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ERECTILE FUNCTION IN TWO KIDNEY-ONE CLIP HYPERTENSIVE RATS IS MAINTAINED BY A POTENTIAL INCREASE IN NITRIC OXIDE PRODUCTION

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Abstract

Introduction—Hypertension is closely associated with erectile dysfunction (ED) as it has been observed in many experimental models of hypertension. Additionally, epidemiological studies show that approximately a third of the hypertensive patients have ED.

Aim—To test the hypothesis that the two-kidney one-clip (2K-1C) rat model of hypertension displays normal erectile function due to increased nitric oxide (NO) production in the penis.

Methods—Ganglionic-induced increase in intracavernosal pressure (ICP)/mean arterial pressure (MAP) ratio was used as an index of erectile function in 2K-1C and in normotensive sham-operated (SHAM) anesthetized rats. Cavernosal strips from hypertensive and normotensive rats were used for isometric tension measurement. The contraction induced by alpha-adrenergic agonist phenylephrine, and the relaxation induced by the nitric oxide (NO) donor sodium nitroprusside (SNP) and by the Rho-kinase inhibitor Y-27632 were performed in the absence and in the presence of the nitric oxide synthase (NOS) inhibitor L-NNA.

Results—Changes in ICP/MAP induced by ganglionic stimulation were not different between 2K-1C and SHAM rats. The contractile response induced by phenylephrine as well as the relaxation induced by SNP or the Y-27632 were similar in cavernosal strips from both groups. However, in the presence of L-NNA, the relaxation induced by Y-27632 was significantly impaired in 2K-1C compared to SHAM).

Conclusions—These data suggest that hypertension and ED could be dissociated from high levels of blood pressure in some animal models of hypertension. Erectile function in 2K-1C hypertensive rats is maintained in spite of the increased Rho-kinase activity by increased NO signaling.

Keywords

Two kidney-one clip hypertension; Rho-kinase; Nitric Oxide; Erectile Function

Introduction

Erectile dysfunction (ED) affects more than 30 million men in the United States and it is estimated to affect more than 150 million men worldwide.¹⁻² Endothelial dysfunction and impaired nitric oxide (NO) production are considered major players in cardiovascular diseases such as hypertension³⁻⁴ and ED.⁵⁻⁶

A pivotal role for NO as the central component of the major signal transduction system that acts in the penis to mediate the erectile response is widely accepted.⁷⁻⁹ NO synthase (NOS)-containing nerve fibers associated with penile arteries and cavernosal tissue, and the endothelium lining the sinusoids are the sources of NO responsible for relaxation and consequently penile erection.⁷⁻⁹

Studies from our group and others have shown that several models of experimental hypertension, including deoxycorticosterone acetate (DOCA)-salt, spontaneously hypertensive rats (SHR), stroke prone SHR (SHRSP), and angiotensin II-induced hypertension,¹⁰⁻¹³ are associated to some extent to endothelial dysfunction due to impaired NO/cGMP signaling pathway. The findings from these experimental models, corroborated by clinical evidence,¹⁴⁻¹⁶ indicate correlation between the incidence of cardiovascular disease and ED.

Hypertension has been also associated with increased RhoA/Rho-kinase pathway activity,¹⁷ a calcium-sensitizing mechanism favoring vasoconstriction.¹⁸ We have demonstrated the contribution of the RhoA/Rho-kinase pathway to the maintenance of the non-erect state/constricted state,¹⁹ and that increased Rho-kinase activity in SHRSP contributes to ED.¹³ One of the mechanisms behind NO-mediated vasodilation involves Rho-kinase inhibition.²⁰ In fact, we have demonstrated that NO inhibits Rho-kinase-induced cavernosal vasoconstriction during erection.²¹

Impaired endothelium-dependent relaxation appears to be involved in many models of hypertension, including the renovascular model two-kidney one-clip (2K-1C).²²⁻²³ However, alterations in smooth muscle membrane potential rather than alteration in NO production seems to be the culprit for such impairment in this model.²² Additionally, a strong body of evidence reveals increased NO production as an attempt to buffer hypertension in 2K-1C animals.²⁴⁻²⁸ Such increase in NO production observed in several vascular beds from 2K-1C hypertensive animals may not completely prevent the high blood pressure, but could serve as a protective vascular mechanism that significantly contributes to preserve the erectile function in this model.

