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Function and Protein Expression of Potassium Channels in Mesenteric Resistance Arteries Isolated from 2K-1C Hypertensive Rats

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Abstract

The present study aimed to evaluate the K^+ channels activation in vascular relaxation induced by the nitric oxide (NO) donors ruthenium-derived complex (Terpy) and sodium nitroprusside (SNP), as well as its protein expression, on mesenteric resistance arteries (MRA) isolated from renal hypertensive rats (2K-1C) and sham-operated rats (Sham). The NO donors Terpy and SNP induced relaxation with similar efficacy in isolated MRA from both 2K-1C and Sham rats, although SNP was more potent than Terpy. The maximum relaxation induced by Terpy was decreased when the voltage-gated potassium channels were blocked in MRA from Sham, but not in 2K-1C rat arteries. The blockade of ATP-sensitive (K_{ATP}), big and small conductance Ca^{2+}-activated (SK_{Ca}) or inward rectifier (K_{IR}) potassium channels decreased the maximum relaxation induced by Terpy in MRA from Sham and 2K-1C rats. However, the maximum relaxation induced by SNP was inhibited in Sham but not in 2K-1C rats when the big conductance calcium-activated potassium channel was blocked. However, it remained the same when the other potassium channels were blocked. The protein expression of the SK_{Ca} and K_{ATP} were not altered in 2K-1C hypertensive rat MRA whereas the expression of KV and BK_{Ca} were augmented in MRA from 2K-1C rats. Therefore, the potassium channels play different role on the relaxation induced by SNP and Terpy. The activation of different potassium channels and the protein expression of potassium channels may be differently modulated in arteries from 2K-1C hypertensive rats when compared to normotensive rats.

Keywords: Hypertension; Mesenteric resistance arteries; Potassium channels; NO donor

Introduction

Potassium channels play a central role in the maintenance of the electrical potential across the plasma membrane in smooth muscle cells [1]. In arterial smooth muscle, the potassium channel activity is linked to contractile tone. The factors that regulate the activity of potassium channels may have major influences on tone and also on blood vessel diameter and consequently on vascular resistance, blood flow and blood pressure [1].

At least four different subtypes of potassium channels have been identified in arterial smooth muscle cells, which include the inward rectifier (K_{IR}), voltage-gated (K_V), ATP gated (K_{ATP}) and Ca^{2+}-gated potassium (K_{Ca}) channels. Potassium channels from different vascular beds, different blood vessels size (i.e. conduit *versus* resistance blood vessels) or even different segments of the same artery have different properties [2-5]. These differences may account for the differences in their responses to physiological, pharmacological, or pathological stimuli [6]. Therefore, ion channels in the cells which make up the walls of vessels in the microcirculation display unique function or expression patterns that remain largely unexplored [7].

Diseases like hypertension may induce changes in the function and/or expression pattern of the potassium channels. As recently shown by Matsumoto et al. [8], the defective activation of β-adrenoceptors-mediated relaxation in mesenteric arteries from DOCA-salt hypertensive rats could be attributed to impairment in KCa channels. Moreover, the resting membrane potential of vascular smooth muscle cells is reported to be more depolarized in arteries from hypertensive *versus* normotensive animals [9,10]. The effect of hypertension on resting membrane potential may be more profound in smaller resistance vessels, especially in vascular beds that play a significant role in the regulation of peripheral resistance [11]. It suggests that small arteries (<200 μm in diameter) from physiologically relevant vascular beds (renal, mesenteric, and cerebral) are altered in hypertension and play a significant role in the regulation of peripheral resistance [11].

Therefore, generalizing the role of potassium channels is extremely dangerous. It is clear that more information is needed on potassium channel expression profiles in different arterial preparations before general conclusions can be drawn about channel alterations during hypertension [12]. In this study, we aimed to verify the functional and expressional pattern of potassium channels in mesenteric resistance arteries isolated from 2K-1C hypertensive rats.

Methods

Animals

Male rats (180-200g) were maintained in standard conditions, including 12h light/dark cycle and free access to standard rat chow and water. Experimental protocols followed standards and policies of the Animal Care and Use Committee of University of São Paulo (044/2008). Briefly, rats were anesthetized with tribromoethanol (2.5 $mg.kg^{-1}$, ip). A small midline laparotomy was then performed and a silver clip (0.2 mm internal diameter) was placed on the left renal artery. The abdomen was closed in two layers, and the animals were allowed to recover. Sham-operated animals were submitted to laparotomy only. After the surgery, the animals were treated with a unique dose of oxytetracyclin (200 $mg.kg^{-1}$, i.m.) in order to minimize the risk of infection. Six weeks after the surgery, the systolic arterial pressure (SAP) was measured by

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tail-cuff method and the rats were considered to be hypertensive when SAP was higher than 160 mmHg.

