Adrenal and Vascular Tyrosine Hydroxylase Activity in Goldblatt Hypertension

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SUMMARY To examine the role of the sympathetic nervous system in hypertension, the in vitro activity of tyrosine hydroxylase was examined in one-kidney, one clip (1K1C) and two-kidney, one clip (2K1C) hypertensive rabbits and their respective controls 2 weeks after surgical procedures. The in vitro activity of tyrosine hydroxylase provides a measure of catecholamine synthesis and serves as a biochemical index of activity of noradrenergic neurons and the adrenal medulla. Mean arterial pressure rose from 91.5 ± 1.0 to 128.5 ± 5.6 mm Hg (p < 0.01) in the 1K1C group and from 91.8 ± 1.3 to 106.5 ± 5.0 mm Hg (p < 0.02) in the 2K1C group, whereas no change in blood pressure was found in their respective controls. Adrenal tyrosine hydroxylase activity was increased 85% in the 1K1C group, as compared with values in one-kidney controls (from 11.8 ± 1.5 to 21.8 ± 1.1 pmol CO2/min/mg; p < 0.0002), and was increased 49% in the 2K1C group, as compared with values in two-kidney controls (from 8.01 ± 1.2 to 11.9 ± 1.1 pmol CO2/min/mg; p < 0.02). In the 1K1C group, proximal mesenteric tyrosine hydroxylase activity was decreased 46% compared with values in one-kidney controls (from 23.5 ± 5.0 to 12.8 ± 2.5 pmol CO2/min/mg; p < 0.03) and distal mesenteric tyrosine hydroxylase activity was decreased 42% (from 7.73 ± 1.2 to 4.46 ± 0.8 pmol CO2/min/mg; p < 0.03). In the 2K1C group, neither proximal nor distal mesenteric tyrosine hydroxylase activity was altered. Tyrosine hydroxylase activity was not detectable in the femoral arteries, or in the thoracic and abdominal aorta. These results indicate that the adrenal medulla contributes to the pathophysiology of both 1K1C and 2K1C forms of hypertension. These results also indicate that alterations in the sympathetic nervous system in 1K1C hypertension are not mediated by an activation of tyrosine hydroxylase in the mesenteric arteries. (Hypertension 12: 434-442, 1988)

KEY WORDS • tyrosine hydroxylase • sympathetic nervous system • catecholamine synthesis • adrenal medulla • Goldblatt hypertension

HYPERTENSION represents a diverse group of diseases that are collectively characterized by an elevated systemic blood pressure. Efforts to reduce the risk of cardiovascular complications in hypertension have focused on the treatment of the elevated blood pressure; however, blood pressure is not the sole determinant for cardiovascular complications.1 In experimental models of hypertension, the elevation in blood pressure cannot predict the occurrence of necrotizing arteritis2-5 and cerebral hemorrhages6 or the degree of vascularp,7 and cardiac hypertrophy.8-11 The identity of other determinants for cardiovascular complications in experimental models of hypertension is unknown. Identification of these determinants would aid not only in understanding the pathogenesis of cardiovascular disease but also in developing more efficacious treatments for hypertension.

The sympathetic nervous system (SNS) participates in the regulation of blood pressure and plays a key role in the pathophysiology of hypertension. The development of one-kidney, one clip (1K1C) hypertension, a model of hypertension with a high incidence of cardiovascular complications,5 is associated with a variety of alterations in the SNS. In this model, the cardiovascular turnover of norepinephrine (NE) is increased,12 plasma levels of NE and its metabolites are elevated,13,14 and the level of NE metabolites is increased in the heart.13 In two-kidney, one clip (2K1C) hypertension, a model with a low incidence of cardiovascular complications,5 no alteration in SNS function is uncovered by examination of plasma levels of NE14 or of the cardiovascular turnover of NE.12 Since SNS alterations are present in the model of hypertension with a high incidence of cardiovascular complications, these alterations may be associated with determinants for cardiovascular complications. Thus, understanding the mechanism of these alterations in the
SNS may provide insight into the identity of the determinants for cardiovascular complications in 1K1C hypertension.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of catecholamines. The activity of TH is regulated in vivo by feedback inhibition by cytoplasmic NE and other catechols and by alterations in the kinetic characteristics of TH. These alterations are related to the level of neuronal activity and can be determined by measuring the in vitro activity of TH. Physiological, pharmacological, and electrical stimulation of catecholaminergic neurons and the adrenal medulla increases the in vitro activity of TH. Although both brief and prolonged periods of stimulation increase the in vitro activity of TH, only a prolonged decrease in neuronal activity of several days produces a decrease in the in vitro activity of TH. Thus, the in vitro activity of TH not only functions as a measure of catecholamine synthesis but also serves as a biochemical index of neuronal activity in catecholaminergic neurons. In neurogenic hypertension, a model of hypertension mediated by an increase in activity of the SNS, mesenteric TH activity has been demonstrated to be increased.

