CYP450 4A Inhibition Attenuates O_2 Induced Arteriolar Constriction in Chronic but not Acute Goldblatt Hypertension

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Abstract

We explored the role of 20-hydroxy-5Z, 8Z, 11Z, 14Z-eicosatetraenoic acid (20-HETE) in oxygen-induced vasoconstriction in a normal renin form of hypertension [the 1 kidney-1 clip Goldblatt hypertensive rat (1K1C)] and a high renin form of hypertension [the 2 kidney-1 clip Goldblatt hypertensive rat (2K1C)]. A silver clip was placed around the left renal artery of adult Sprague-Dawley males. The right kidney was removed in the 1K1C group and left intact in the 2K1C group. Arteriolar responses to elevation of O_2 concentration in the superfusion solution from 0% O_2 to 21% O_2 were determined in the *in situ* cremaster muscle before and after inhibition of cytochrome P450 4A ω-hydroxylase (CYP450 4A) with N-methyl-sulfonyl-12, 12-dibromododec-11-enamide (DDMS). Arteriolar constriction to elevated PO_2 was enhanced in the chronic 1K1C but not the acute 1K1C or 2K1C. DDMS eliminated O_2-induced arteriolar constriction in the 9 week 1K1C, but had no effect in the 2 wk 1K1C, and only partially inhibited O_2-induced constriction of arterioles in the 4 wk 2K1C rat. These findings indicate that although the CYP4A/20-HETE system contributes to arteriolar constriction in response to elevated PO_2 in the established stage of 1K1C renovascular hypertension, physiological alterations in other mechanisms are the primary determinants of O_2-induced constriction of arterioles in the early and developing stages of 1K1C and 2K1C hypertension.

Keywords

renovascular hypertension; 20-HETE; cytochrome P450 ω-hydroxylase; oxygen; cremaster muscle

INTRODUCTION

Arterioles of many vascular beds constrict when exposed to elevated oxygen levels (Bak et al., 2007; Gilmore et al., 2005; Kolbitsch et al., 2002; Mak et al., 2002; Rossi and Boussuges, 2005; Rousseau et al., 2007; Sirinyan et al., 2006; Tsai et al., 2003; Yamazaki, 2007). While multiple chemical mediators have been proposed to contribute to vascular responses to changes

in O_2 availability (Cabrales et al., 2006; Drenjancevic-Peric et al., 2003; Frisbee et al., 2001; Frisbee et al., 2002; Jackson et al., 1986; Mak et al., 2002; Messina et al., 1994; Rubanyi and Vanhoutte, 1986; Sirinyan et al., 2006), a number of recent reports suggest that 20-hydroxy-5Z, 8Z, 11Z, 14Z-eicosatetraenoic acid (20-HETE), a vasoactive metabolite of arachidonic acid produced by the enzymatic activity of the cytochrome P450-4A ω-hydroxylase (CYP450 4A) family of enzymes, is a likely candidate to serve as a tissue oxygen sensor (Harder et al., 1996; Kunert et al., 2001a; Kunert et al., 2001b; Lombard et al., 2004; Lombard et al., 1999).

There are multiple reports of enhanced vasoconstrictor sensitivity to elevated PO_2 in arterioles of animals with genetic and experimentally-induced hypertension compared to their respective normotensive controls (Drenjancevic-Peric et al., 2003; Kunert et al., 2001b; Lombard et al., 1984; Lombard et al., 1986; Lombard and Stekiel, 1988; Rafi and Boegehold, 1993). Because angiotensin II (ANG II) has been proposed to increase the formation and release of 20-HETE (Alonso-Galicia et al., 2002; Carroll et al., 1997; Carroll et al., 1996; Croft et al., 2000), the present study investigated the role of 20-HETE in O_2-induced constriction of cremasteric arterioles in non-genetic models of normal renin hypertension ([the 1 kidney-1 clip Goldblatt hypertensive rat (1K1C)] and high-renin hypertension [the 2 kidney-1 clip Goldblatt hypertensive rat (2K1C)].

