Sympathetic Activity and Cardiac Adrenergic Receptors in One-Kidney, One Clip Hypertension in Rats

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SUMMARY The activity of the sympathetic nervous system, as measured by levels of plasma and cardiac catecholamines and catecholamine metabolites and the function of cardiac α- and β-adrenergic receptors, was evaluated at 3 days and 4 weeks after induction of one-kidney, one clip hypertension (1K1C) in the rat. At 3 days, the plasma level of norepinephrine (NE) was lower in the 1K1C group than the control group (p < 0.01), whereas epinephrine (E) and the metabolites dihydroxymandelic acid (DOMA), dihydroxyphenylglycol (DOPEG), and normetanephrine (NMN) were similar in both groups. In addition, cardiac content of catecholamines, their metabolites, and adrenergic receptors were similar in both groups. At 4 weeks, plasma levels of NE and DOPEG were lower (p < 0.01), whereas levels of DOMA and NMN were higher (p < 0.02 and p < 0.001, respectively) in the 1K1C group than the control group. Cardiac content of NE (p < 0.01), and DOPEG (p < 0.05) was significantly lower, whereas DOMA and NMN were significantly higher (p < 0.01) in the 1K1C group as compared to controls. In addition, cardiac density of both α- and β-adrenergic receptors was reduced in the 1K1C group, whereas receptor affinities were unchanged.

We conclude that: 1) the decreased level of plasma NE at 3 days and 4 weeks in the 1K1C model may indicate reduced activity of the sympathetic nervous system during the developmental and established phases of 1K1C hypertension; or 2) the increased levels of the extraneuronal catecholamine metabolites DOMA and NMN and the decreased density of the cardiac adrenergic receptors at 4 weeks may indicate increased amounts of NE at postsynaptic receptor sites, which, in turn, may contribute to the maintenance of hypertension in 1K1C model. (Hypertension 6: 654-659, 1984)

Key Words • sympathetic nervous system • catecholamines • adrenergic receptors • one-kidney, one clip hypertension • dihydroxymandelic acid • dihydroxyphenylglycol • normetanephrine • metabolites • norepinephrine

Several lines of evidence indicate that the renin-angiotensin system participates in the pathogenesis of elevated blood pressure in renal-artery-clip hypertension in the rat to a varying degree depending on the type and stage of the renovascular hypertension. The finding that pharmacologic blockade of angiotensin II (ANG II) receptors decreases blood pressure in two-kidney, one clip (2K1C) Goldblatt hypertension of 8 weeks’ duration but not in the one-kidney, one clip (1K1C) model led to the conclusion that the pathophysiology of the established phase of hypertension is different in these two types of hypertension. Such an interpretation also was supported by other studies indicating that various indices of sympathetic nervous system activity are abnormal in the 1K1C but not in 2K1C rat. The number of both α- and β-adrenergic receptors in the heart was reduced in the 1K1C rat. Since an inverse relationship between receptor density and amount of agonist has been reported, the decreased number of cardiac receptors has been interpreted as evidence of enhanced sympathetic activity in 1K1C hypertension. However, there are no studies in which the function of both the sympathetic system and the adrenergic receptors were simultaneously investigated in clip hypertension.
In the present study, we investigated the activity of the sympathetic nervous system by measuring plasma and cardiac catecholamines and catecholamine metabolites. In addition, cardiac adrenergic receptors were measured during the initial and established phases of 1K1C hypertension.

**Material and Methods**

Male Wistar rats (Simonsen's Laboratories, Gilroy, California), weighing 180 to 220 g were anesthetized with ether, and a silver clip (width 0.22 mm) was placed around the left renal artery followed by contralateral nephrectomy (1K1C model); in control rats a broad nonconstricting sliver clip was placed around the left renal artery followed by contralateral nephrectomy. Forty to 50 rats were randomly allocated to either the 1K1C or control group. Some rats were used for the subacute studies (3 days) and others for the chronic experiment (4 weeks). Rats were numbered and housed four per cage. They were maintained on regular chow with free access to water.