In the present study, the activity of TH was used to elucidate the role that SNS plays in the pathophysiology of two models of Goldblatt hypertension. The activity of TH was examined in the femoral arteries, thoracic and abdominal aorta, two segments of the mesenteric arteries, and the adrenals of 1K1C and 2K1C hypertensive rabbits and their respective controls (1K1Cc and 2K1Cc).

Materials and Methods

Male, white New Zealand rabbits (H&D, Pell City, AL, USA) weighing 2.1 to 3.0 kg were used in these studies. Rabbits were caged singly and received a commercial rabbit diet (Purina Laboratory rabbit chow, Richmond, IN, USA) and water ad libitum. Room lights were controlled by an automatic time switch that provided illumination from 0600 to 1800 daily. All rabbits were maintained for at least 1 week before surgical procedures. Direct blood pressure readings were recorded from a surface branch of the posterior tibial artery of the rabbit after necropsy and removal of the brain and spinal cord. The irises were scored as follows: 0 to 0.5 (no change in diameter), 1 to 2 (1.5 to 2 times the control diameter), 2.5 to 4 (2 to 2.5 times the diameter), and 4.5 to 5 (2 to 3 times the control diameter, with progression from the appearance of satellite capillary petechiae to the development of miliary aneurysms). The aneurysms were scored as follows: 0 to 0.5 (no change in diameter or tortuosity), 1 to 2 (1.5 to 2 times the arterial diameter), 2.5 to 4 (2 to 2.5 times the diameter), and 4.5 to 5 (2 to 3 times the control diameter, with progression from the appearance of satellite capillary petechiae to the development of miliary aneurysms).

Results were examined using the Microstat (EcoSoft, Indianapolis, IN, USA) statistical program. Significant differences between groups were analyzed using a paired or unpaired t test, and relationships between groups were analyzed using correlation (r) or linear regression (r²) coefficients. For the correlation coefficients, higher degrees of significance than a p value below 0.05 were not determined.
Results

In the 1K1C group, the parameters of the direct blood pressure readings (diastolic, mean arterial, and systolic pressure) were uniformly elevated 12 to 13 days after application of the constricting clip to the renal artery as compared with the direct blood pressure measurements obtained before the constriction of the renal artery (Figure 1, upper panel). Diastolic pressure was increased 40.0% (from 76.8 ± 1.3 to 107.5 ± 5.1 mm Hg; p < 0.01); mean arterial pressure, 40.4% (from 91.5 ± 1.0 to 128.5 ± 5.6 mm Hg; p < 0.01); and systolic pressure, 37.3% (from 121.3 ± 1.5 to 166.5 ± 5.1 mm Hg; p < 0.01). In the 1K1Cc group, the parameters of direct blood pressure were not influenced by the surgical procedure. The diastolic, mean, and systolic pressures were, respectively, 74.6 ± 2.0, 91.4 ± 0.9, and 125.4 ± 2.5 mm Hg before the nephrectomy and the application of the nonconstricting clip and 74.6 ± 1.4, 92.6 ± 1.5, and 127.6 ± 3.4 mm Hg, 12 to 13 days afterward.

