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Ca^{2+}-Activated K^+ Channels Underlying the Impaired Acetylcholine-Induced Vasodilation in 2K-1C Hypertensive Rats

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ABSTRACT

We tested the hypothesis that an abnormal function of K^+ channels in vascular smooth muscle cells plays a key role in the impaired acetylcholine (ACh) vasodilation in aortas from two kidney-one clip (2K-1C) hypertensive rats and further investigated the K^+ channel subtype involved in this altered response. ACh-induced endothelium-dependent relaxation was assessed in aortic rings from 2K-1C and normotensive two kidney (2K) rats. Glibenclamide, an ATP-sensitive K^+ channel blocker, did not inhibit ACh-induced relaxation in aortic rings from 2K or 2K-1C rats. The voltage-dependent K^+ channels inhibitor 4-aminopyridine attenuated ACh-induced relaxation in both groups. Charybdotoxin and iberiotoxin, blockers of Ca^{2+}-sensitive (K_{Ca}) and large-conductance K_{Ca} (BK_{Ca}) channels, respectively, reduced ACh-induced relaxation in aortic rings from 2K rats without affecting this response in those from 2K-1C rats, abolishing the differences between groups. ACh-induced relaxation in vessels from both 2K and 2K-1C rats was unaffected by apamin, a small-conductance K_{Ca} blocker. NS1619 [1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2*H*-benzimidazol-2-one], an activator of K_{Ca}, induced a smaller vasodilation in endothelium-denuded aortic rings from 2K-1C rats compared with those from 2K rats. Iberiotoxin reduced sodium nitroprusside-induced relaxation in endothelium-denuded aortic rings from 2K without affecting this response in those from 2K-1C rats. The inhibition of Na^+,K^+-ATPase with ouabain had no effects on ACh-induced relaxation in aortic rings from 2K-1C or 2K rats. These data indicate that a deficient functional activity of BK_{Ca} channels plays a key role in the impaired ACh vasodilation in aortas from 2K-1C rats.

Several agonists, including acetylcholine (ACh), hyperpolarize the vascular smooth muscle cell membrane in an endothelium-dependent manner by the release of mediators such as nitric oxide (NO) (Tare et al., 1990), endothelium-derived hyperpolarizing factor (Chen et al., 1988, 1991), and prostacyclin (Parkington et al., 1995). ACh-induced hyperpolarization is not generated in high extracellular K^+ concentration ($[K^+]_o$) solution, indicating that vascular smooth muscle hyperpolarization by endothelial factors is mediated by K^+ channels activation, which in turn closes voltage-dependent Ca^{2+} channels, reduces cytosolic Ca^{2+} concentration, and induces vasodilation (Chen et al., 1991; Feletou and Vanhoutte, 1999; Coleman et al., 2001). NO and endothelium-derived hyperpolarizing factor-mediated responses can also involve the Na^+,K^+-ATPase, which is found in the plasma membrane of vascular smooth muscle cells (Busse et al., 2002) and is thought to be critically involved in the maintenance of cellular ionic homeostasis needed to regulate membrane potential and vascular smooth muscle tone (Marín and Redondo, 1999).

Altered vascular tone, a characteristic feature of most forms of experimental and human hypertension, has been associated with impaired endothelium-dependent vasodilation and reduced NO signaling (Puddu et al., 2000; Schiffrin, 2001; Taddei and Salvetti, 2002). We previously reported that endothelium-dependent vasodilation and smooth muscle cell hyperpolarization are impaired in aortic segments from two kidney-one clip (2K-1C) hypertensive rats (Callera et al.,

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ABBREVIATIONS: ACh, acetylcholine; NO, nitric oxide; $[K^+]_o$, extracellular K^+ concentration; 2K-1C, two kidney-one clip; 2K, two kidney; K_V, voltage-dependent K^+ channel; K_{ATP}, ATP-sensitive K^+ channels; BK_{Ca} large-conductance Ca^{2+}-sensitive K^+ channel; SK_{Ca}, small-conductance Ca^{2+}-sensitive K^+ channel; OUA, ouabain; K_{Ca}, Ca^{2+}-sensitive channel; SBP, systolic blood pressure; PSS, physiological salt solution; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; ChTX, charybdotoxin; IbTX, iberiotoxin; SNP, sodium nitroprusside; dAUC, difference(s) of the area under the concentration-response curve(s).

2000). However, no predisposing deficiency in NO has been demonstrated in 2K-1C renal hypertension, and NO seems to be particularly important in preserving normal renal hemodynamics in this model by opposing the influence of constrictor factors (Sigmon and Beierwaltes, 1998; Dedeoglu and Springate, 2001). Indeed, we also observed that the NO-cGMP pathway is not altered in aortic rings from 2K-1C rats (Callera et al., 2000). Therefore, reduced aortic vasodilation in renal hypertension may also be related to an abnormal function of vascular smooth muscle cells in this vessel.

Consistent with the central role of K^+ channels in the regulation of vascular tone, altered function and expression of K^+ channels has been observed in vascular smooth muscle cells of different models of hypertension (Cox, 2002). Because high $[K^+]_o$ solution abolishes the differences in ACh-induced relaxation between aortic segments from 2K-1C and 2K rats (Callera et al., 2000), we hypothesized that an abnormal function of vascular smooth muscle K^+ channels plays a key role in the impaired ACh-induced relaxation in aortas from 2K-1C rats and may explain the decreased vascular smooth muscle membrane hyperpolarization and the blunted endothelium-dependent vasodilation previously observed in aortas from renal hypertensive rats.