MATERIALS AND METHODS

Experimental Animal Groups

Male Sprague Dawley rats were anesthetized with sodium pentobarbital (60mg/kg). The left kidney was accessed through a left lateral incision and a 0.20 mm silver clip was placed around the left renal artery. In the 1K1C rat, the right kidney was accessed through a right lateral incision and removed after tying off the renal artery and vein. 1K1C rats and their sham operated controls (SOC) were studied 2 wks post surgery (1K1C, 11±0.2 wks, 311±7 g, n=17 and SOC, 11±0.3 wks, 317±7 g, n=18) and 9 weeks post surgery (1K1C 19±0.4 wks, 413±10 g, n=23 and SOC 18±0.3 wks, 423±7 g, n=18). 2K1C rats and their SOC were studied 4 weeks post clipping (2K1C 11±0.4 wks, 349±9 g, n=13 and SOC 12±0.4 wks, 364±9 wks, n=13). All rats were housed with free access to food and water in an animal care facility at the Medical College of Wisconsin (MCW), which is approved by the American Association for Accreditation of Laboratory Animal Care. All protocols were approved by the MCW IACUC.

In Vivo Experiments

On the day of the experiment, the rats were anesthetized with sodium pentobarbital (60mg/kg, i.p.), and an *in situ* transilluminated cremaster muscle was prepared for measurement of internal diameters of third-order arterioles via television microscopy, as described previously (Baez, 1973; Hill et al., 1990). The tissue was superfused at 35°C at a rate of 3–5ml/minute with a bicarbonate-buffered (pH 7.35) physiological salt solution (PSS), equilibrated with 0% O_2, 5% CO_2, 95% N_2 gas mixture, to ensure that O_2 delivery to the tissue was controlled entirely by the microcirculation and that no O_2 was delivered from the superfusate. Under these conditions, PO_2 in the rapidly flowing layer of the superfusate is 3–5mmHg, while tissue PO_2 is higher, due to O_2 supply from the microcirculation. Arterioles for study were selected by indentifying a second-order arteriole in a clearly visible region of the cremaster muscle and tracking along its length to find a third order arteriolar branch that was located in a region of the muscle that was away from any incision, had clearly discernible vessel walls, a brisk flow velocity, and active tone, as verified by the occurrence of a brisk dilation following topical application of 10^{-4} M adenosine.

Evaluation of Vascular O_2 Sensitivity

After a 30 minute to 1 hour equilibration period, control measurements of arteriolar diameter and mean arterial pressure (carotid artery cannula) were obtained each minute for 5 minutes during 0% O_2 superfusion. Arteriolar responses to increased O_2 availability were then tested by measuring arteriolar diameters for 10 minutes after equilibrating the superfusion solution with a 21% O_2, 5% CO_2, 74% N_2 gas mixture. This gas mixture causes a significant elevation of tissue and periarteriolar PO_2, although not to the same extent as the elevation in superfusate PO_2 (Duling and Berne, 1970), and has been used in previous studies testing arteriolar O_2 sensitivity (Frisbee et al., 2000). In those studies, arteriolar responses to smaller elevations in superfusate oxygen concentration (5% O_2 and 10% O_2) were also potentiated in animals with reduced renal mass hypertension, and were sensitive to inhibition of 20-HETE production (Frisbee et al., 2000). After exposure to the 21% O_2 solution, the superfusate was re-equilibrated with the control (0% O_2) gas mixture until vessel diameters recovered to their control values. The preparation was then superfused for 30 minutes at 0.33 mL per minute with warmed PSS containing a 50 µM solution of the selective cytochrome P450 4A ω-hydroxylase inhibitor, N-methylsulfonyl-12, 12-dibromododec-11-enamide (DDMS) (Alonso-Galicia et al., 1997) or its vehicle (a 0.1% solution of absolute ethanol added to PSS), followed by continuous superfusion with PSS containing a 1 µM maintenance concentration of DDMS (DDMS treated animals only) for the remainder of the experiment. After application of the DDMS, the preparation was superfused again with 0% O_2 solution at the control rate of 3–5 ml/min, after which arteriolar responses to elevated PO_2 were re-evaluated. Vessel responses to 10^{-7} M norepinephrine were also tested to verify the ability of the arteriole to respond to vasoconstrictor stimuli, e.g., in vessels where inhibition of the CYP4A system eliminated O_2-induced constriction of the arterioles.