In the 2K1C group, the parameters of the direct blood pressure readings were uniformly elevated 12 to 13 days after application of the constricting clip to the renal artery as compared with the direct blood pressure measurements before the constriction of the renal artery (Figure 1, lower panel). Diastolic pressure was increased 15.0% (from 77.2 ± 2.1 to 88.8 ± 3.6 mm Hg; p < 0.02); mean arterial pressure, 16.1% (from 91.8 ± 1.3 to 106.5 ± 5.0 mm Hg; p < 0.02); and systolic pressure, 16.0% (from 123.4 ± 1.8 to 143.2 ± 8.6 mm Hg; p < 0.02). In the 2K1Cc group, the parameters of direct blood pressure were not influenced by surgical procedure. The diastolic, mean, and systolic pressures were, respectively, 74.0 ± 1.8, 89.6 ± 2.3, and 121.2 ± 3.5 before application of the nonconstricting clip and 77.6 ± 2.9, 93.6 ± 4.2, and 125.6 ± 7.0 mm Hg 12 to 13 days afterward.

No significant differences in body weight, carcass weight, and adrenal size index were found in 1K1C and 2K1C groups when compared with values in their respective controls (Table 1). No differences were found in the size indices of the proximal or distal mesenteric arteries of 1K1C rabbits when compared with values in their controls (proximal mesenteric, 66.5 ± 6.1 vs 69.0 ± 9.5 mg/kg; distal mesenteric, 752 ± 97.6 vs 843 ± 138 mg/kg) or of 2K1C rabbits as compared with values in their controls (proximal mesenteric, 60.2 ± 4.9 vs 63.9

![Figure 1. Parameters of direct blood pressure before (open columns) and after application of clip (shaded columns) in 1K1C and control (1K1Cc) rabbits (upper panel) and 2K1C and control (2K1Cc) rabbits (lower panel). Values are expressed as means ± SEM. Asterisk (p < 0.05) indicates significant difference compared with values before clipping.](http://hyper.ahajournals.org/)

| Table 1. Body Weight, Carcass Weight, Organ Size Indices, and the Presence of Indoarteriopathy in 1K1C and 2K1C Groups and Their Respective Controls |
|---------------------------------|----------------|----------------|----------------|----------------|
| Variable                        | 1K1C           | 1K1Cc          | 2K1C           | 2K1Cc          |
| Body weight (kg)                | 2.93 ± 0.05    | 2.84 ± 0.10    | 2.91 ± 0.07    | 2.73 ± 0.08    |
| Carcass weight (kg)             | 2.23 ± 0.07    | 2.06 ± 0.11    | 2.29 ± 0.09    | 2.04 ± 0.09    |
| Cardiac index (g/kg)            | 3.47 ± 0.34*   | 2.38 ± 0.08    | 2.20 ± 0.09    | 2.17 ± 0.43    |
| Left renal index (g/kg)         | 5.02 ± 0.48†   | 4.55 ± 0.10†   | 2.74 ± 0.26†   | 3.75 ± 0.48†   |
| Right renal index (g/kg)        | 4.09 ± 0.40    | 3.71 ± 0.19    | 3.93 ± 0.33    | 3.55 ± 0.46    |
| Adrenal index (g/kg)            | 0.10 ± 0.01    | 0.15 ± 0.03    | 0.14 ± 0.02    | 0.14 ± 0.02    |
| Presence of indoarteriopathy    | 3/6            | 0/5            | 0/5            | 0/5            |

Values are means ± SEM. 1K1Cc and 2K1Cc are control groups. Organ index = organ weight/carcass weight.

* p < 0.05, compared with value in respective control.