Vascular reactivity studies

The rats were anesthetized and killed by decapitation. The mesentery was removed and immediately placed in a Petri plate containing cold (4ºC) Krebs solution with the following composition (in mM): NaCl 118.0, KCl 5.9, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, dextrose 11.0, pH 7.4. The second or third order mesenteric arteries (internal diameter 200-300 μm) were dissected, cleaned of fat and connective tissue, cut into 2 mm long rings and mounted in a Mulvany-Halpern Myograph for isometric tension changes recordings. The arteries were normalized to a transmural pressure of 100 mmHg [13]. To test the viability of the preparations, the contraction of the arterial segments was stimulated twice with 120 mM KCl. The endothelium was removed by gently rubbing the internal surface of the arteries with a human hair and the removal was confirmed by the absence of relaxation to acetylcholine (10 μM) in preparations contracted with phenylephrine (PE 10 μM).

Cumulative concentration-effects curves for the NO donors sodium nitroprusside (SNP) and $[Ru(terpy)(bdq)NO]^{3+}$ (Terpy) were constructed in Sham and 2K-1C rat mesenteric resistance arteries contracted with 10 μM PE. In order to verify the potassium channels subtypes involved in this relaxation, the concentration-effect curves for SNP or Terpy were constructed in the presence of the following potassium channel blockers: 4-Aminopiridine (1 mM 4-AP for K_V); Glibenclamide (3 μM, for K_{ATP}); Apamin (1 μM, for SK_{Ca}); paxilline (1 μM, for BK_{Ca}) and barium chloride (30 μM $BaCl_2$ for K_{IR}).

The NO donor $[Ru(terpy)(bdq)NO]^{3+}$ (Terpy) was synthesized by the Analytical Chemistry Laboratory at the Department of Physics and Chemistry of the Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, as previously described by de Lima et al. [14].

Western blotting

The rats were anesthetized and killed by decapitation. The mesenteric bed was removed and placed immediately in a Petri plate containing cold (4ºC) Krebs solution with the following composition (in mM): NaCl 118.0, KCl 5.9, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, dextrose 11.0, pH 7.4. The mesenteric bed was dissected and cleaned of fat and connective tissue. The mesenteric artery, as well as the first-order branch, was discarded. The remaining tissue was immediately frozen in liquid nitrogen. Each sample was homogenized in protein ice-cold lysis buffer RIPA (Tris-HCl 65.2 mM; NaCl 154 mM; NP-40 1%; sodium deoxycolate 0.25%; EDTA 0.8 mM). Homogenates were centrifuged at 10,000 rpm at 4°C for 10 min, to remove tissue debris. Protein concentrations in the samples were determined by the Bradford method (Bio-Rad Protein Assay). Protein from the tissue samples (30 μg/ well) were separated on 8-10% SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were blocked for 60 min with 5% nonfat milk in tris-buffered solution, at room temperature. Membranes were incubated overnight with goat polyclonal primary antibody against the subunit $K_{V1.5}$ of K_V (1:5000, Chemicon, catalog number AB5182), or BK_{Ca} (1:5000, BD Biosciences, catalog number 611248), or SK_{Ca} (1:5000, Santa Cruz Biotechnology, catalog number SC-28621), or subunits SUR 2B (1:1000, Santa Cruz Biotechnology, catalog number SC-5793) or K_{IR} 6.1 of K_{ATP} (1:1,000, Santa Cruz Biotechnology, catalog number SC-11224), at 4oC. Membranes were then incubated with a HRP-conjugated secondary antibody (rabbit, 1:1,000 for K_V; mouse, 1:2,000 for BK_{Ca}; rabbit 1:2,000 for SK_{Ca} or goat 1:4,000 for the subunits of K_{ATP}) for 2 h at room temperature, followed by chemiluminescence labeling (ECL, GE Healthcare) for 2 min. Bands were detected by using a film developer (Image Quant 350 GE). To determine loading consistencies, each membrane was incubated with antibody against mouse β-actin (Santa Cruz Biotechnology, catalog number SC-47778), and data were normalized by β-actin values. The abundance of the proteins was quantified by densitometry (Image J).

Drugs and reagents

NaCl, KCl, $MgSO_4$, KH_2PO_4, $CaCl_2$ and $NaHCO_3$ were purchased from Labsynth. D-glucose was purchased from Vetec Química. The drugs phenylephrine, acetylcholine, 4-aminopiridine, glibenclamide, apamin, paxilline, and barium chloride were purchased from Sigma-Aldrich (Sigma-Aldrich Co.).

The drugs phenylephrine, acetylcholine, 4-amminopiridine, glibenclamide, apamin and barium chloride were dissolved in distilled water. Paxilline was dissolved in DMSO. Terpy was diluted in a solution of DMSO 10%. The final concentration of DMSO did not exceed 0.01% in the incubation bath.