Many subtypes of K^+ channels have been identified in smooth muscle cells (Standen and Quayle, 1998; Korovkina and England, 2002), and the contribution of the different subtypes of K^+ channels to vasodilator responses has been estimated from the effects of selective inhibitors. To evaluate which subtype of K^+ channel contributes to the decreased ACh-induced relaxation in 2K-1C aortas, blockers of voltage-dependent K^+ channels (K_V), ATP-sensitive K^+ channels (K_{ATP}), large and small conductance Ca^{2+}-sensitive K^+ channels (BK_{Ca} and SK_{Ca}, respectively) were used. In the present study, we also considered that an altered activity of the Na^+,K^+-ATPase may contribute to changes in aortic vasodilation in 2K-1C hypertensive rats. Therefore, we also evaluated the effects of ouabain (OUA), an inhibitor of Na^+,K^+-ATPase, on ACh-induced relaxation.

Materials and Methods

Experimental Animals. Experimental protocols followed standards and policies of the University of São Paulo's Animal Care and Use Committee. Renovascular hypertension was induced in rats as described previously (Callera et al., 2001). Briefly, male Wistar rats (180–200 g) were anesthetized and after a midline laparotomy, a silver clip with an internal diameter of 0.20 mm was placed around the left renal artery. The 2K rats were submitted to laparotomy only. Animals were maintained on standard rat chow with a 12-h light/dark cycle and given free access to both food and water. Systolic blood pressure (SBP) was measured weekly in nonanesthetized animals by an indirect tail-cuff method (MLT125R pulse transducer/pressure cuff coupled to the PowerLab 4/S analog-to-digital converter; AD Instruments Pty Ltd., Castle Hill, Australia), and rats were considered hypertensive when SBP was higher than 160 mm Hg.

Vascular Reactivity Studies. Six weeks after surgery, rats were killed by decapitation, and the thoracic aortas were isolated. Aortic rings, 4 mm in length, were placed in bath chambers (10 ml) for isolated organs containing physiological salt solution (PSS) at 37°C, continuously bubbled with 95% O_2 and 5% CO_2, pH 7.4. Two fine stainless steel holders were placed through the lumen of the aortic rings; one of the holders was fixed to the tissue chamber and the other was connected to a F-60 force-displacement transducer and the contractile/relaxant responses were recorded on a polygraph (Narco Biosystems Inc., Houston, TX). The aortic rings were submitted to a tension of 1.5 g, which was readjusted every 15 min during a 60-min equilibration period before the addition of a given drug. Optimal basal tension, 1.5 g in aortic rings from both 2K and 2K-1C, was previously standardized by exposing the vessels to 90 mM KCl under various resting tensions (0.25–2.5 g).

At the beginning of the experiments, vessels were stimulated with 0.1 μM norepinephrine to test their functional integrity. In some experiments, the endothelium was removed by gently rubbing the lumen side of the aortic rings and integrity or removal of the endothelium was tested by the presence or absence, respectively, of relaxation in response to 1 μM ACh.

Endothelium-dependent relaxation was evaluated in both 2K-1C and 2K groups by the response to ACh (1 nM–10 μM) in aortas precontracted with phenylephrine. The contribution of K^+ channels to ACh-induced relaxation was assessed in aortas previously incubated, for a 30-min period, with K^+ channel blockers as follows: 3 μM glibenclamide (blocker of K_{ATP}), 5 mM 4-aminopyridine (4-AP, blocker of many K_V), 1 mM tetraethylammonium (TEA, nonselective blocker of K^+ channels), 0.1 μM charybdotoxin (ChTX, blocker of K_{Ca} and K_V), 30 nM iberiotoxin (IbTX, selective blocker of BK_{Ca}), 0.5 μM apamin (selective blocker of SK_{Ca}), and the association of IbTX and apamin (Nelson and Quayle, 1995; Satake et al., 1997; Ceron et al., 2001). The K^+ channel blockers were left in the incubation bath throughout the entire experiment.

Additional experiments were performed in endothelium-denuded aortas to determine changes in the relaxant function of vascular smooth muscle. Responses to the exogenous NO donor sodium nitroprusside (SNP, 0.1 nM–1 μM) was examined in aortas contracted with phenylephrine or 60 mM KCl. The effect of 30 nM IbTX on SNP relaxation as well as the relaxing effect of 3 μM NS1619, an activator of BK_{Ca} channels, was evaluated in endothelium-denuded phenylephrine-contracted aortic rings.

In another set of experiments, we evaluated the functional activity of the sarcolemmal Na^+,K^+ATPase as well as its contribution to endothelium-dependent and -independent relaxation in aortas from 2K-1C and 2K rats. Aortic rings without endothelium were incubated in a K^+-free medium for 30 min and stimulated with phenylephrine at the concentration that produces 50% magnitude of 90 mM KCl-induced contraction. K^+ (1–10 mM) was subsequently added to the bath, producing relaxation by activation of the Na^+,K^+ATPase followed by hyperpolarization and Ca^{2+} influx blockade (Rossoni et al., 2002). This protocol was repeated in the presence of 100 μM ouabain, an inhibitor of the sarcolemmal Na^+,K^+-ATPase, and the magnitude of K^+-induced relaxation was used as an index of Na^+,K^+-ATPase activity because it is inhibited by ouabain. The effect of 30-min preincubation of 100 μM ouabain on ACh and SNP-induced relaxation was observed in aortas with or without endothelium, respectively.