Statistical Analysis

In order to determine the influence of the treatment factor and the surgical factor on the results, data was statistically analyzed with a two way ANOVA with repeated measures and a Bonferroni post hoc test (Figure 2 and Figure 3, GraphPad Prism) and were summarized as means ±SEM. Data for Figure 1 (arteriolar constriction to 21% oxygen in all groups before any treatment) was statistically analyzed with a one way ANOVA with a Student Newman-Keuls post hoc test. A $p < 0.05$ was considered to be statistically significant.

RESULTS

Arterial blood pressures in the 1K1C (2 and 9 wk) and 2K1C (4 week) rats were significantly higher than those of their respective sham-operated controls (Table 1). Treatment with vehicle only or DDMS (Table 1) had no effect on the resting diameters (0% O_2 superfusion) of arterioles in the sham or experimental rats, 2, 9 or 4 weeks post surgery (Table 1). Treatment with vehicle alone also had no effect on arteriolar responses to elevated PO_2 in any of the experimental groups (data not shown for clarity).

Arteriolar constriction in response to elevated PO_2 in 1K1C rats (prior to vehicle or any treatment) was similar to sham-operated controls 2 weeks post-clipping, but was significantly larger in the hypertensive animals 9 weeks post-clipping compared to their age-matched sham-operated controls (Figure 1). DDMS eliminated arteriolar constriction during 21% O_2 superfusion in the 1K1C rats 9 weeks post clipping and in all sham operated controls, but had no effect on O_2-induced constriction of arterioles in 1K1C rats 2 weeks post clipping (Figure 2). The alternative CYP-4A inhibitor 17-octadecynoic acid (17-ODYA, 10 µM) had identical effects on arteriolar O_2 responses in both groups of 1K1C rats and their sham operated controls, verifying that the failure of DDMS to eliminate O_2-induced constriction in arterioles of 2 week 1K1C rats was not due to ineffectiveness of the inhibitor (data not shown). In 2K1C rats,

arteriolar constriction in response to elevated PO_2 was similar to that in the sham-operated controls (Figure 1). DDMS abolished arteriolar constriction in response to 21% O_2 superfusion in the sham-operated controls, and reduced, but did not eliminate O_2-induced constriction of arterioles in the 2K1C rats 4 weeks post-clipping (Figure 3).

DISCUSSION

Plasma renin activity (PRA) is normal in 1K1C hypertensive rats after a brief rise immediately post clipping (Ayers et al., 1977; Murphy et al., 1984), but is substantially elevated (2.5–7 times normal) 4 weeks after clipping in the 2K1C model (Amiri and Garcia, 1997; Murphy et al., 1984; Russell et al., 1983; Welch et al., 2007; Wilcox et al., 1996). Therefore, the design of the present study can provide insight into the possible influence of the renin-angiotensin system on vascular responses to elevated PO_2, and the ability of cytochrome P450 inhibition to modify that response in 1K1C and 2K1C renovascular hypertension. This is important because circulating ANG II levels have been shown to play a crucial role in maintaining normal vascular relaxation in response to reduced PO_2 and other vasodilator stimuli (Lombard et al., 2003; McEwen et al., 2009; Phillips and Lombard, 2005).

In this study, arteriolar responses to elevated PO_2 were enhanced in 1K1C rats 9 weeks after clipping (Figure 1). However, cremasteric arterioles of 2 week 1K1C (Figure 1) and 4 week 2K1C (Figure 2) rats did not show an enhanced O_2 response compared to their sham-operated controls, even though arterial blood pressure was significantly elevated in both models of hypertension (Table 1). The lack of an enhanced response to elevated PO_2 in these groups is surprising, and contrasts with the enhanced constriction to elevated PO_2 in multiple models of hypertension including SHR with developing hypertension (Kunert et al., 2001b;Lombard et al., 1986), Dahl SS rats (Rafi and Boegehold, 1993), and rats in the early and established stages of reduced renal mass (RRM) hypertension (Lombard et al., 1989). To the best of our knowledge, this is the first documented case of a hypertensive rat model in which O_2-induced constriction of arterioles is not enhanced early in hypertension. While the reason for this difference is not clear, it is possible that the neural, hormonal, or local mechanisms that potentiate O_2-induced constriction of arterioles in other forms of hypertension are not altered sufficiently to enhance arteriolar O_2 responses in either the 1K1C or 2K1C rats.