Data analysis

Data are expressed as mean ± SEM, with n indicating the number of animals.

For the functional studies, the maximum effect (ME) was considered as the maximal amplitude response reached in the concentration-effect curves for the relaxant agent. Data are represented as percentage of the maximum effect, in order to normalize the relaxation. The concentration of the agent producing half-maximal relaxation amplitude (EC_{50}) was determined after logarithmic transformation of the normalized concentration-effect curves, and EC_{50} values are reported as the negative logarithm (pD_2) of the mean of individual values for each tissue. Statistical analysis was performed by one-way ANOVA with Newman Keul's post-hoc test, using the software Prism Graphpad 5.0.

For the western blotting studies, the statistical analysis was performed by unpaired t Student test, using the Prism Graphpad 5.0.

In all the cases, a p value < 0.05 was considered statistically significant.

Results

Vascular reactivity

The NO donors SNP and Terpy induced relaxation with similar efficacy (ME) in isolated mesenteric resistance arteries from both sham and hypertensive 2K-1C rats (SNP, Sham: 96.2 ± 0.9%, n=5; 2K-1C 87.3 ± 3.5%, n=7; Terpy Sham: 93.9 ± 1.3%, n=7; 2K-1C: 93.2 ± 1.6%, n=7). However, SNP was more potent in inducing relaxation than Terpy as shown by the pD_2 values (SNP, Sham: 6.71 ± 0.12, n=5; 2K-1C: 6.90 ± 0.30, n=7; Terpy Sham: 4.76 ± 0.14, n=7; 2K-1C: 4.68 ± 0.15, n=7). It can be noticed in the Figure 1 that the curve profile is also different. The maximum relaxation induced by SNP was not altered by the voltage-dependent potassium channel blocker, 4-aminopiridine (4-AP), both in Sham (ME: 94.5 ± 2.8%, pD_2: 6.40 ± 0.30, n=7, Figure 1A) and 2K-1C rat arteries (ME: 92.3 ± 7.2%, pD_2: 7.72 ± 0.51, n=4, Figure 1C).

The maximum relaxation induced by Terpy was decreased by the voltage-dependent potassium channel blocker 4-aminopiridine (4-AP), in mesenteric resistance arteries from Sham (ME: 70.9 ± 6.6%,

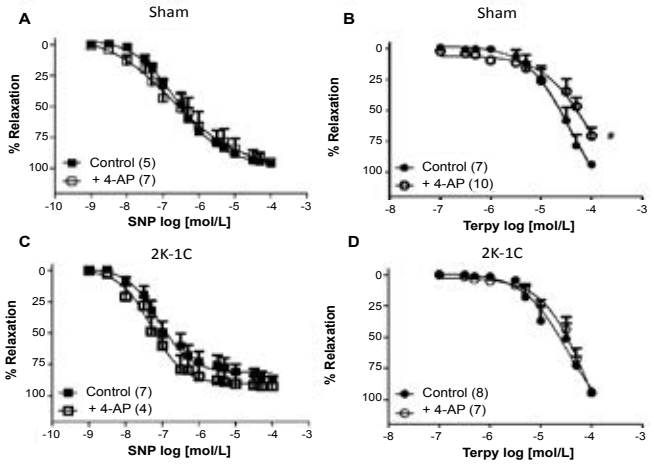

Figure 1: Effect of the selective blockade of the voltage-dependent potassium channels on the relaxation induced by the NO donors SNP and Terpy in mesenteric resistance arteries from Sham (A and B) and hypertensive 2K-1C rats (C and D). The curves represent the relaxation response induced by the NO donor in contracted arterial rings with 10 μM phenylephrine, in the absence (control) or in the presence of 4-aminopyridin (4-AP 1 mM). The points represent mean ± SEM of experiments performed in preparations of different animals. The number in parenthesis indicates the n of the experiment. #statistically different (p<0.01), one-way ANOVA, followed by Newman-Keuls.

pD_2: 4.08 ± 0.26, n=10, Figure 1B), but not in 2K-1C rat arteries (ME: 94.6 ± 2.7%, pD_2: 4.04 ± 0.4, n=7, Figure 1D).

The relaxation induced by SNP was not significantly changed by the ATP-sensitive potassium channels blocker glibenclamide, in arteries from Sham (ME: 91.9 ± 2.8%, pD_2: 6.60 ± 0.30, n=4, Figure 2A) or from 2K-1C rats (ME: 80.4 ± 6.2%, pD_2: 7.20 ± 0.50, n=5, Figure 2C). However, the maximum relaxation induced by Terpy was decreased by the ATP-sensitive potassium channel blocker glibenclamide, in mesenteric resistance arteries from Sham (ME: 69.5 ± 5.6%, pD_2: 4.25 ± 0.18, n=8, Figure 2B) as well in 2K-1C rat (ME: 72.9 ± 9.3%, pD_2: 4.35 ± 0.13, n=6, Figure 2D).