Inhibition of Ca^{2+}-sensitive and voltage-sensitive K^+ channels as well as endothelium removal usually augmented basal tone and the contractile responses to phenylephrine in both groups of aortas. At these conditions, phenylephrine concentration was adjusted, so that the contractile tone was the same as that obtained with 0.3 μM phenyleprine (EC_{80}) in aortic rings with intact endothelium from 2K in the absence of blockers (around 1.5 g of tension). The concentration range of phenylephrine used was 10 nM to 0.3 μM. To avoid the possibility of time-dependent changes in vascular responsiveness, a single concentration-effect curve was performed in each aorta.

Solutions and Drugs. Composition of PSS was the following: 130.0 mM NaCl, 1.6 mM $CaCl_2$, 4.7 mM KCl, 1.17 mM $MgSO_4$, 1.18 mM KH_2PO_4, 14.9 mM $NaHCO_3$, 0.026 mM EDTA, and 5.5 mM dextrose. In experiments with high $[K^+]_o$, an isotonic 60 mM KCl PSS was prepared by equimolar replacement of NaCl with KCl. Norepinephrine, phenylephrine, SNP, TEA, 4-AP, ChTX, IbTX, and ouabain were obtained from Sigma-Aldrich (St. Louis, MO); ACh, NS1619, and glibenclamide were from Sigma/RBI (Natick, MA), and Apamin was from Calbiochem-Novabiochem (La Jolla, CA).

Data Analysis. Results are expressed as means ± S.E.M., and n indicates the number of animals. Responses to ACh and SNP are expressed as a percentage of the preceding contraction induced by phenylephrine or KCl. The concentration of the agonist producing a half-maximal response (EC_{50}) was determined after logit transformation of the normalized concentration-response curves and is reported as the negative logarithm ($-\log EC_{50} = pD_2$ values) of the mean of individual values for each tissue by the use of the Prism GraphPad 4.04 software (GraphPad Software Inc., San Diego, CA). The maximum effect was considered as the maximal amplitude response reached in concentration-effect curves for relaxant agents. For statistical analysis, multiple comparisons were made by one-way variance followed by the Bonferroni post test, and single comparisons were made using Student's unpaired t test, as appropriate. P value <0.05 was taken as significant.

Results

Six weeks after surgery, SBP was significantly increased in 2K-1C (203 ± 4 mm Hg, $n = 28$, $P < 0.001$) versus 2K rats (116 ± 3 mm Hg, $n = 24$). Relaxation to ACh was decreased in aortic rings from 2K-1C (maximum effect 75.3 ± 6.5%, $P < 0.01$; $pD_2 = 7.1 \pm 0.1$, $P < 0.001$; $n = 9$) compared with those from 2K rats (maximum effect 94.4 ± 1.9%; $pD_2 = 7.5 \pm 0.1$; $n = 7$).

Blockade of K_{ATP} channels with 3 μM glibenclamide failed to produce any inhibitory effect on ACh-induced relaxation in aortic rings from both 2K (maximum effect 91.6 ± 4.1%; $pD_2 = 7.7 \pm 0.1$; $n = 8$) and 2K-1C rats (maximum effect 75.4 ± 6.5%; $pD_2 = 7.1 \pm 0.2$; $n = 7$) (Fig. 1A). As shown in Fig. 1B, blockade of K_V channels with 5 mM 4-AP markedly attenuated ACh-induced relaxation in aortic rings from both 2K (maximum effect 73 ± 2.8%, $P < 0.001$; $pD_2 = 6.3 \pm 0.1$, $P < 0.001$; $n = 8$) and 2K-1C rats (maximum effect 29.4 ± 3.8%, $P < 0.001$; $pD_2 = 5.8 \pm 0.1$, $P < 0.001$; $n = 9$). The addition of 4-AP increased the resting tone above the basal level in a greater magnitude in aortic rings from 2K-1C rats (1.5 ± 0.4 g, $P < 0.001$) than in vessels from 2K rats (0.6 ± 0.12 g).

Incubation with 1 mM TEA significantly reduced ACh-induced relaxation in aortic rings from 2K rats (maximum effect 79.9 ± 6.7%, $P < 0.05$; $pD_2 = 6.9 \pm 0.2$, $P < 0.01$; $n = 7$) without affecting ACh response in aortas from 2K-1C rats (maximum effect 77.1 ± 4.6%; $pD_2 = 6.7 \pm 0.1$; $n = 9$; Fig. 2A). A blocker of K_{Ca} channels, ChTX (0.1 μM), also inhibited ACh response in aortic rings from 2K rats (maximum effect 75.7 ± 5.7%, $P < 0.001$; $pD_2 = 6.8 \pm 0.2$, $P < 0.001$; $n = 7$), but not in those from 2K-1C rats (maximum effect 82.2 ± 3.3%; $pD_2 = 7.0 \pm 0.1$; $n = 8$; Fig. 2B). Similar results were obtained using 30 nM IbTX, a BK_{Ca} blocker, which reduced the relaxation to ACh in aortic rings from 2K rats (maximum effect 64.1 ± 10.4%, $P < 0.01$; $pD_2 = 7.1 \pm 0.2$, $P < 0.05$; $n = 7$) and failed to alter this response in vessels from 2K-1C rats (maximum effect 63.8 ± 5.2%; $pD_2 = 7.3 \pm 0.1$; $n = 7$; Fig. 2C). Therefore, in the presence of these blockers there were no differences in the relaxation to ACh between aortic rings from 2K and 2K-1C rats. Only TEA induced an increase in basal tone of aortic rings, which was greater in 2K-1C (0.6 ± 0.2 g; $P < 0.05$) than in 2K (0.04 ± 0.02 g).