† p < 0.05, compared with values in the contralateral kidney; in 1K1C and 1K1Cc rabbits, right kidneys were surgically removed.
± 7.0 mg/kg; distal mesenteric, 412 ± 46.7 vs 344 ± 74.8 mg/kg). In the 1K1C group, a 46% increase in the cardiac size index was found when compared with that in the 1K1Cc group (p < 0.01). No significant correlation was found between any parameter of blood pressure and the cardiac size index. No increase in the cardiac size index was found in the 2K1C group when compared with values in the 2K1Cc group. However, in the 2K1C group, linear regression of final arterial pressure on cardiac size index yielded a regression coefficient ($r^2$) of −0.997 (p < 0.0001). In the 2K1C group, the renal size index of the kidney with the constricting clip was decreased 30% compared with that of the contralateral kidney. In the 2K1Cc group, the renal size index of the kidney with the nonconstricting clip was not changed compared with that of the contralateral kidney. In the 1K1C and 1K1Cc groups, removal of the right kidney increased the renal size index of the contralateral kidney by 21 and 23%, respectively, as compared with that of the removed kidney (p < 0.05 and p < 0.01, respectively). In the 1K1C group, three of the six rabbits were found to have eye lesions that were graded as 0.5, 1.5, and 2.0. No eye lesions were found in their respective controls (1K1Cc) or in the 2K1C hypertensive rabbits and their respective controls (2K1Cc).

The activity of adrenal TH was increased in both 1K1C and 2K1C groups as compared with values in their respective controls (Figure 2, upper panel). In the 1K1C group, the activity of adrenal TH was increased 85% as compared with that in one-kidney controls (from 11.8 ± 1.5 to 21.8 ± 1.1 pmol CO$_2$/min/mg; p < 0.0002). In the 2K1C group, the activity of adrenal TH was increased 49% compared with that in two-kidney controls (from 8.01 ± 1.2 to 11.9 ± 1.1 pmol CO$_2$/min/mg; p < 0.02). In 1K1C hypertension, the adrenal TH was negatively correlated with the final mean arterial pressure ($r = -0.919$, $p < 0.05$; Figure 3, upper panel). In contrast, in 2K1C hypertension, linear regression of adrenal TH activity on final MAP yielded a regression coefficient ($r^2$) of 0.840 ($p < 0.02$; Figure 3, lower panel).

Figure 2. Activity of tyrosine hydroxylase in the adrenal (upper panel), proximal mesenteric artery (middle panel), and distal mesenteric artery (lower panel) from 1K1C and 2K1C hypertensive rabbits and their respective controls (1K1Cc and 2K1Cc). Values are expressed as means ± SEM. Asterisk ($p < 0.01$) and dagger ($p < 0.05$) indicate significant difference compared with values in respective controls.

No alteration in the activity of proximal and distal mesenteric TH was found in the 2K1C group compared with activity in the 2K1Cc group (Figure 2, middle and lower panels). However, in the 1K1C group, the activity of both proximal mesenteric and distal mesenteric TH was reduced compared with that in the 1K1Cc group. The activity of proximal mesenteric TH was reduced 46% (from 23.5 ± 5.0 to 12.8 ± 2.5 pmol CO$_2$/min/mg; $p < 0.03$). The regression coefficient of the linear regression of proximal mesenteric TH activity on final mean arterial pressure was −0.741 ($p < 0.03$; Figure 4, upper panel). The activity of distal mesenteric TH was reduced 42% (from 7.73 ± 1.2 to 4.46 ± 0.8 pmol CO$_2$/min/mg; $p < 0.03$). The regression coefficient of the linear regression of distal mesenteric TH activity on final mean arterial pressure was 0.946 ($p < 0.03$; Figure 4, lower panel). The activity of distal mesenteric TH was also negatively correlated with activity of adrenal TH and proximal mesenteric TH ($r = -0.908$, $p < 0.05$ and $r = -0.999$, $p < 0.05$, respectively), whereas the activity of adrenal TH and proximal mesenteric TH was positively correlated ($r = 0.922$, $p < 0.05$).

Tyrosine hydroxylase activity was undetectable in the femoral artery and thoracic aorta. In the abdominal aorta, the activity of TH was 50% over background, but this value was too low for accurate determination of activity.
FIGURE 3. Relationship of adrenal tyrosine hydroxylase activity and final mean arterial pressure in 1K1C hypertensive rabbits (upper panel) and 2K1C hypertensive rabbits (lower panel).