The maximum relaxation induced by SNP was not altered by the Small conductance Ca2+-activated potassium channel blocker apamin, both in arteries from Sham (ME: 85.3 ± 4.3%, pD_2: 6.80 ± 0.30, n=4, Figure 3A) and 2K-1C rats (ME: 80.9 ± 5.4%, pD_2: 6.52 ± 0.20, n=5, Figure 3C).

The maximum relaxation induced by Terpy was inhibited by the Small conductance Ca2+-activated potassium channel blocker apamin, both in arteries from Sham (ME: 65.6 ± 6.2%, pD_2: 4.27 ± 0.34, n=7, Figure 3B) and 2K-1C rats (ME: 74.2 ± 2.8%, pD_2: 4.28 ± 0.10, n=4, Figure 3D).

The maximum relaxation induced by SNP was inhibited by the Large conductance Ca2+-activated potassium channel blocker paxilline, in arteries from Sham (ME: 43.1 ± 7.6%, pD_2: 6.03 ± 0.19, n=4, Figure 4A), but not in arteries from 2K-1C rats (ME: 84.1 ± 5.4%, pD_2: 6.85 ± 0.30, n=7, Figure 4C).

The maximum relaxation induced by Terpy was inhibited by the Large conductance Ca2+-activated potassium channel blocker paxilline, both in arteries from Sham (ME: 13.4 ± 4.1%, pD_2: 5.43 ± 0.65, n=5, Figure 4B) and 2K-1C rats (ME: 69.5 ± 9.3%, pD_2: 4.04 ± 0.20, n=5, Figure 4D).

The maximum relaxation induced by SNP was not changed by the inward rectifier potassium channel blocker BaCl2, in arteries from Sham (ME: 53.8 ± 6.1%, pD_2: 4.32 ± 0.03, n=5, Figure 5A) and from 2K-1C rats (ME: 76.5 ± 8.5%, pD_2: 6.52 ± 0.30, n=5, Figure 5C).

The maximum relaxation induced by Terpy was inhibited by the inward rectifier potassium channel blocker BaCl2, both in arteries from Sham (ME: 53.8 ± 6.1%, pD_2: 4.32 ± 0.03, n=5, Figure 5B) and 2K-1C rats (ME: 77.5 ± 7.5%, pD_2: 5.09 ± 0.50, n=6, Figure 5D).

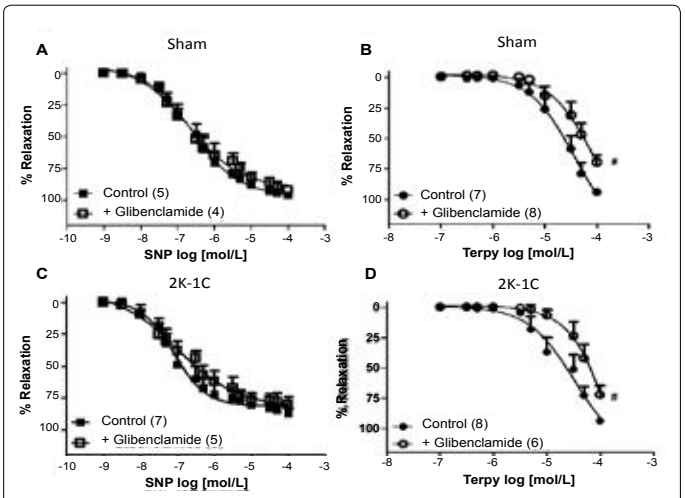

Figure 2: Effect of the selective blockade of the ATP-sensitive potassium channels on the relaxation induced by the NO donors SNP and Terpy in mesenteric resistance arteries from Sham (A and B) and hypertensive 2K-1C rats (C and D). The curves represent the relaxation response induced by the NO donor in contracted arterial rings with 10 μM phenylephrine in the absence (control) or in the presence of glibenclamide (3 mM). The points represent mean ± SEM of in experiments performed in preparations isolated from different animals. The number in parenthesis indicates the n of experiments. #statistically different (p<0.01), one-way ANOVA, followed by Newman-Keuls.