Apamin (0.5 μM), a selective SK_{Ca} channels blocker, had no effect on the response to ACh in aortic rings from either 2K (maximum effect 90.0 ± 5.0%; $pD_2 = 7.7 \pm 0.1$; $n = 8$) or 2K-1C rats (maximum effect 74.2 ± 4.0%; $pD_2 = 7.2 \pm 0.1$; $n = 6$; Fig. 3A). The combination of apamin with IbTX reduced ACh response in aortic rings from 2K (maximum effect 66.4 ± 7.9%; $pD_2 = 7.2 \pm 0.2$; $n = 7$; Fig. 3B) and this inhibitory effect was similar to that produced by IbTX alone. However, in aortic rings from 2K-1C rats apamin plus IbTX decreased ACh-induced relaxation (maximum effect 45.1 ± 9.0%; $P < 0.05$; $pD_2 = 6.9 \pm 0.2$; $n = 5$; Fig. 3B) compared with IbTX alone that had no effect in ACh response.

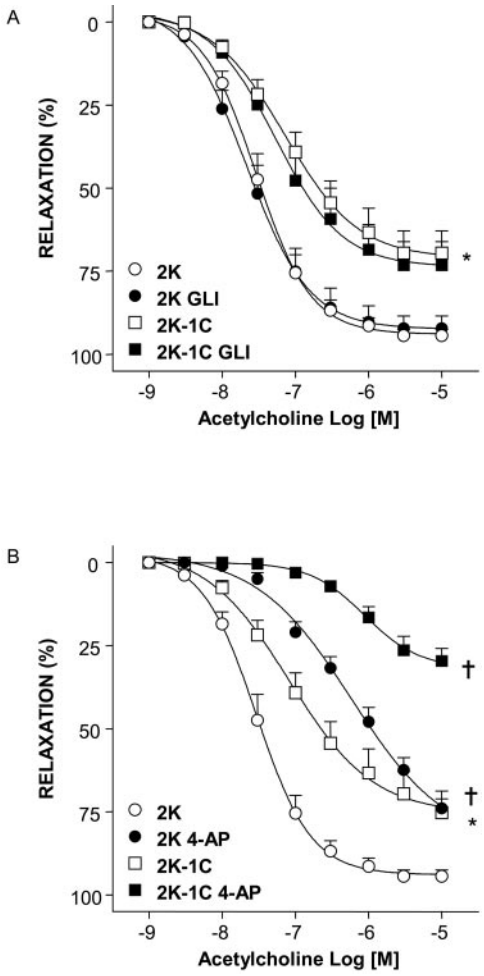

Fig. 1. Effects of 3 μM glibenclamide (GLI) (A) and 5 mM 4-AP (B) on ACh-induced relaxation of phenylephrine-contracted aortic rings from 2K and 2K-1C rats. Values are means ± S.E.M. and are expressed as percentage of relaxation. Differences in the maximum relaxation to ACh are indicated as *, $P < 0.05$, 2K-1C versus 2K in the absence of inhibitors; †, $P < 0.05$, presence versus absence of 4-AP in 2K and 2K-1C.

As shown in Fig. 4, NS1619 (3 μM), an activator of BK_{Ca} channels, induced relaxation in endothelium-denuded aortas contracted with phenylephrine. This response was greater in aortic rings from 2K rats (75.1 ± 10.7%; $n = 4$) than in aortas from 2K-1C rats (31.6 ± 5.9; $n = 4$; $P < 0.05$).

SNP induced a concentration-dependent relaxation in endothelium-denuded aortas contracted with either phenylephrine (Fig. 5A) or KCl (Fig. 5B). In phenylephrine-contracted aortic rings, sensitivity to SNP was greater in 2K ($pD_2 = 9.2 \pm 0.1$; $n = 8$) than in 2K-1C ($pD_2 = 8.3 \pm 0.1$; $n = 8$; $P < 0.001$), whereas maximum relaxation was similar in 2K (118.0 ± 9.2%) and 2K-1C (121.5 ± 6.6%). When aortic rings were contracted with 60 mM KCl, relaxation to SNP was similar in 2K (maximum effect 101.8 ± 4.7%; $pD_2 = 7.7 \pm 0.1$; $n = 7$) and 2K-1C (maximum effect 100.9 ± 0.9%; $pD_2 =$