Discussion

In the present study, animals were anesthetized with sodium pentobarbital before decapitation to avoid activation of TH and SNS. With the use of this procedure, no TH activity was detected in the thoracic and abdominal aorta or the femoral arteries. This finding is compatible with the lack of a role for these elastic conduit blood vessels in the maintenance of peripheral resistance. The high activity of TH in the mesenteric arteries indicates a major contribution of the SNS in the regulation of this vascular bed and is compatible with the major contribution that the splanchnic circulation makes to the peripheral resistance. Although the structure of the distal mesenteric artery—small and highly branched arteries—is compatible with a role in the regulation of blood pressure, the structural nature of the proximal mesenteric artery—a simple conduit with no branches—is not. However, the splanchnic circulation is also a very important vascular bed in the regulation of plasma volume. The ability of this blood vessel to determine blood flow into the splanchnic circulation suggests that it may play an important role in the regulation of plasma volume.

In 2K1C hypertension, the activity of TH in proximal and distal mesenteric arteries was unchanged. This observation and the previous report of no change in NE turnover in the mesenteric artery of 2K1C hypertensive rabbits at the same time point indicate the lack of a role for these sympathetic neurons in the pathophysiology of this model of hypertension. These results are compatible with the dependency of this model of hypertension on the renin–angiotensin II (Ang II) system. In 1K1C hypertension, TH activity was decreased in proximal and distal mesenteric arteries. The decreased TH activity in the distal mesenteric artery was positively correlated with final mean arterial pressure. This finding is compatible with a contribution by the small arteries of the mesenteric vasculature to the vascular resistance and the major role of this vascular bed in the determination of blood pressure. In contrast, the decreased TH activity in the proximal mesenteric artery was negatively correlated with final mean arterial pressure. This result suggests that the proximal mesenteric artery is not a major factor in the determination of blood pressure.
and that the proximal mesenteric artery may be involved in the regulation of plasma volume. Since increases in blood pressure produce a pressure-natriuresis that decreases plasma volume, plasma volume and blood pressure are inversely related.45 The negative relationship of proximal mesenteric TH activity with blood pressure and the importance of the splanchnic circulation in plasma volume regulation49 suggest that proximal mesenteric TH activity may be related to alterations in plasma volume changes. Thus, the decreases in proximal and distal mesenteric TH activity in 1K1C hypertension may be mediated by the interplay between blood pressure and plasma volume regulation. The inverse correlation between the proximal and distal mesenteric TH activity supports this conclusion.

Adrenal TH activity was increased in both 1K1C and 2K1C hypertension. In 2K1C hypertension, adrenal TH activity was positively correlated with final mean arterial blood pressure. The dependency of 2K1C hypertension on the renin-Ang II system41-44 and the ability of Ang II to depolarize the chromaffin cells of the adrenal medulla46, 47 suggest that this activation of TH is mediated by Ang II. In 1K1C hypertension, the negative correlation of adrenal TH with final mean arterial blood pressure suggests that activation of the adrenal medulla does not play a major role in the increase in blood pressure in this model of hypertension; however, this activation may make a substantial contribution to the pathophysiology of 1K1C hypertension. The unchanged or decreased activity of the renin-Ang II system43, 44, 48 suggests that the activation of TH is mediated by a mechanism that does not involve Ang II. Although the mechanism for increased adrenal TH activity may be different in 1K1C and 2K1C hypertension, the increase in adrenal TH activity may represent a common feature of hypertension. This hypothesis is supported by data demonstrating an increase in the activity of adrenal TH in a variety of the other models of hypertension (e.g., salt-sensitive Dahl rat,49 spontaneously hypertensive rat,50, 51 mineralocorticoid-salt hypertension,51 and hypertension produced by section of the aortic and carotid baroreceptor nerves).52 The ability of many forms of hypertension to develop without the adrenal medulla53-57 indicates that the adrenal medulla is not necessary for the development of hypertension; however, it does not preclude a role for the adrenal medulla in the development of the elevated blood pressure in hypertension because of the compensatory influence of other vasopressor systems. Adrenalectomy produces a marked activation of the SNS58 and the plasma renin-Ang II system.57 Removal of these compensatory mechanisms reveals a marked dependency of the cardiovascular system on the adrenals.55, 57, 59, 60 These findings suggest that the adrenal medulla contributes to the pathophysiology of a wide variety of forms of hypertension. However, when this contribution is eliminated, it can be successfully compensated by other vasopressor systems.