**Initial Phase of One-Kidney, One Clip Hypertension (3 Days Postoperative)**

Rats designated for the subacute studies were brought into the laboratory each day for measurement of body weight and tail artery blood pressure. They were warmed at 38° C for 5 minutes and placed in a clean lucite restrainer for the recording of arterial pressure with a tail sphygmomanometer unit and a water-filled pulse pick up. Blood was obtained by venipuncture with a 21-gauge needle from the lateral tail vein and was dripped into a chilled tube that contained glutathione and ethylenebis (oxyethylene-nitriilo) tetra-acetic acid solution as preservative. The tubes were gently inverted, and plasma was separated by centrifugation at 10,500 g for 4 minutes in a Brinkman microcentrifuge at 4°C. The plasma was removed and stored in a freezer at -70°C until assayed. NE and E were measured with the radioenzymatic assay of Peuler and Johnson. We incubated 50 μl of plasma or 30 μl of heart homogenate (obtained from the same tissues used for measurement of adrenergic receptors, see below, diluted at 1:150 vol/vol), in the presence of catechol-O-methyltransferase and S-adenosyl-L-methionine-

**Established Phase of One-Kidney, One Clip Hypertension (4 Weeks Postoperative)**

Rats designated for these studies were brought to the laboratory every week for measurement of body weight and tail artery systolic blood pressure. However, approximately 25% of the rats either died or did not develop hypertension. At the end of the 4th postoperative week, blood was collected by lateral tail vein venipuncture from 21 rats per group. The rats were then killed and the hearts collected. In addition, since venipuncture and restraint of the rats increase plasma catecholamines, an indwelling catheter was inserted into the left femoral artery of seven 1K1C and eight control rats, and 1 day later blood was collected while the rats were in home cages and undisturbed.

**Assays of Catecholamines and Catecholamine Metabolites**

The content of norepinephrine (NE) and epinephrine (E) and of their major metabolites normetanephrine, 3,4-dihydroxyphenylglycol (DOPEG) and 3,4-dihydroxyphenylmandelic acid (DOMA) was determined in both plasma and heart. Of the six metabolic products of NE and E resulting from the action of catecholamine-degradative enzymes, two are O-methylated (normetanephrine [NMN] and metanephrine [MN]), two are deaminated (DOPEG and DOMA), and two are deaminated and methylated (3-methoxy-4-hydroxyphenylglycol and 3-methoxy-4-hydroxymandelic acid). Although NMN and MN are metabolites of NE and E, respectively, DOPEG and DOMA derive from both catecholamines.

Plasma and tissue NE and E were measured with the radioenzymatic assay of Peuler and Johnson as modified by us. We incubated 50 μl of plasma or 30 μl of heart homogenate (obtained from the same tissues used for measurement of adrenergic receptors, see below, diluted at 1:150 vol/vol), in the presence of catechol-O-methyltransferase and S-adenosyl-L-methionine-

For DOPEG and DOMA we used two radioenzymatic assays that have been developed in our laboratory. Details of both assay procedures have been described elsewhere.

**Membrane Preparation**

On the day of the receptor-binding studies, three hearts from each group were thawed, minced with scissors, pooled together, and homogenized with a Potter/Elvehjem homogenizer (Fisher Scientific, Pittsburgh, Pennsylvania) in 7 volumes of buffer containing 50 mM Tris-HCL, pH 7.7, 10 mM MgCl₂, and 0.25 M sucrose. From each homogenate, an amount of 0.5 ml was transferred into a plastic tube, 0.25 ml of 0.6 N perchloric acid was added, vortexed for 10 seconds twice, and the sample was centrifuged at 5000 g for 10 minutes in a refrigerated centrifuge. The clear supernatant was transferred into a small container, stored in a freezer at -70°C, and used for analysis of the cardiac content of catecholamines and catecholamine metabolites. Protein content of the specimens was determined by the method of Lowry et al., with bovine serum albumin used as standard.