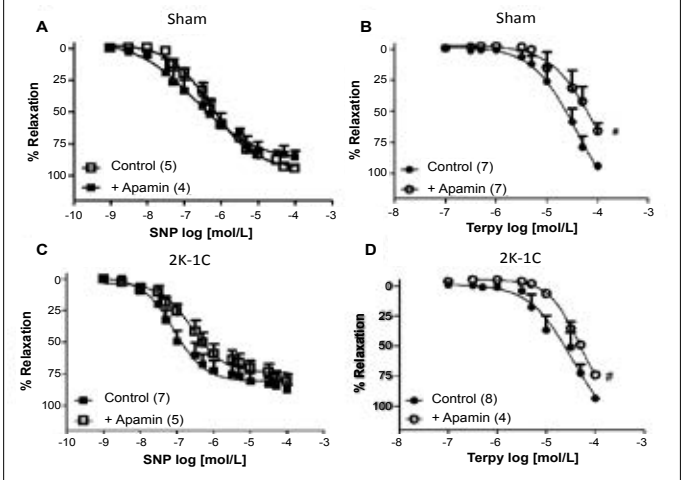

Figure 3: Effect of the selective blockade of the small conductance calcium-activated potassium channels on the relaxation induced by the NO donors SNP and Terpy in mesenteric resistance arteries from Sham (A, B) and hypertensive 2K-1C rats (C,D). The curves represent the relaxation response induced by the NO donor in contracted arterial rings with 10 μM phenylephrine in the absence (control) or in the presence of apamin (1 mM). The points represent mean ± SEM of experiments performed in preparations of different animals. The number in parenthesis indicates the n of the experiment. #statistically different (p<0.01); one-way ANOVA, followed by Newman-Keuls.

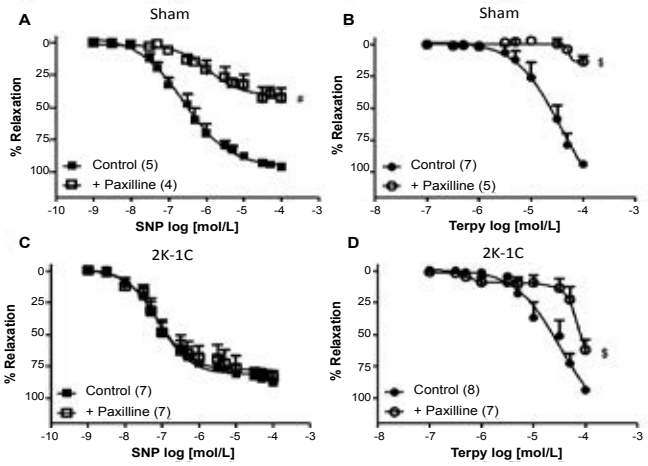

Figure 4: Effect of the selective blockade of the high conductance calcium-activated potassium channels on the relaxation induced by the NO donors SNP and Terpy in mesenteric resistance arteries from Sham (A.B) and hypertensive 2K-1C (C.D) rats. The curves represent the relaxation response induced by the NO donor in contracted arterial rings with 10 μM phenylephrine in the absence (control) or in the presence of paxilline (1 mM). The point represents mean ± SEM of experiments performed in preparations of different animals. The number in parenthesis indicates the n of the experiment. #statistically different (p<0.01); $statistically different (p<0.001); one-way ANOVA, followed by Newman-Keuls.

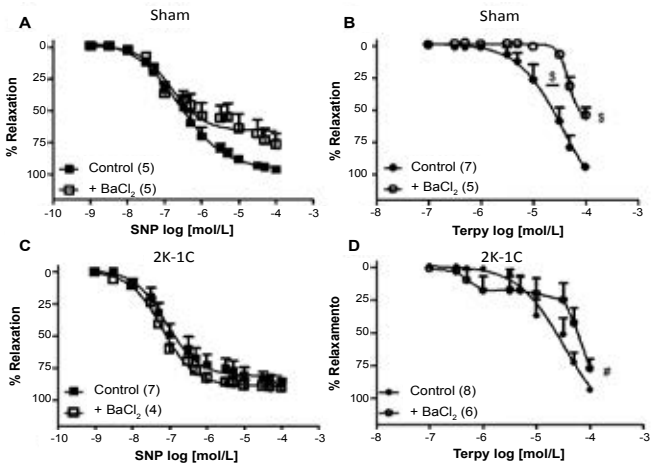

Figure 5: Effect of the selective blockade of inward rectifier potassium channels on the relaxation induced by the NO donors SNP and Terpy in mesenteric resistance arteries from Sham (A,B) and hypertensive 2K-1C rats (C,D). The curves represent the relaxation induced by the NO donor in contracted arterial rings with 10 μM phenylephrine in the absence (control) or in the presence of BaCl2 (30 mM). The point represent mean ± SEM of experiments performed in preparations isolated from different animals. The number in parenthesis indicate the n of the experiment. #statistically different (p<0.01); $statistically different (p<0.001); one-way ANOVA, followed by Newman-Keuls.

