Decrease in Hypothalamic Norepinephrine Content Following Renal Denervation in the One-Kidney, One Clip Goldblatt Hypertensive Rat

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SUMMARY Peripheral and central sympathetic mechanisms have been shown to contribute to the development and maintenance of increased blood pressure in the one-kidney model of renal hypertension in the rat. Previous studies from our laboratory have demonstrated that the renal sympathetic nerves, in particular, contribute to the maintenance of hypertension in this model. In those studies, renal denervation, performed 2 weeks after renal artery clipping, resulted in a significant decrement in blood pressure that was associated with a decrease in peripheral sympathetic activity. To further define the role of the renal nerves in the pathogenesis of hypertension in this model, we determined the systolic blood pressure and norepinephrine and dopamine content of the hypothalamus, midbrain, pons medulla, and spinal cord at 1 week following renal denervation or sham operation of rats with early established one-kidney one clip hypertension. Age-matched uninephrectomized rats were controls. The blood pressure of denervated animals decreased significantly from 189 ± 9.21 to 151 ± 6.5 mm Hg (p < 0.001), while that of sham-operated animals did not change. Hypothalamic norepinephrine content of sham-operated animals was significantly greater than that of controls (2.24 ± 0.8 μg/g sham vs 1.84 ± 0.12 μg/g controls, p < 0.01). Renal denervation resulted in a decrease in hypothalamic norepinephrine content to control levels (1.72 ± 0.11 μg/g). There was a significant (r = 0.65, p < 0.01) positive correlation between systolic blood pressure and hypothalamic norepinephrine content of renal-denervated and sham-operated animals. The norepinephrine content of other brain regions was not different between groups. The results suggest that the renal nerves contribute to the maintenance of hypertension in the one-kidney one clip rat by modulating central sympathetic nervous system activity.

KEY WORDS • renal hypertension • renal afferent nerves • central noradrenergic nervous system

Evidence from a number of laboratories indicates that increased activity of the sympathetic nervous system is important in the pathogenesis of hypertension in the one-kidney one clip Goldblatt model of renal hypertension in the rat. Plasma norepinephrine, used as an index of peripheral sympathetic activity, has been found to be elevated during the established phase of hypertension in this model. Further, central sympatholytic interventions, including treatment with intracisternal 6-hydroxydopamine (6-OHDA) or lesioning of the periventricular tissue surrounding the anteroventral third ventricle (AV3V) or ventromedial-median eminence regions of the hypothalamus, have been reported to prevent the development of hypertension, and posterior hypothalamic lesions have also been shown to lower blood pressure in the chronic established phase of hypertension in the one-kidney model.

Support for the hypothesis that central noradrenergic mechanisms are involved in the pathogenesis of hypertension in this model comes from the recent findings of Eide et al. that norepinephrine content and tyrosine hydroxylase activity of the hypothalamus are increased 3 weeks after renal artery clipping in uninephrectomized rats. An important finding of that study was the absence of changes in the hypothalamic norepinephrine content or tyrosine...
hydroxylase activity in the two-kidney one clip hypertensive rat despite elevation in blood pressure comparable to that seen in the one-kidney model. This suggests that the elevated hypothalamic norepinephrine levels observed in the one-kidney model are not a compensatory response to increased blood pressure. Further, the observation by the same authors that the changes in norepinephrine content seen in the one-kidney model are paralleled by increases in tyrosine hydroxylase activity indicates that in this model increased norepinephrine stores reflect increased synthesis.

In a recently completed study, we found that renal denervation performed 2 weeks after clipping of the renal artery of uninephrectomized rats resulted in a significant decrease in blood pressure. There was no evidence that the attenuation of hypertension was secondary to interruption of the renal afferent nerves. Later experiments in the same model demonstrated an increase in peripheral sympathetic activity 3 weeks after clipping. Peripheral sympathetic activity returned to control levels following renal denervation. These observations led us to hypothesize that the renal afferent nerves influence the development and maintenance of increased blood pressure in one-kidney renal hypertension by modulating central and thereby peripheral sympathetic activity. The purpose of the present study was to further examine this hypothesis by investigating the effects of renal denervation on blood pressure and central catecholamine stores in the early established phase of hypertension in this model.