Aims

To test the hypothesis that two-kidney one-clip (2K-1C) hypertensive rats do not exhibit impaired erectile response due to increased nitric oxide (NO) signaling in the penis to counteract the Rho-kinase activity, experimentally dissociating hypertension from ED.

Methods

Animals

2K-1C rats were prepared as described previously.²⁹ Briefly, Sprague Dawley rats (200-224 g; Harlan, IN-USA) were anesthetized by ketamine/xylazine (87 and 13 mg/Kg, respectively) and a silver clip (0.2 mm internal diameter) was placed on the left renal artery. Control age-matched rats were submitted to similar procedures with the exception of the silver clip placement. Rats were maintained on a 12h light/dark cycle with food and water *ad libitum* for 6 weeks when *in vivo* erectile function and isolated cavernosal contractility

studies were performed. Systolic blood pressure (SBP) was measured weekly by tail-cuff, and the animals were considered hypertensive if SBP was higher than 160 mmHg at the end of 6th week of implantation of the silver clip or sham surgery. All procedures were conducted in accordance with the Medical College of Georgia's Animal Use for Research and Education Committee.

In Vivo Measurement of Erectile Responses

To compare the erectile function of 2K-1C hypertensive with SHAM normotensive rats, the *in vivo* model of erection in anesthetized animals was used. Rats were anesthetized by ketamine/xylazine and maintained on supplemental doses as needed. Mean arterial pressure (MAP) and intracavernosal pressure (ICP) were continuously and simultaneously monitored as previously described.⁵ Briefly, the left carotid artery was cannulated for MAP recording. To monitor ICP, the shaft of the penis was denuded of skin and fascia, and a 30-gauge needle was inserted into the right corpus cavernosum. The abdominal cavity was opened, exposing the major pelvic ganglion (MPG). Bipolar electrodes connected to a Grass stimulator (Astro-Med, USA) were positioned on the MPG. Electrical stimulation of the MPG (5-ms pulse, 30 s, 12 Hz) at different voltages (1 to 5 V) was performed. All pressure data were collected for analysis using a PowerLab/8SPTM data acquisition system.

Isometric Force Generation in Cavernosal Strips

Under pentobarbital anesthesia (50 mg/Kg, i.p.), rats were killed by pneumothorax and the penis was excised, as previously described.⁵ After removal of the ventral corpus spongiosum, dorsal vein and surrounding connective tissue, the right and left corpora cavernosa were obtained. Each strip was longitudinally mounted in an organ chamber for isometric tension recordings and bathed in physiological salt solution (PSS) of the following composition in mM: NaCl 130.0, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 1.6, NaHCO₃ 14.9, EDTA 0.03 and glucose 5.5, which was maintained at 37°C and bubbled with 95% O₂ and 5% CO₂. Cavernosal strips were stretched to 300 mg passive tension and changes in isometric force were recorded using a PowerLab/8SPTM data acquisition system (AD Instruments, USA). After 1-hour equilibration period in the presence of indomethacin (10⁻⁵M) the contractility studies were performed. Cavernosal smooth muscle contraction to alpha adrenergic receptor activation was tested through concentration-responses curves to phenylephrine (10⁻⁷ to 3×10⁻⁴M). NO-dependent relaxation was evaluated via concentration-response curves to sodium nitroprusside (SNP, 10⁻⁸ to 10⁻⁴M) in strips previously contracted by phenylephrine (10⁻⁵M). Rho-kinase signaling and/or nitric oxide formation were compared through relaxation curves to a Rho-kinase inhibitor (Y-27632, 10⁻⁸ to 10⁻⁵M), performed in strips contracted by phenylephrine (10⁻⁵M), either in the absence or in the presence of a NOS inhibitor [N^ω-nitro-L-arginine (L-NNA), 10⁻⁴M]. The cavernosal strips weight/length ratio from SHAM and 2K-1C rats were similar (data not shown). Each strip was only used once for a single EFS or drug-induced relaxation.

Drugs

Indomethacin, phenylephrine, sodium nitroprusside (SNP), and N^ω-nitro-L-arginine (L-NNA) were purchased from Sigma Chemicals, USA. The Y-27632 compound [(R)-(+)-trans-N-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide] was obtained from Calbiochem, USA.