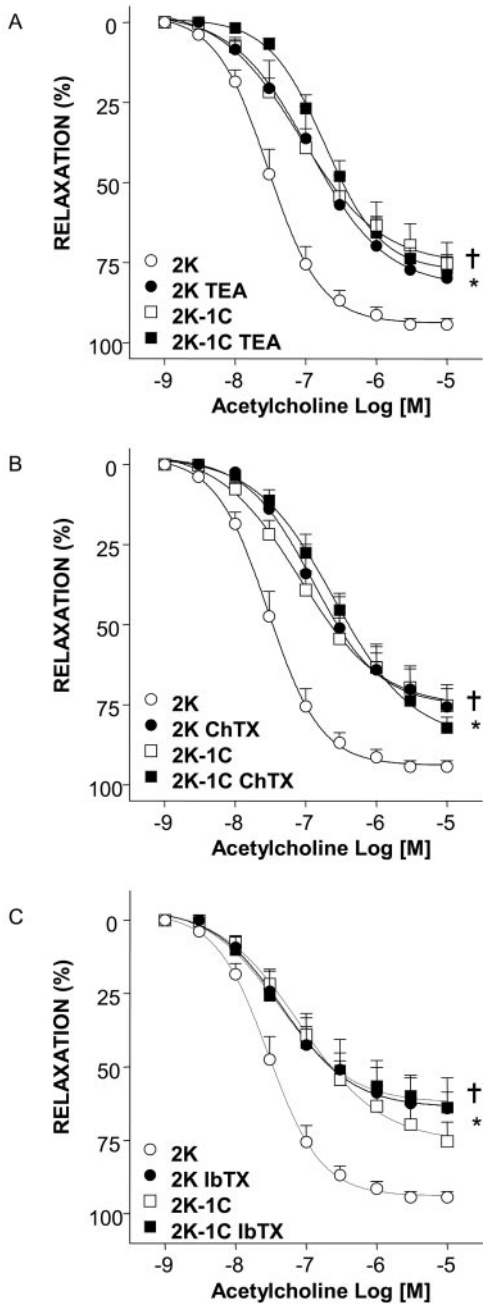

Fig. 2. Effects of 1 mM TEA (A), 0.1 μM ChTX (B), and 30 nM IbTX (C) on ACh-induced relaxation of phenylephrine contracted aortic rings from 2K and 2K-1C rats. Values are means ± S.E.M. and are expressed as percentage of relaxation. Differences in the maximum relaxation to ACh are indicated as *, $P < 0.05$, 2K-1C versus 2K in the absence of inhibitors; †, $P < 0.05$, presence versus absence of K^+ channel blockers in 2K.

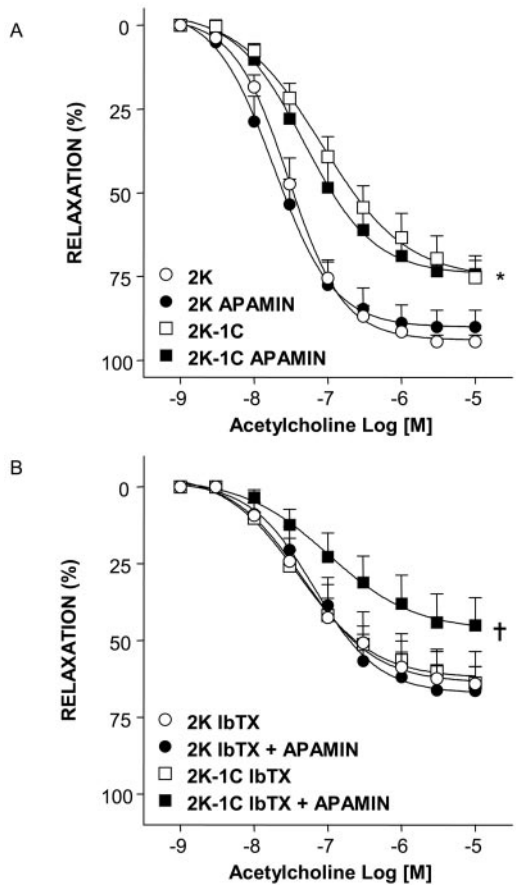

Fig. 3. Effects of 0.5 μM apamin (A) and 0.5 μM apamin plus 30 nM IbTX (B) on ACh-induced relaxation of phenylephrine-contracted aortic rings from 2K and 2K-1C rats. Values are means ± S.E.M. and are expressed as percentage of relaxation. Differences in the maximum relaxation to ACh are indicated as *, $P < 0.05$, 2K-1C versus 2K in the absence of inhibitors; †, $P < 0.05$, presence of apamin + IbTX versus IbTX alone in 2K-1C.

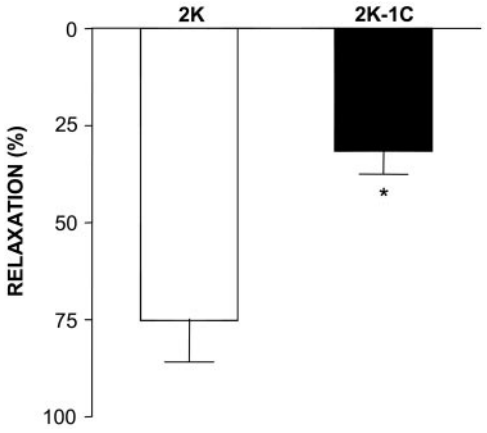

Fig. 4. Relaxation to 3 μM NS1619 of phenylephrine-contracted aortic rings from 2K and 2K-1C. Values are means ± S.E.M. and are expressed as percentage of relaxation. *, $P < 0.05$, difference in the relaxation between 2K and 2K-1C.

7.7 ± 0.1; $n = 7$) rats (Fig. 5B). As shown in Fig. 5A, SNP relaxation was also evaluated in phenylephrine-contracted aortic rings in the presence of IbTX. Whereas IbTX significantly shifted to the right the concentration-response curve to SNP in aortic rings from 2K rats (maximum effect 106.0 ± 5.5%; $pD_2 = 8.7 \pm 0.1$; $P < 0.01$; $n = 5$), the response in vessels from 2K-1C rats was not affected (maximum effect 104.7 ± 1.62%; $pD_2 = 8.3 \pm 0.1$; $n = 6$). In aortic rings without endothelium, addition of IbTX to the bath solution produced a similar contractile effect in 2K (0.28 ± 0.1 g) and 2K-1C rats (0.26 ± 0.07 g).