In contrast to sham operated controls and 1K1C rats 9 weeks post clipping, inhibition of the CYP450 4A/20-HETE system with DDMS did not affect O_2-induced constriction of arterioles in the 2 week 1K1C rat (Figure 2) and only partially inhibited O_2 induced constriction of arterioles in the 4 week 2K1C rats (Figure 3). These observations suggest that factors other than the CYP-4A/20-HETE system contribute to O_2-induced vasoconstriction of arterioles in the earlier phase of 1K1C or 2K1C Goldblatt hypertension, while this metabolic pathway eventually plays a role in the enhanced response to elevated PO_2 in the established stage of 1K1C Goldblatt hypertension. This finding is similar to that of a previous study showing that inhibition of cytochrome P-450 4A ω-hydroxylase with two mechanistically different inhibitors, 17-octadecynoic acid (17-ODYA) and N-methyl-sulfonyl-12, 12-dibromododec-11-enamide (DDMS) attenuated arteriolar constriction to elevated PO_2 in mature (12 week old) SHR, but not in young (6 week old) SHR (Kunert et al., 2001b), suggesting that 20-HETE mediates the oxygen-induced constriction of arterioles in SHR with established hypertension, but not in young SHR prior to the development of established hypertension.

The differential response to 20-HETE inhibition after different durations of 1K1C hypertension appears to be related to vascular alterations occurring during the development of hypertension, rather than age *per se*, because cytochrome P450 inhibition eliminated O_2-induced constriction of arterioles in age-matched sham-operated controls for all the groups (Figure 2 and Figure 3).

This reduced contribution of CYP-4A enzymes and 20-HETE to arteriolar constriction in the 2 week 1K1C rats and the 2K1C rats may involve mechanisms similar to those reported by Marvar et al. (Marvar et al., 2007), who found that chronic exposure to elevated dietary salt intake led to a reduction in arteriolar sensitivity to elevated PO_2 that was mediated by a reduced responsiveness of the arterioles to 20-HETE.

Since plasma renin activity is normal in the early 1K1C rat but high in the 4 week post clipping 2K1C rat (Amiri and Garcia, 1997), plasma renin levels do not seem to explain the differential response to inhibiting 20-HETE production in these models. The latter finding is consistent with studies of SS rats versus consomic and congenic controls, where normalization of PRA and circulating ANG II levels by introgression of the Brown Norway (Drenjancevic-Peric et al., 2005) or Dahl R renin gene (Drenjancevic-Peric et al., 2004) does not affect vascular oxygen responses or prevent the enhanced constriction of cremasteric arterioles in response to elevated PO_2 in salt-fed animals (even though it restores normal vascular relaxation in response to acetylcholine).

In summary, the CYP450 4A/20-HETE system plays a role in the enhanced O_2-induced vasoconstriction in 1K1C rats with established hypertension. However, O_2-induced vasoconstriction is not enhanced in the acute phases of the 1K1C (normal renin) and the 2K1C (high renin) models of renovascular hypertension. Inhibition of cytochrome P450 ω-hydroxylase does not affect O_2-induced constriction of skeletal muscle arterioles of rats in the acute phase of 1K1C renovascular hypertension and only partially inhibits arteriolar constriction in response to elevated PO_2 in animals with 4 weeks of 2K/1C hypertension, but eliminates arteriolar O_2 responses in the established phase of 1K1C hypertension. Taken together, these findings indicate that 20-HETE plays a role in the oxygen response during steady state renovascular hypertension, but that other factors dominate during the transition to a stabilized hypertensive state.