Protein expression by Western blotting analysis

There was no difference on the protein expression of the small conductance calcium-activated potassium channel (SK_{Ca}) and on both subunits (SUR-2B and KIR 6.1) of the ATP-sensitive potassium channel (K_{ATP}) between the mesenteric bed of Sham and 2K-1C rats. However, the protein expression of the $K_{V1.5}$ subunit of the voltage-dependent potassium channel (KV) and of the big conductance calcium-activated potassium channel (BK_{Ca}) were augmented in the mesenteric bed of 2K-1C rats when compared to Sham rats (Figure 6).

Discussion

The microvessels undergo extensive adaptation during the pathogenesis of systemic hypertension. The major finding of this study was to show the functional and expressional pattern of the potassium channels in resistance vessels in 2K-1C renal hypertensive rats.

Potassium channels play a central role in the maintenance of the electrical potential across the plasma membrane in smooth muscle cells [1]. Voltage-dependent potassium channels (K_v) open to allow the efflux of potassium in response to depolarization of the membrane potential, resulting in repolarization and the return to the resting membrane potential [15]. It has been shown that Kv current from arteries is reduced during SHR, DOCA-salt [11] and Dahl salt-sensitive [16] hypertensive rats. In humans, decreased KV channel function was also shown [17]. Specifically in mesenteric resistance arteries, it was observed a smaller current through KV in SHR than in Wistar Kyoto rats (WKY) [18]. In the same way, in our study, the blockade of KV inhibited the response induced by Terpy in mesenteric resistance arteries from Sham, but not from 2K-1C rats, suggesting an impaired function of these channels.

Regarding the protein expression, Cox et al. [19] have shown that the protein expression of $K_{V1.5}$ subunit of KV was not different between aortas from SHR and WKY rats, but it was higher in mesenteric resistance arteries from SHR. They have also shown that the K_V currents associated with Kv1.X and Kv2.1 channels were both larger in mesenteric arteries myocytes from SHR, but less than expected based upon differences in KV protein expression. On the other hand,

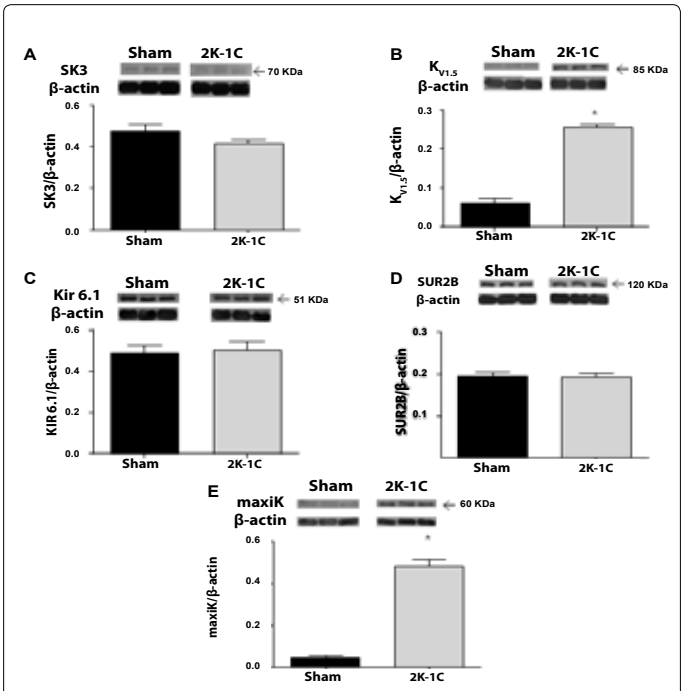

Figure 6: Protein expression of the SK_{Ca} (A), $K_{V1.5}$ subunit of KV (B), KIR6.1 (C) and SUR-2B (D) subunits of KATP and BK_{Ca} (E) in the mesenteric bed isolated from Sham and 2K-1C rats. Determinations were made by Western blotting technique. Each lane represents the protein obtained from a sample from (n=6) different animals. The bars represent the densitometry of the blots corrected by β-actin. *statistically different of Sham (p<0.05) by unpaired t Student test.

[20] have observed that the $K_{V1.5}$ subunit expression is smaller in hypertensive rats induced by administration of N-nitro-arginine. In our study, although the function of KV seems to be impaired, we have observed that protein expression of the $K_{V1.5}$ subunit was increased in the mesenteric bed from 2K-1C rats, when compared to Sham rats. Cox and Rusch [21] have also shown a functional downregulation of smooth muscle KV channels in hypertension, despite evidence that expression of the channel protein is increased. We may suggest that this augmented protein expression could be a compensatory mechanism in this vessels in this experimental model of hypertension.

Another type of potassium channels expressed in the vascular smooth muscle cells are the calcium-activated potassium channels, which may be of small conductance (SK_{Ca}) or big conductance (BK_{Ca}). BK_{Ca} are activated by membrane depolarization or by augmented intracellular calcium concentration [22-24]. A number of vasodilators, including NO, seems to activate BK_{Ca} channels in some systems either directly [25,26], or by activation of protein kinases [27].