As shown in Fig. 6A, Na^+,K^+-ATPase inhibition with 100 μM ouabain had no effects on ACh-induced relaxation in aortic rings from either 2K-1C (maximum effect 63.8 ± 10,1%; $pD_2 = 6.9 \pm 0.2$; $n = 5$) or 2K rats (maximum effect 96.5 ± 3.5%; $pD_2 = 7.1 \pm 0.2$; $P < 0.05$; $n = 5$). Ouabain inhibited the relaxation to SNP in aortic rings from 2K-1C

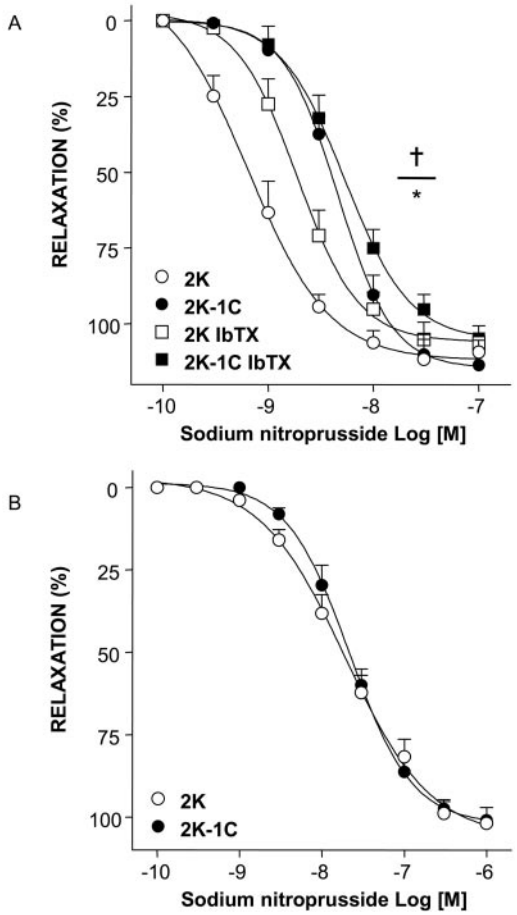

Fig. 5. Concentration-response curves to sodium nitroprusside in aortas without endothelium from 2K and 2K-1C contracted with phenylephrine (A) or 60 mM KCl (B). The effects of 30 nM IbTX on sodium nitroprusside-induced relaxation is also shown (A). Values are means ± S.E.M. and are expressed as percentage of relaxation. Differences in the pD_2 values to ACh are indicated as ∗, $P < 0.05$, 2K-1C versus 2K in the absence of IbTX; †, $P < 0.05$, presence versus absence of IbTX in 2K.

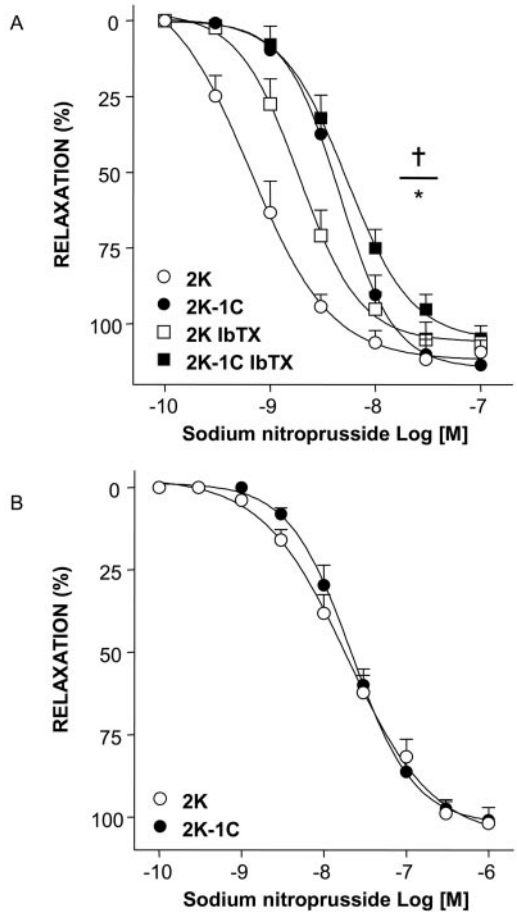

Fig. 6. Effects of 100 μM OUA on ACh- (A) and sodium nitroprusside (B)-induced relaxation of phenylephrine-contracted aortic rings from 2K and 2K-1C. Values are means ± S.E.M. and are expressed as percentage of relaxation. Differences in the pD_2 values or in the maximum relaxation to SNP are indicated as ∗, $P < 0.05$, 2K-1C versus 2K in the absence of OUA; †, $P < 0.05$, presence versus absence of OUA in 2K-1C.

rats (maximum effect 89.8 ± 4.0%, $P < 0.001$; $pD_2 = 7.8 \pm 0.3$; $n = 6$) and failed to alter that in vessels from 2K rats (maximum effect 121.11 ± 4.48%; $pD_2 = 9.1 \pm 0.3$; $n = 5$; Fig. 6B). The concentration-dependent relaxing effect of KCl in phenylephrine-contracted aortic rings is shown in Fig. 7. Sensitivity to KCl was decreased in aortic rings from 2K-1C rats ($pD_2 = 2.6 \pm 0.2$; $n = 6$; $P < 0.01$) compared with that in vessels from 2K rats ($pD_2 = 2.9 \pm 0.1$; $n = 6$). Ouabain-induced inhibition of KCl effects was greater in aortic rings from 2K-1C rats (Fig. 7A). Ouabain-sensitive functional activity of the Na^+,K^+-ATPase was increased in aortic rings from 2K-1C rats [difference of the area under the concentration-response curve (dAUC) 96.0 ± 0.8%; $n = 6$; $P < 0.05$ versus 2K, dAUC 80.9 ± 1.7%; $n = 6$].