Acknowledgments

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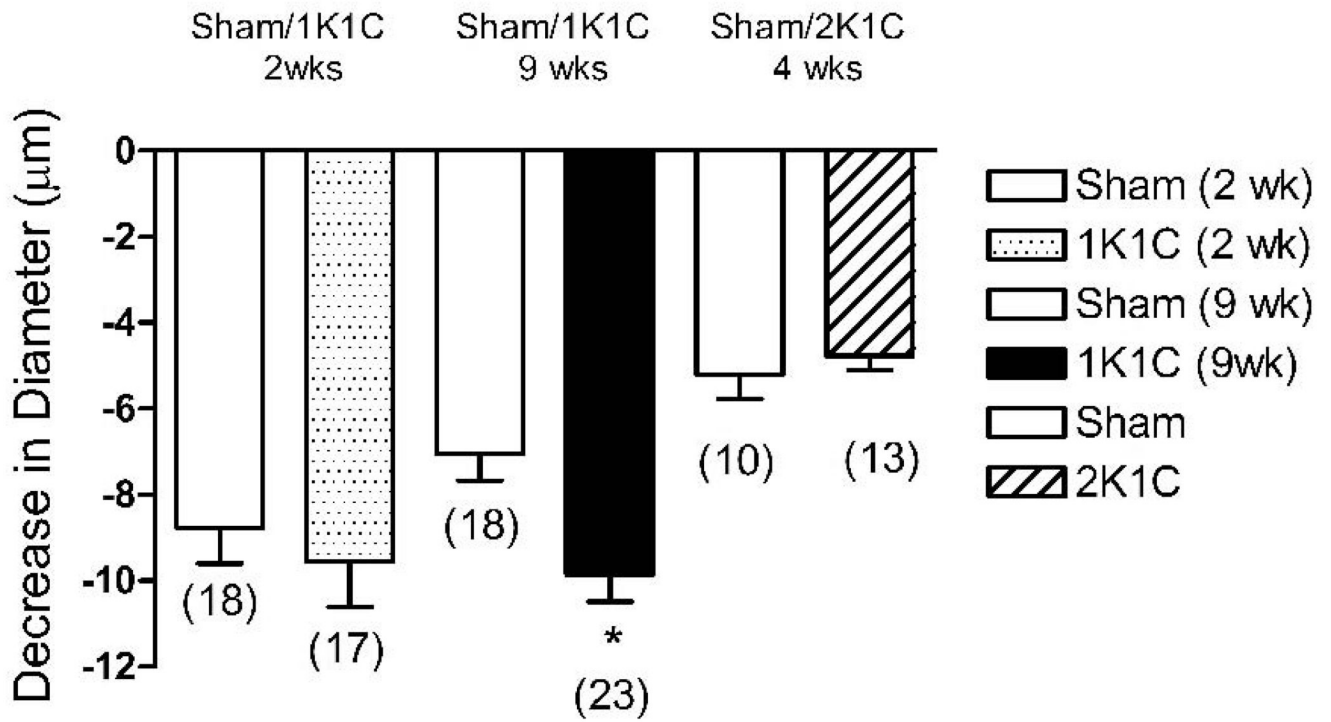**Figure 1.**
Decrease in cremasteric arteriolar diameter from control (0% O_2 superfusion) in response to superfusion with 21% O_2 PSS before any treatment in all 1K1C rats 2 weeks post clipping (stippled bar), all 1K1C rats 9 weeks post clipping (black solid bar) and all 2K1C rats 4 weeks post clipping (slanted line bar) versus all their respective sham-operated controls (open bars). Parentheses indicate number of animals in each group; * = $p < 0.05$, significantly different from matched sham prior to any treatment.
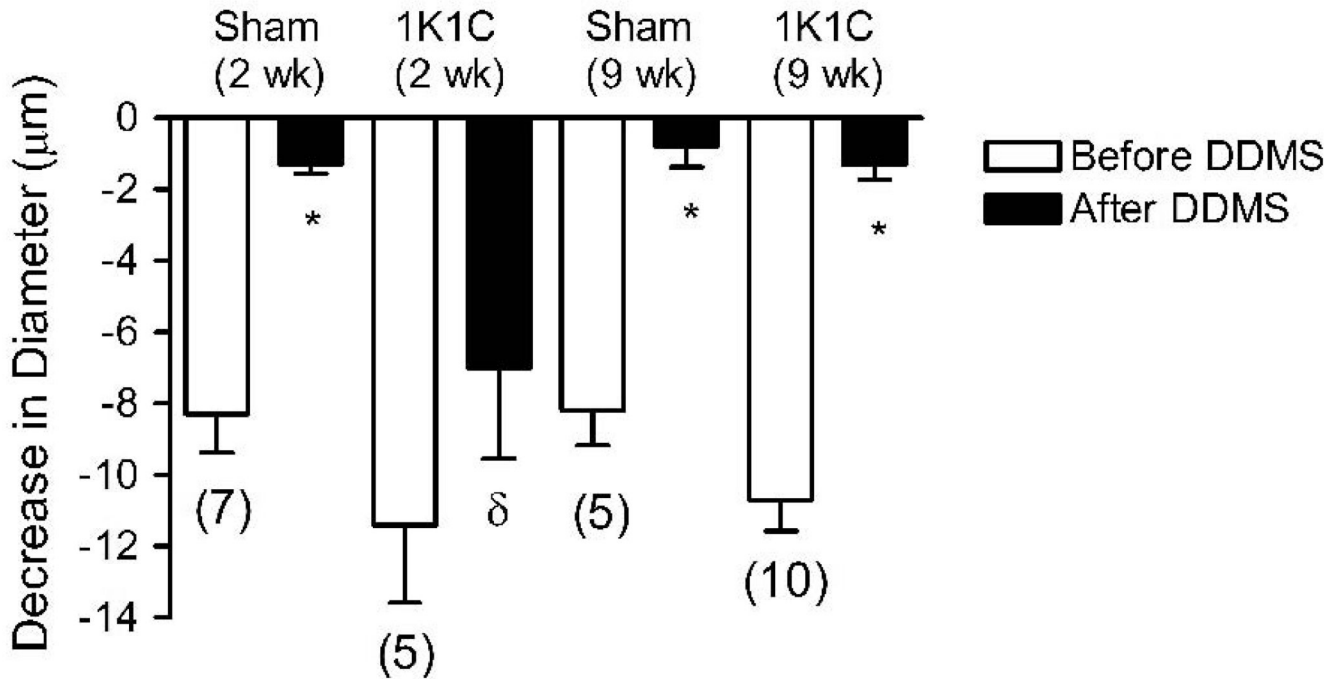
Figure 2.
Decrease in cremasteric arteriolar diameter during superfusion with 21% O_2 PSS before (open bars) and after (black solid bars) the application of DDMS in 1K1C rats 2 weeks post clipping and 9 weeks