Resistance arteries exist physiologically in a partially constricted state, from which they constrict further or dilate, to respond to the perfusion needs of the tissue or organ. A major physiological stimulus for constriction is intravascular pressure [28]. Resistance arteries respond to an elevation in intravascular pressure by a graded membrane depolarization, elevation in $[Ca^{2+}]i$, and constriction, which is dependent on the increased calcium entry through voltage-dependent calcium channels [29]. A number of negative feedback mechanisms are linked to the increase in VSMC $[Ca^{2+}]i$, including the activation of BK_{Ca} channels [30].

The function of the BK_{Ca} has been shown to be augmented in SHR [31-33]. Rusch et al [34] and England et al. [35] demonstrated that SHR aortic smooth muscle cells have more K_{Ca} current than WKY cells. Then, other studies [36,37] provided evidence that the increased BK_{Ca} current in aortic smooth muscle cells from SHR and salt-sensitive hypertensive rats was the result of increased Ca^{2+} influx and/or transmembrane Ca^{2+} cycling and suggested that it may be a compensatory mechanism to counterbalance the augmented contraction that occurs in hypertension and prevent organ damage. This phenomenon occurs similarly throughout the vasculature, including the aorta [34] (Rusch et al., 1992) carotid, mesenteric, femoral [38], and cerebral vascular beds [39]. Therefore, increased BK_{Ca} channel function in arterial smooth muscle cells may provide a protective mechanism against progressive increases in blood pressure. This negative-feedback mechanism would modulate increased pressure and vascular tone, and subsequently limit pressure-induced vasoconstriction and preserve local blood flow.

However, Kang et al. [40] have shown that the function of BK_{Ca} is impaired in mesenteric resistance arteries from various models of hypertension (angiotensin II-infused, associated or not to an augmented ingestion of salt, and DOCA-salt hypertension), although the nitric oxide pathway compensates to maintain vasorelaxation in these arteries through NOS-mediated cGMP and H_2O_2 production. Similarly, we have previously observed that the relaxation induced by Terpy is not impaired in the mesenteric resistance arteries from 2K-1C hypertensive rats [41]. However, in the present study, the blockade of BK_{Ca} inhibited the mesenteric resistance arteries relaxation induced by SNP in Sham, but not in 2K-1C rats and the inhibition caused by the blockade of BK_{Ca} is smaller in 2K-1C when compared to Sham rats. These data suggest an impaired function in these channels in mesenteric resistance arteries in this model of hypertension.

Some studies have reported an augmented expression of the BK_{Ca} during hypertension, but most of them were conducted in large vessels [42,43]. Similarly to what happens to KV, in our study, the protein expression of BK_{Ca} is augmented in the mesenteric bed of 2K-1C hypertensive rats. This augmented expression with decreased effect of the blocker paxilline may suggest that there is a functional downregulation of this subtype of potassium channels.

It is important to notice that although the molecular weight of BK_{Ca} is 125kD, we have observed in the Western blotting studies a band in 60 kD. Similarly, Garcia-Calvo et al. [44] have observed that the BK_{Ca} on tracheal smooth muscle appeared in 60 kD. Knaus et al. [45] have shown that it occurs due to the proteolysis caused by the extraction process.

There is a paradigm for "ion channel remodeling" in hypertension, which may be regarded as the change in surface density of Ca^{2+} and potassium channels in the vascular smooth muscle cells membrane associated with the chronic elevation of blood pressure, according to Cox and Rusch [21]: First, the upregulation of L-Type Ca^{2+} channels and/or loss of KV channels have been proposed as key excitatory events that result in membrane depolarization and increased voltage-gated Ca^{2+} influx in arteries exposed to high blood pressure. Subsequently, the compensatory overexpression of BK_{Ca} channels is thought to provide a counter-regulatory mechanism to help prevent local vasospasm and ischemic episodes in hypertensive disease. Taking into account these findings, we may suggest that these channels are more expressed and do not have their function impaired in mesenteric resistance arteries during 2K-1C hypertension.

Some microvascular smooth muscle cells may also express small conductance K_{Ca} (SK_{Ca}) channels [46]. The physiological function of these channels in vascular smooth muscle is not fully understood. SK_{Ca} may be presented as SK1, SK2 and SK3. SK3 is the most expressed in vascular smooth muscle cells [47]. In our study, we have observed that these channels do not participate of the relaxation induced by SNP but they participate of the relaxation induced by Terpy. However, there is no difference between hypertensive 2K-1C and normotensive rat arteries. Moreover, the protein expression of these channels was not different between 2K-1C and Sham arteries.