Discussion

The principal new finding of the present study is that the impaired acetylcholine-induced relaxation in aortic segments from 2K-1C rats is due to a decreased functional activity of vascular smooth muscle BK_{Ca} channels. This is the first characterization of the subtype of K^+ channels involved in the impaired aortic vasodilation in this model of hypertension.

TEA was preliminarily used to evaluate the overall contribution of K^+ channels on ACh-induced relaxation. We observed that TEA significantly reduced ACh vasodilation in aortic rings from 2K rats and failed to alter this response in vessels from 2K-1C rats, abolishing the differences between groups. Considering that K_{Ca} channels have a dynamic role in the control of arterial smooth muscle tone, by serving as a feedback pathway to regulate the level of cell membrane potential, and anticipating an altered activation of K_{Ca} channels in 2K-1C aortas, we evaluated the effects of other blockers of K_{Ca} channels, such as ChTX and IbTX, a highly selective blocker of BK_{Ca}. An overlapping pattern of inhibition was observed with all three blockers in 2K-1C and 2K aortas, indicating that decreased activity of BK_{Ca} channels contributes to the impaired relaxation induced by ACh in aortas from 2K-1C.

Membrane hyperpolarization and increased cytosolic Ca^{2+} concentration have been reported in endothelial cells stimulated by ACh (Busse et al., 1988; Chen and Cheung, 1992). However, BK_{Ca} blockers have only a marginal effect on cytosolic Ca^{2+} in endothelial cells, indicating that these blockers most likely act on K^+ channels of the vascular smooth muscle cells (Yamanaka et al., 1998; Ghisdal and Morel, 2001). To attribute the effects of BK_{Ca} channel blocker on ACh re-

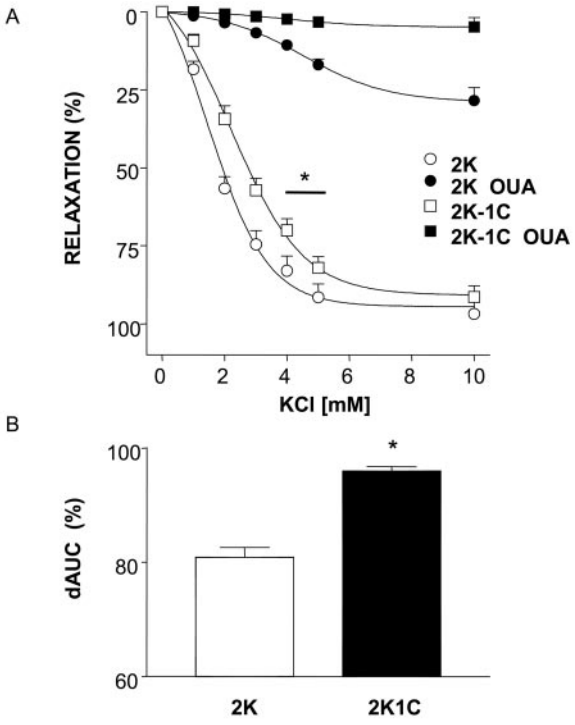

Fig. 7. A, effects of 100 μM OUA on the concentration-response curves to potassium (KCl, 1–10 mM) in phenylephrine-contracted aortas from 2K and 2K-1C rats, after incubation in $[K^+]_o$-free solution. B, dAUC to KCl in 2K and 2K-1C aortas in the presence of OUA. dAUC are means ± S.E.M. and expressed as a percentage of the corresponding AUC in absence of OUA. Differences in the pD_2 values to KCl, in the absence of OUA, or in the AUC are indicated as *, $P < 0.05$, 2K-1C versus 2K.

sponses to actions on aortic vascular smooth muscle, we have shown that IbTX inhibits SNP vasodilation only in endothelium-denuded aortic rings from 2K rats without affecting the response in vessels from 2K-1C rats. Although responses induced by relaxant endothelial factors and nitrovasodilators do not necessarily involve the same population of K^+ channels, our data strongly support the suggestion that changes in BK_{Ca} channels in vascular smooth muscle cells play a role in the impaired relaxation to ACh in aortas from 2K-1C rats. In fact, a reduced vasodilation in response to the activation of K_{Ca} channels with NS1619 was observed in aortas without endothelium from 2K-1C rats.

Changes in the activity of K_{Ca} channels, represented either by increased gene expression or enhanced maximal currents through K_{Ca} channels, have been reported in arterial smooth muscle cells from several hypertensive rat models (Liu et al., 1995, 1997, 1998; Cox et al., 2001a,b). Moreover, Ca^{2+} sensitivity of these channels seems to be increased in hypertension (England et al., 1993). From these observations, it has been postulated that an overactivation of K_{Ca} channels produces hyperpolarization and relaxation of vascular smooth muscle, which may be a compensatory mechanism to regulate cell membrane depolarization and vasoconstriction during acute increases in arterial pressure. However, in the present study, we observed that the vascular changes related to the functional activity of K_{Ca} channels are not similar to those described for most of the experimental models of hypertension. Instead of an increased functional activity of K_{Ca} channels, we found a decreased functional activity of these channels in aortic segments from 2K-1C rats.