Statistics

The data are expressed as mean (SEM). Single comparisons between groups (SBP and - LogEC₅₀) were performed using two-tailed unpaired *t* test. Multiple comparisons among

groups were analyzed by one-way ANOVA followed by post hoc Student-Newman-Keuls tests. Differences were considered significant at $P < 0.05$.

Results

Blood Pressure

Figure 1A shows the weekly SBP measurements obtained by tail-cuff method during the period of 6 weeks after the surgery. At the 6th week, the BP was significantly higher in 2K-1C (210 ± 9 mmHg, $n=6$) than in SHAM (146 ± 4 mmHg, $n=5$; $P < 0.05$) rats.

Erectile responses

Electrical stimulation of the MPG induced a voltage-dependent (1-5 V) and sustained increase in ICP/MAP ratio as shown in Figure 1B. Changes in ICP/MAP upon ganglionic stimulation in 2K-1C hypertensive was similar to that obtained in SHAM normotensive rats (Figure 1C).

Cavernosal smooth muscle reactivity studies

Phenylephrine induced concentration-dependent contraction observed in cavernosal strips from 2K-1C and SHAM-operated rats were similar (Figure 2A). SNP induced comparable concentration-dependent relaxation curves in cavernosal smooth muscle from 2K-1C and SHAM rats (Figure 2B). Treatment with L-NNA did not affect the contractile response to phenylephrine or the relaxation induced by SNP in strips from both SHAM and 2K-1C rats (data not shown).

Rho-kinase and nitric oxide signaling studies

In the absence of L-NNA, Y-27632 induced similar concentration-dependent relaxation in cavernosal strips from 2K-1C and SHAM rats ($-\text{LogEC}_{50}$ 5.84 ± 0.10 vs 5.63 ± 0.07 , Figure 3A). L-NNA had no significant effect on Y-27632-induced relaxation in corpus cavernosum from SHAM rats ($-\text{LogEC}_{50}$ 5.63 ± 0.07 and 5.49 ± 0.06 , control vs L-NNA treated, Figure 3B). On the other hand, L-NNA treatment significantly inhibited Y-27632-induced relaxation in 2K-1C rat cavernosal smooth muscle ($-\text{LogEC}_{50}$ 5.84 ± 0.10 and 5.43 ± 0.03 , control vs L-NNA treated $p < 0.05$, Figure 3C).

Discussion

We are the first to present experimental evidence that ED does not occur in all experimental forms of hypertension. Cardiovascular risk factors have been shown to be correlated with impaired sexual function. In fact, most of the studies in the literature using experimental models of hypertension associate this vascular pathology with ED.^{10-13, 30-32} However, in the human population, only a third of hypertensive patients display significant degree of ED.^{14, 15, 33} Our findings show that renovascular hypertensive 2K-1C rats developed normal erectile responses.

The functional states of penile flaccidity, tumescence, erection and detumescence are dependent on the balance between contractile and relaxant factors, controlled by central and peripheral mechanisms.³⁴ Most of the time the penis is in the flaccid state. Contraction of the cavernosal smooth muscle has an important role in maintaining the penis in the flaccid state by minimizing intracavernosal blood flow. Carneiro et al.³⁵ have shown that contractile responses induced by electrical field stimulation or phenylephrine are enhanced, whereas the myosin light chain phosphatase activity is reduced (suggestive of activated Rho-kinase signaling) in cavernosal tissues from DOCA-salt hypertensive rats, contributing to the severe ED observed in this particular model of hypertension. Interestingly, in the present

study, we observed that the contractile responses to the alpha-adrenergic agonist phenylephrine were not altered in 2K-1C rats, justifying in part the preservation of normal erectile function observed in these renovascular hypertensive rats.

Despite the importance of several substances, such as noradrenaline, angiotensin II and endothelin-1, it has been increasingly recognized that the RhoA/Rho-kinase signaling pathway plays a crucial role in the control of the contracted (flaccid) state of the penis.^{13, 34, 36} We observed corpus cavernosum smooth muscle relaxation induced by the Rho-kinase inhibitor Y-27632 in both groups, indicating the importance of this signaling pathway to the control of cavernosal smooth muscle tone. This is in agreement with our previous findings showing the importance of the Rho-kinase pathway to the contraction of rat aorta,³⁷ caeliac artery,³⁸ anococcygeus smooth muscle³⁹ and cavernosal smooth muscle.^{19, 40}

The RhoA/Rho-kinase signaling pathway has been shown to be increased in certain pathological states such as hypertension, diabetes and aging.^{6, 13, 41-45} Administration of Y-27632 to different experimental models of hypertensive rats, such as SHR, DOCA-salt and 2K-1C lowered blood pressure,¹⁷ indicating the contribution of the RhoA/Rho-kinase pathway to the pathophysiology of hypertension.