The increase in adrenal medullary activity in 1K1C and 2K1C hypertension cannot be extrapolated to the rest of the SNS. In many physiological states—fasting,41, 61 hypoglycemia,63 moderate hypoxia,64 and acute trauma65—the activity of the sympathetic neurons and the adrenal medulla is not only dissociated but inversely related. The decreased activity of mesenteric TH in 1K1C hypertension and the unchanged activity in 2K1C hypertension are compatible with this dissociation. The inverse relationship between proximal and distal mesenteric TH activity in 1K1C hypertension suggests that sympathetic function can also be dissociated in the same vascular bed.

If the kinetic characteristics of TH can be assumed to be an indicator of the firing of noradrenergic neurons in hypertensive states, then the decrease in vitro activity of mesenteric TH in 1K1C hypertension indicates that the noradrenergic neuronal activity of the mesenteric arteries is chronically depressed. This finding suggests that the increase in turnover of cardiovascular NE12 and the other alterations in sympathetic function in 1K1C hypertension5, 14 are mediated by an increase in the release of NE per neuronal impulse and a decrease in neuronal uptake of NE. This hypothesis is supported by evidence indicating that electrical stimulation produces a greater release of NE per neuronal impulse66 and that uptake of intravenously administered radiolabeled NE is decreased in 1K1C hypertension.67 Plasma and tissue levels of NE metabolites in 1K1C hypertension are also compatible with this hypothesis. Extraneuronal metabolites of NE (normetanephrine and dihydroxymandellic acid) are increased, while intraneuronal metabolites of NE (dihydroxyphenylglycol) are decreased in plasma and cardiac tissue, suggesting a greater release of NE, with a decrease in its neuronal uptake and metabolism.13 These alterations in sympathetic function may be related to the volume-dependent nature of 1K1C hypertension.47-50 Plasma volume expansion increases the plasma levels of a sodium transport inhibitor69, 70 and activates the cardiopulmonary baroreceptor reflex.71, 72 Activation of this reflex produces a withdrawal of sympathetic activity to resistance and capacitance vessels such as the mesenteric arteries.72-74 Inhibitors of sodium transport increase the release of NE and inhibit its neuronal uptake.75, 76 These findings suggest that the increase in a sodium transport inhibitor in 1K1C hypertension77 could mediate the alterations in sympathetic function, but they do not provide any information about the role of these alterations in the maintenance of the elevated blood pressure in 1K1C hypertension. Inhibitors of sodium transport are not capable of raising blood pressure by themselves.78, 79 Additionally, if the alterations in sympathetic neurons in 1K1C hypertension were to contribute to the increase in blood pressure, the increase in turnover
of NE in the mesenteric arteries should increase the vascular resistance of the mesenteric vasculature. A decrease in splanchnic resistance in 1K1C hyperten­sion suggests that the alterations in sympathetic neurons do not contribute to the elevated blood pressure.

The decrease in mesenteric TH in 1K1C hypertension may be related to the increased tendency of this model of hypertension to have cardiovascular complications. A sodium transport inhibitor, is associated with an increase in cerebral hemorrhages in one-kidney, one wrapped hypertension. Additionally, stimuli for increasing the plasma levels of a sodium transport inhibitor are associated with an increase in cardiovascular complications. These results suggest that alterations in the sympathetic neurons in 1K1C hypertension may be related to its increased incidence of cardiovascular disease rather than to the increased blood pressure.

In conclusion, current evidence suggests that the alterations in sympathetic function in 1K1C hypertension are mediated by an increase in the release of NE per neuronal firing and a decrease in neuronal uptake of NE. No evidence was found in this study to suggest that an increase in neuronal activity contributed to these alterations in sympathetic function. Further investigation is required to clarify the mechanism of these alterations in the function of the sympathetic neurons and its possible role in the development of cardiovascular disease in hypertension.

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