channels, which results from down-regulation of SK_{Ca} channels and an imbalance in the expression of the BK_{Ca} subunits. Therefore, K_{Ca} channels may represent new targets for the treatment of arterial hypertension-associated vascular dysfunction. Additional studies addressing the molecular mechanisms that regulate IK_{Ca} , SK_{Ca} , and BK_{Ca} will help to understand the interaction of β -AR with this system and their role on blood pressure regulation.

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Fig. 1.

β -Adrenoceptor-mediated relaxation, under nitric oxide synthase and cyclooxygenase inhibition, is impaired in DOCA-salt small mesenteric arteries. Concentration-response curves for isoprenaline (ISO) (A), formoterol (B), CL316243 (C) and ISO in the presence of 10^{-5} M atenolol (D) in mesenteric arteries from DOCA-salt hypertensive, and control Uni rats. All concentration-response curves were performed in the presence of 10^{-4} M L-NNA and 10^{-5} M indomethacin. Details are given under Section 2. Data are means $\pm$ SEM. Number of determinations is shown within parentheses. * $P < 0.05$, DOCA-salt vs. Uni group. # $P < 0.05$, Uni vs. Uni atenolol group. † $P < 0.05$, DOCA-salt vs. DOCA-salt atenolol group. ‡ $P < 0.05$, Uni atenolol vs. DOCA-salt atenolol group.

Fig. 2. cAMP/PKA pathway does not contribute to the impaired ISO-induced relaxation in DOCA-salt small mesenteric artery. The effect of cAMP/PKA pathway on ISO-induced relaxation, under nitric oxide synthase and cyclooxygenase inhibition, in small mesenteric arteries from DOCA-salt and Uni rats. The effect of 10⁻⁴ M SQ22536 (A), 5 × 10⁻⁵ M Rp-cAMPS (B), and 10⁻⁷ or 10⁻⁶ M H89 on ISO-induced relaxation. (D) Concentration-response curves for forskolin (FSK) in mesenteric arteries from DOCA-salt and Uni rats. All concentration-response curves were performed in the presence of 10⁻⁴ M L-NNA and 10⁻⁵ M indomethacin. Details are given under Section 2. Data are means ± SEM. Number of determinations is shown within parentheses. *P < 0.05, DOCA-salt vs. Uni group. †P < 0.05, DOCA-salt SQ22536 (A), DOCA-salt Rp-cAMPS (B), or DOCA-salt 10⁻⁶ M H89 (C) vs. Uni SQ22536 (A), Uni Rp-cAMPS (B), or Uni 10⁻⁶ M H89 (C), respectively.

Fig. 3.

K_{Ca} channels, but not K_{ATP} channels, contribute to impaired ISO-induced relaxation in small mesenteric arteries from DOCA-salt rats. The effects of IK_{Ca} (10^{-5} M TRAM-34) and SK_{Ca} (10^{-7} M UCL1684) channel inhibitors (A), BK_{Ca} channel inhibitor (10^{-7} M IbTX) (B), and K_{ATP} channel inhibitor (10^{-6} M glibenclamide) (C) on ISO-induced relaxation, under inhibition of nitric oxide synthase and cyclooxygenase in small mesenteric arteries from DOCA-salt and Uni rats. Details are given under Section 2. Data are means $\pm$ SEM. Number of determinations is shown within parentheses. (A) * $P < 0.05$, DOCA-salt vs. Uni group. # $P < 0.05$, Uni vs. Uni TRAM-34 UCL1684. (B) * $P < 0.05$, DOCA-salt vs. Uni group. # $P < 0.05$, Uni vs. Uni IbTX. (C) * $P < 0.05$, DOCA-salt vs. Uni group. # $P < 0.05$,

Uni vs. Uni glibenclamide. †P < 0.05, DOCA-salt vs. DOCA-salt glibenclamide. ‡P < 0.05, DOCA-salt glibenclamide vs. Uni glibenclamide group.

Fig. 4.

Altered expression of SK3 and BK_{Ca} channel subunits in small mesenteric arteries from DOCA-salt rats. Analysis of protein expression for SK3 (A) and pore forming α (B) and regulatory β 1 (C) subunits of BK_{Ca} channel in mesenteric arteries from DOCA-salt and Uni rats. *Upper*: Representative Western blots of SK3 or BK_{Ca} channel subunits. *Lower*: Bands were quantified as described in Section 2. Data are mean $\pm$ SEM of 4 (A) or 6 (B, C) determinations (SK3 or BK_{Ca} subunits/ β -actin). Number of determinations is shown within parentheses. * $P < 0.05$ vs. Uni group.

Fig. 5. Mesenteric artery from DOCA-salt hypertension rats exhibit increased expression of receptor for activated C-kinase 1 (RACK1). Western blots analysis for RACK1 in mesenteric arteries from DOCA-salt and Uni rats. *Left:* Representative Western blots of RACK1. *Right:* Bands were quantified as described in Section 2. Ratios were calculated for the optical density of RACK1 over that of β -actin. Data are mean $\pm$ SEM of 6 determinations. Number of determinations is shown within parentheses. * $P < 0.05$ vs. Uni group.