Another type of K^+ channels is the ATP-sensitive potassium channels (K_{ATP}), They are inhibited by cytosolic ATP and they also appear to play a role in the mechanism of action of vasodilators [24,48] (Jackson, 1993, JACKSON ET AL 1993), including NO [22]. They are voltage-independent channels. In hypertensive states, the K_{ATP} may have their function impaired, as shown by Gosh et al. [49] in aortas from DOCA-salt hypertensive rats. Synthetic K_{ATP} channel activators are less potent dilators *in vivo* in both large [50] and small cerebral vessels [51] of chronically hypertensive rats. On the other hand, Blanco-Rivero et al. [52] have shown that the protein expression of K_{ATP} was lower in aorta from SHR when compared to WKY, although the function of these channels were preserved. Moreover, one study reported enhanced K_{ATP} channel function in SHR [53]. In the present study, the blockade of K_{ATP} alone did not alter the relaxation induced by SNP and the participation of K_{ATP} on the relaxation induced by Terpy was not different between arteries from Sham and 2K-1C rats, suggesting that their function is not impaired in 2K-1C hypertension. The K_{ATP} from vascular smooth muscle cells are composed of a tetramer of KIR 6.1 subunits that form the ion conductive pore, and complementary regulatory sulfonylurea receptor (SUR) subunits, SUR 2B [1,54]. The subunit KIR6.2 has also been found [55]. The protein expression of the subunits of KATP was not changed in arteries from 2K-1C rats in our study.

The inward rectifier family of potassium channels (K_{IR}) is divided into seven subfamilies (K_{IR} 1.0 to K_{IR} 7.0), but the KIR 2.0 subfamily is the most relevant to the blood vessel wall. In the vascular wall, K_{IR} channels are expressed in both the endothelial and in the smooth muscle cells [56]. KIR channels are abundant in the smooth muscle of small-diameter resistance vessels [3,57]. For example, the currents through KIR in the smooth muscle cells from coronary arteries augment from the conductance until the small resistance arteries [3]. This difference in the protein expression explains the fact that the conductance arteries present small response to small augments in extracellular potassium, while the resistance arteries present great dilatation [3,58].

There is evidence that vascular K_{IR} channels function may be impaired in hypertension. McCarron and Halpern [59] reported that the barium-sensitive vasodilator responses to potassium were impaired in posterior cerebral arteries from SHR when compared to WKY. However, in our study, the blockade of K_{IR} impaired the relaxation induced by Terpy but not to SNP, in a similar way in Sham and 2K-1C hypertensive rats.

As can be seen, the inhibition of none of the channels alone was able to inhibit the relaxant response induced by SNP. However, the blockade of the channels in a non-selective way with TEA, the relaxation was impaired (data not shown). We suggest that there should be a compensatory mechanism to guarantee the relaxation even if one channel subtype is impaired. Taking into account that it did not happen to the relaxation induced by Terpy, we may also conclude that the activation of potassium channels is a mechanism more important to the relaxation induced by Terpy than it is for SNP.

Experimental hypertension models enable development of targeted interventions in order to decrease blood pressure [60]. The animal model for hypertension research should have human-like disease characteristics and complications [61]. Renovascular hypertension 2K-1C is a non-genetic model that is influenced by renin-angiotensin-aldosterone system. Renovascular hypertension is among the most common causes of secondary hypertension [62].

There are several experimental hypertension models developed which simulate the impaired NO production/availability together with another vascular alterations reported to endothelial dysfunction, including in 2K-1C. The classic nitrovasodilators organic nitrate and nitrite esters, including nitroglycerin, amyl nitrite and isosorbide dinitrate have been employed for many years in cardiovascular diseases treatment, but their use is limited due to tolerance and/or side effects.

Considering the relevance of the resistance vessels in regulating arterial pressure, it was of great interest to investigate the vascular relaxation induced by the new NO donor (Terpy) in mesenteric resistance artery from hypertensive and normotensive rats. Activation of potassium (K^+) channels is one of the major mechanisms of NO-induced vasodilation. For our surprise, the vascular relaxation was not impaired in renal hypertensive as compared to normotensive as it was observed in conductance vessels. It could be explained by the fact that several types of K+ channels are activated in both hypertensive and normotensive rats mesenteric arteries. However, only in hypertensive mesenteric artery, voltage-dependent K^+ channels (K_V) are activated. It can be relevant for human hypertension, since K^+ channels (K_V) could be a target for nitrovadilators clinically used to treat hypertension. In addition, Terpy does not induce citotoxity to vascular smooth muscle cells in the concentrations used to induce vasorelaxation (unpublished data).

In conclusion, SNP and Terpy induce relaxation of mesenteric resistance arteries by activation of different potassium channels. Moreover, the function and protein expression of different potassium channels may be differently changed during hypertension.

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