Methods

Animals used in this experiment were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. Throughout the study they were housed in a room that had constant temperature (24° ± 1°C) and humidity (60% ± 5%) and was lighted from 6:00 a.m. to 6:00 p.m. Systolic blood pressures were measured in conscious, prewarmed restrained rats using an electrophysymomanometer and physiograph recorder (Narco Biosystems, Incorporated, Houston, Texas).

Male Sprague-Dawley rats (n = 24) underwent unilateral right nephrectomy at 4 weeks of age. The right adrenal gland was left in place. Two weeks were allowed for compensatory hypertrophy of the left kidney. Following determination of baseline blood pressure, 16 animals underwent renal artery clipping. Eight uninephrectomized rats were used as controls. Clipping was accomplished by approaching the kidney through a flank incision and placing a 0.4 mm silver clip on the left renal artery. Systolic blood pressures and weights of all animals were determined weekly thereafter.

Two weeks following clipping, animals were randomly assigned to one of two groups, receiving either renal denervation or sham operation. Renal denervation was performed through a flank incision by stripping the renal adventitia and painting the renal artery with 20% phenol (weight/volume) in absolute alcohol. Sham operation consisted of a flank incision only.

After the final blood pressure determination 1 week after operation, animals were sacrificed by decapitation without anesthesia. Brains and spinal cords were removed and hypothalamus, midbrain, and pons medulla were dissected from the brain according to the method of Glowinski and Iversen. Tissues were frozen immediately in liquid nitrogen and stored at −80°C until the time of assay.

When ready for assay, tissue samples were weighed, homogenized in 0.4M perchloric acid, and spun at 4000 g in a refrigerated centrifuge for 30 minutes. Aliquots of the supernatant were eluted onto alumina at an alkaline pH, desorbed with 0.4M perchloric acid, and the eluate analyzed for norepinephrine and dopamine content by means of high performance liquid chromatography with electrochemical detection (Bioanalytical Systems LC-304) using a modification of the method of Keller et al. Results were expressed as μg/g tissue.

Analysis of differences in blood pressure and tissue catecholamine content between groups was performed using Student’s unpaired t test and within groups using the paired t test. Results were expressed as means ± SEM, and differences were considered significant if the p value was less than 0.05. Linear regression analysis was used to correlate blood pressure with hypothalamic norepinephrine content.

Results

Figure 1 shows the systolic blood pressures for each group of animals during the 3 weeks of the study. Clipping of the renal artery produced a rapid increase in blood pressure from a mean of 124 ± 2.2 mm Hg to a mean of 189 ± 9.4 mm Hg within 2 weeks. Renal denervation (n = 7) performed 2 weeks following renal artery clipping resulted in a significant decrease in blood pressure from 189 ± 9.4 mm Hg preoperatively to 151 ± 6.5 mm Hg postoperatively (p < 0.001). Sham operation produced no change (189 ± 9.4 mm Hg preoperatively vs 195 ± 9.9 mm Hg postoperatively, n.s.). Postoperative mean blood pressure of denervated animals was significantly less than that of shams (p < 0.05) but remained greater than that of uninephrectomized controls (130 ± 3.6 mm Hg, p < 0.01).

When measured 3 weeks after renal artery clipping, hypothalamic norepinephrine content of sham-operated rats was significantly greater than that of uninephrectomized control animals (2.24 ± 0.08 μg/g sham vs 1.84 ± 0.12 μg/g control, p < 0.02) (fig. 2). Renal denervation resulted in a decrease in mean hypothalamic norepinephrine content to control levels (1.72 ± 0.11 μg/g). Figure 3 shows the significant positive correlation between systolic blood pressure at
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FIGURE 1. Effects of renal denervation on blood pressure of the one-kidney one clip rat. Arrow indicates the time of renal denervation or sham operation. Values are means ± SEM.

FIGURE 2. Hypothalamic norepinephrine content of renal-denervated, sham-operated, and control animals. Values are means ± SEM. *p < 0.01, difference from control.

FIGURE 3. Regression analysis comparing systolic blood pressure at the time of sacrifice with hypothalamic norepinephrine content of renal-denervated and sham-operated animals.

Table 1 shows the results of measurements of the norepinephrine content of the midbrains, pons medullas, and spinal cords of the three groups of animals. No significant differences in norepinephrine content between groups was seen in any of these regions.