post clipping versus their respective sham-operated controls (open and slant-lined bar, respectively). Parentheses indicate number of animals in each group; * = within group, significantly different (p<0.01) from the response prior to treatment with DDMS; δ=between groups, significantly different (p<0.05) from sham-operated control with same treatment.
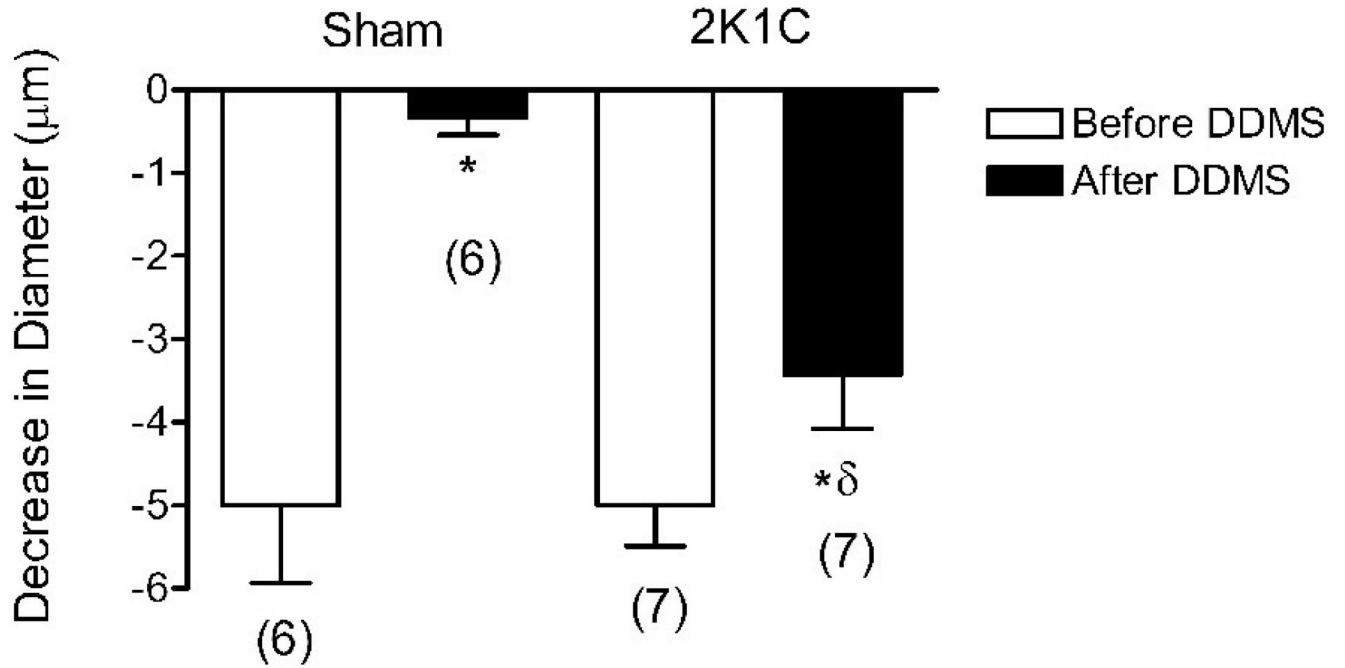
Figure 3.
Decrease in cremasteric arteriolar diameter during superfusion with 21% O_2 PSS before and after application of DDMS in 2K1C rats 4 weeks after clipping the left renal artery versus their sham-operated controls. Parentheses indicate number of animals in each group; * = within group, $p<0.05$ (2K1C); $p<0.001$(sham), significantly different from response prior to treatment with DDMS, $\delta = p<0.01$, between groups, significantly different from sham with same treatment.