The activity of K_{Ca} channels is modulated by membrane potential and intracellular Ca^{2+} concentration, and these two conditions are altered in vessels from 2K-1C rats: 1) cell resting membrane potential is more depolarized in 2K-1C aortic smooth muscle cells than in 2K cells (Callera et al., 2000, 2001); and 2) basal intracellular Ca^{2+} concentration and Ca^{2+} influx are increased in cultured vascular smooth muscle cells from 2K-1C compared with 2K cells (Callera et al., 2001). A decreased expression of the Ca^{2+} sensor, represented by the β1 subunit, in BK_{Ca} channels or a greater threshold for Ca^{2+} represent possible mechanisms involved in the impaired activity of BK_{Ca} channels in aortas from 2K-1C, and further studies are needed to test this hypothesis.

Because small-conductance K_{Ca} channels seem to be involved in endothelium-dependent relaxation, we evaluated the contribution of these channels to ACh vasodilation by using apamin. No inhibitory effects of apamin in ACh relaxation were observed either in 2K or 2K-1C aortas. Considering that BK_{ca} channels play a pivotal role in regulating vascular tone, we evaluated whether the blockade of these channels was required to underscore the contribution of SK_{ca} channels to ACh response in aortic segments from 2K and 2K-1C rats. Surprisingly, the association of apamin and IbTX produced an inhibition of ACh relaxation, compared with the effect of IbTX alone, only in aortic rings from 2K-1C rats. Interestingly, we have observed that the blockade of K_{Ca} channels induces a similar increase in intracellular Ca^{2+} concentration in isolated aortic vascular smooth muscle cells from 2K and 2K-1C rats (data not shown). However, basal intracellular Ca^{2+} levels are higher in cells from 2K-1C. A possible explanation for the results from the apamin and IbTX association is that the higher total amount of intracellular Ca^{2+} after K_{Ca} blockade contributes to the activation of SK in 2K-1C aortas. Based on these results, we suggest that apamin-sensitive K^+ channels contribute to ACh-induced relaxation, instead of being responsible for the impaired ACh response, in aortas from 2K-1C.

4-AP-sensitive K^+ channels are not involved in the decreased ACh-induced relaxation in aortic segments from 2K-1C, because a similar inhibitory effect was observed in both 2K and 2K-1C groups. Another interesting result of our study was that, among the K^+ channel blockers, 4-AP displayed the most potent constrictor activity, evaluated by changes in the arterial basal tone. This observation suggests an increased functional activity of K_V channels in 2K-1C aortas. Our finding was surprising in view of other studies showing that the K_V currents are decreased (Cox et al., 2001b, 2003; Wellman et al., 2001) or not altered (Liu et al., 1995, 1997) in hypertension. An increase in K_V channels activity/expression may be a compensatory response to normalize changes in cell membrane potential and intracellular Ca^{2+} concentration in vascular smooth muscle cells from hypertensive animals, but this remains to be determined.

Considering that an impairment in the relaxant action of ATP-sensitive K^+ channels activators has been described in different vascular beds from hypertensive animals (Ohya et al., 1996; Takaba et al., 1996), we tested the effects of glibenclamide on ACh-induced relaxation in 2K-1C aortas. Because glibenclamide did not affect ACh relaxation in aortas from 2K or 2K-1C, we suggest that K_{ATP} channels may not be tonically active in aortas from these animals.

The sarcolemmal Na^+,K^+-ATPase also plays an essential

role in the maintenance of vascular smooth muscle tone (Marín and Redondo, 1999), and changes in its activity have been associated with abnormalities in endothelium-dependent vasodilation. However, ACh-induced relaxation was reduced by high $[K^+]_o$ solution (Callera et al., 2000), but not by ouabain, in aortas from 2K and 2K-1C, suggesting that Na^+,K^+-ATPase does not contribute to the decreased endothelium-dependent relaxation in 2K-1C. Interestingly, SNP-induced relaxation was inhibited by ouabain only in 2K-1C, suggesting an increased contribution of Na^+,K^+-ATPase to this response. Further support to this suggestion was obtained by evaluating the functional activity of Na^+,K^+-ATPase, which is increased in 2K-1C aortas. Our data are consistent with other studies that indicate an increased activity of the pump in arterial smooth muscle cells from renal hypertensive rats (Overbeck and Grissette, 1982; Myers et al., 1987). Furthermore, the relaxant effect of K^+, after incubation in $[K^+]_o$ free solution, indicates an impaired ability for repolarization in 2K-1C aortas, which is not related to a decreased Na^+,K^+-ATPase activity.

In summary, the present study emphasizes that the processes underlying impaired vasodilation in hypertension extend beyond the endothelium and that the vascular smooth muscle is of importance in aortas from 2K-1C hypertensive rats. Our results provide the first evidence that a decreased activity of BK_{Ca} channels plays a critical role in the reduced vascular smooth muscle relaxation in aortas from 2K-1C rats. Because K^+ channels display a critical role in cellular signaling processes regulating vascular smooth muscle function, further characterization of the molecular changes involved in the altered activity of BK_{Ca} channels in 2K-1C aorta rats is of importance.

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