FIGURE 4. Hypothalamic dopamine content of renal-denervated, sham-operated, and control animals. Values are means ± SEM.
FIG.


demonstrated in the kidney. A variety of stimuli, including alterations in renal artery pressure, renal venous occlusion, ureteral occlusion, compression of the kidney, ischemia, and changes in the ionic composition of the pelvic urine, have been shown to produce alterations in renal afferent nerve activity. \(^{20-29}\) Stimulus of the renal afferents of one kidney has been shown to produce a reflex increase in efferent nerve activity and arteriolar vasoconstriction in the contralateral kidney. \(^{25,26}\) These responses appear to be mediated via supraspinal neurons as they are abolished by spinal cord sectioning.

Further evidence that the changes produced by renal afferent nerve stimulation are mediated via the central sympathetic nervous system comes from electrophysiologic studies demonstrating that stimulation of the renal afferent nerves produces changes in the electrical activity of neurons in several regions of the hypothalamus. \(^{27,28}\) In addition, electrical stimulation of the renal afferent nerves has been shown by several investigators to produce a change in systemic arterial pressure. \(^{29,29,50}\) There is disagreement among laboratories as to whether the response to stimulation

**Discussion**

This study demonstrates that: 1) the norepinephrine content of the hypothalamus is increased in the early established phase of one-kidney one clip renal hypertension; 2) renal denervation in this model results in a marked decrease in blood pressure accompanied by a fall in the hypothalamic norepinephrine content to control levels; and 3) there were no significant changes in hypothalamic dopamine content or in the norepinephrine content of other brain regions or of the spinal cord following denervation, indicating that central sympathetic mechanisms may be involved in the development and maintenance of blood pressure response to ganglionic blockade. In a later experiment that plasma norepinephrine levels were increased 3 weeks after renal artery clipping of a one-kidney rat. \(^{19}\) In addition, the decrease in blood pressure in response to ganglionic blockade was significantly greater in clipped animals than in uninephrectomized normotensive controls. These results indicated that there was an increase in peripheral sympathetic activity in this stage of the development of hypertension in this model. Renal denervation performed 2 weeks after renal artery clipping resulted in a return of plasma norepinephrine levels and the blood pressure response to ganglionic blockade to control levels. Taken together, the hypotensive response to renal denervation, the concomitant increases in hypothalamic norepinephrine content and indices of peripheral sympathetic activity following renal artery clipping, their return to normal following renal denervation and the absence of changes expected to result from interruption of the renal efferent nerves following renal denervation provide strong evidence that the renal afferent nerves contribute to the pathogenesis of hypertension in the one-kidney one clip model via an effect on the central and thereby the peripheral sympathetic nervous system.

In support of this hypothesis are a number of lines of evidence indicating that the renal afferent nerves may participate in cardiovascular regulation. Both mechanoreceptors and chemoreceptors have been demonstrated in the kidney. A variety of stimuli, including alterations in renal artery pressure, renal venous occlusion, ureteral occlusion, compression of the kidney, ischemia, and changes in the ionic composition of the pelvic urine, have been shown to produce alterations in renal afferent nerve activity. \(^{20-24}\) Stimulation of the renal afferents of one kidney has been shown to produce a reflex increase in efferent nerve activity and arteriolar vasoconstriction in the contralateral kidney. \(^{25,26}\) These responses appear to be mediated via supraspinal neurons as they are abolished by spinal cord sectioning.

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is pressor or depressor. These apparent discrepancies have been attributed to differences in anesthesia. Additional support for the concept that the renal afferent nerves are involved in the pathogenesis of experimental hypertension comes from the preliminary reports of Kneupfer et al., Brody et al., and Mahoney et al. on the central neural projections of the renal nerves. They found that stimulation of the renal afferent nerves in the rat resulted in alterations in resistance in hindlimb, mesenteric and renal vascular beds, and that this response was abolished by lesioning of the AV3V region. In addition, stimulation of the renal afferent nerves resulted in changes in the unit activity of neurons in the AV3V region. These findings indicate that there are neural connections between the renal afferent nerves and the anterior hypothalamus. Evidence that these connections may be important in the pathogenesis of hypertension comes from experiments that showed that AV3V ablation prevents the development of hypertension in the one-kidney Grollman model.

In summary, this study provides evidence that the central nervous system participates in the maintenance of increased blood pressure in the one-kidney one-clip model of renal hypertension and indicates that the renal afferent nerves may contribute to the pathogenesis of hypertension by modulating hypothalamic noradrenergic activity.

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References

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S R Winternitz, R E Katholi and S Oparil

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