Table 1
Mean arterial pressures and arteriolar diameters in 1K1C and 2K1C rats

First Column: Mean arterial pressure in 1K1C rats (2 and 9 weeks post clipping) and 2K1C rats (4 weeks post clipping) versus mean arterial pressure in their respective sham-operated controls. The pressures represent averages of blood pressure measurements taken via the carotid artery in each anesthetized rat throughout the experimental protocol. Brackets indicate number of animals in each group * = p<0.05, mean arterial pressure significantly different from sham-operated controls. **Third through Fifth Column:** Diameter (µm) in cremasteric arterioles of 1K1C, 2 and 9 weeks post clipping, 2K1C, 4 weeks post clipping and in their respective sham-operated controls at rest (initial post-equilibration diameter) and before and after the application of DDMS with 0% oxygen in the superfusion solution (PSS). Vehicle alone had no effect on resting diameters (data not shown to improve clarity).

Mean Arterial Pressure (mmHg±SE) ["n"]	Animal Group	Arteriolar Diameter (0% O_2 Superfusion)		
		Initial Diameter (µm±SE)	Before DDMS (µm±SE)	After DDMS (µm±SE)
160±6 [17]	1K1C (2 wk)	24±0.5	25 ±1.0	26±1.0
121±4 [17]	Sham (2 wk)	25±0.7	24±1.0	25±1.0
166±3 [23]	1K1C (9 wk)	24±0.6	24±0.7	24±1.0
129±4 [18]	Sham (9 wk)	23±0.6	23±1.0	23±1.0
160±3 [23]	2K1C (4 wk)	20±1.3	20±1.3	20±1.3
132±5 [21]	Sham (4 wk)	20±0.9	19±1.4	20±1.3