In the present study, the relaxation induced by Y-27632 appeared to be unaltered in corpus cavernosum of 2K-1C. This finding is in agreement with normal erectile response observed in 2K-1C hypertensive rats. In contrast, increased Rho-kinase signaling has been reported in cavernosal tissue from DOCA-salt, SHRSP and angiotensin II-induced hypertensive rats.¹⁰⁻¹³ In those models of hypertension, the increase in Rho-kinase activity is closely associated with significant impairment of erectile function.¹⁰⁻¹³ However, NOS blockade by L-NNA significantly impaired the relaxation induced by Rho-kinase inhibition in cavernosal strips from 2K-1C rats. A rightward shift in the relaxation curve induced by the Rho-kinase inhibitor in the presence of L-NNA suggests that an increased in Rho-kinase activity was masked by an augmented NO signaling.

An inverse functional relationship between NO and RhoA/Rho-kinase signaling pathways has been established.²¹ We have previously demonstrated that NO mediates penile erection, in part, through the inhibition of endogenous RhoA/Rho-kinase constrictor activity.²¹ These data support an increased role of NO signaling in 2K-1C, possibly attributed to increases in NO signaling in cavernosal tissue in agreement with previous findings in different vasculatures.^{24, 25, 27} This increased NO signaling would contribute to the maintenance of normal erectile response in spite of the hypertension.

Behr-Roussel et al.¹⁰⁻¹³ have shown increased sensitivity or effectiveness of SNP in cavernosal tissue, but not in aorta, from SHR at 12 and 24 week of age. Such tissue specific compensatory up regulation mechanism of the soluble guanylate cyclase pathway could contribute to preserve erectile function in cases of defective endothelium-dependent relaxations or increased vasoconstrictions. That does not seem to be the case in our study. Here we observed that the guanylate cyclase/cGMP pathway is not altered in cavernosal tissue from 2K-1C hypertensive rats since the relaxation induced by the NO donor SNP was not different between tissues from hypertensive and normotensive rats.

Altogether, these data indicate that the lack of ED in 2K-1C hypertension, in spite of the increased Rho-kinase activity in cavernosal tissue, has to be explained by an improvement in the NO production, since the cGMP pathway seems to be unaltered.

In this study, we provided experimental evidence of the dissociation between hypertension and ED. Our findings using this renovascular model of hypertension compliments the results in the human population demonstrating that a third of the hypertensive patients display

impaired erectile function. Whether this is a matter of progression of the hypertension preceding the appearance of ED needs further investigation. These findings support the need of regular and meticulous clinical evaluation of hypertensive patients, as well as early intervention with appropriate anti-hypertensive therapy to prevent target organ damage and to preserve erectile function.

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Figure 1.

A. Systolic blood pressure (mmHg) measured by tail cuff in 2K-1C and SHAM-operated rats 6 weeks after the surgery. **B.** Representative tracings of the changes of MAP (mmHg) and ICP (mmHg) induced by ganglionic stimulation in anesthetized 2K-1C and SHAM rats. **C.** Increase in ICP/MAP induced by ganglionic stimulation in anesthetized 2K-1C and SHAM rats. * $P < 0.05$, one-way ANOVA.

Figure 2.
A. Phenylephrine-induced contraction (mg) of cavernosal strips from 2K-1C and SHAM rats. **B.** SNP-induced relaxation (% of the phenylephrine contraction) of cavernosal strips from 2K-1C and SHAM rats.

Figure 3.

A. Y-27632-induced relaxation (% of the phenylephrine contraction) of cavernosal strips from 2K-1C and SHAM rats. **B.** Y-27632-induced relaxation (% of the phenylephrine contraction) in the absence and in the presence of L-NNA (10^{-4} M) of cavernosal strips from SHAM, and **C.** from 2K-1C rats. * $P < 0.05$, one-way ANOVA.