The rest of the homogenate was centrifuged at 3000 g for 5 minutes twice. Subsequently the supernatant was centrifuged at 30,000 g for 15 minutes, and the pellet membranes were washed twice. A small portion of the final pellet was resuspended in 1.5 ml of binding buffer (final protein concentration of 2–3 mg/ml) containing 50 mM sodium phosphate, pH 7.4, 4 mM MgSO₄, 0.05% ascorbic acid, and 10⁻¹⁴ M pyrocatechol; this sample was used for analysis of beta receptors. The larger part of the pellet was resuspended in 1.5 ml of binding buffer (final protein concentration of 5–10 mg/ml) containing 50 mM Tris-HCL, pH 7.7, 10 mM MgCl₂, 0.05% ascorbic acid, and 10⁻¹⁴ M pyrocatechol; this sample was used for analysis of α-receptors.
Analysis of Alpha- and Beta-Adrenergic Receptors

Cardiac membranes (50 μl) were incubated, in duplicate, with 100 μl of the appropriate binding buffer containing increasing concentrations of the appropriate radioligand (\(^{3}H\)-WB4101, specific activity 35 Ci/mmol, for analysis of α-receptors or \(^{125}I\)-iodohydroxybenzylpindolol = \(^{123}I\)-HYP, specific activity 2500 mCi, for analysis of β-receptors). For determination of nonspecific binding, separate incubations were carried out in the presence of 10\(^{-3}\) M isoproterenol or E. The tubes designated for the binding of α-receptors were incubated for 22 minutes at 25° C, and their content was rapidly filtered through Whatman (Fisher Scientific) glass fiber filters (GF/C 2.2 mm). Filters were immediately washed with 10 ml of Tris buffer, dried, and counted by using Aquasol (New England Nuclear, Boston, Massachusetts) counting solution. For analysis of β-receptors, the appropriate tubes were incubated for 45 minutes at 37° C, and the content was then rapidly filtered through Whatman filters. Filters were washed with 20 ml of phosphate buffer, dried, and counted in a LKB autogamma scintillation spectrometer (Gaithersburg, Maryland). Specific binding was taken as total minus nonspecific binding. Results were analyzed by the method of Scatchard\(^{19}\) with a Texas Instruments 59 programmable calculator (Dallas, Texas). Studies for characterization of adrenergic receptor sites have been published before.\(^{7}\)

In each group, the data of 21 rats were analyzed for determination of body weight, tail arterial pressure, and tail venous plasma catecholamines and catecholamine metabolites. (The rest of the rats died or were sick or did not develop hypertension.) Receptor-binding studies require three hearts for each analysis. Therefore, the data are averages of seven measurements in each group. In addition, at 4 weeks postoperatively, plasma levels of catecholamines and their metabolites were determined in blood obtained via an indwelling arterial catheter from seven 1K1C rats group and eight control rats while they were freely moving and undisturbed in their home cages. For statistical analysis, analysis of variance (ANOVA) was performed, if ANOVA was significant, Student’s \(t\) test was used for comparison between the two groups.\(^{20}\) Results are expressed as means ± standard error of mean (SEM).

Results

Initial Stage of One-Kidney, One Clip Hypertension

Three days after operation, body weight was 315 ± 7 g, mean ± sem, in the 1K1C hypertension group and 310 ± 6 g in the control group (NS), whereas tail artery blood pressure was 132 ± 3 mm Hg and 109 ± 2 mm Hg, respectively (\(p < 0.001\), Figure 1). The concentrations of plasma catecholamines and their metabolites are depicted in Figure 2. Whereas plasma levels of E were similar in both groups, the concentration of NE was reduced in the 1K1C group (194 ± 18 vs 277 ± 23 pg/ml, \(p < 0.01\)). Plasma levels of all three metabolites were similar in the two groups of rats.

Table 1 gives the cardiac density of adrenergic receptors, catecholamines, and catecholamine metabo-
lites. The dissociation constant of \(^{125}\)I-HYP was 0.24 ± 0.1 nM in the 1K1C group and 0.22 ± 0.05 nM in the control group, whereas the total concentration of \(\beta\)-receptors was 76 ± 5 fmol/mg protein and 82 ± 7 respectively (NS). Likewise, both dissociation constant and total binding of \(^3\)H-WB4101 were similar in both groups. The heart weight/body weight ratio was 3.2 ± 0.3 g/kg, in the 1K1C group and 3.3 ± 0.3 g/kg in the control (NS). Cardiac content of catecholamines and catecholamine metabolites was also similar in both groups.

**Established Phase of One-Kidney, One Clip Hypertension**

Four weeks after operation, body weight was 359 ± 7 g in the 1K1C group and 339 ± 7 g in the control group, whereas tail artery blood pressure was 161 ± 3 mm Hg in the former and 114 ± 1.5 mm Hg in the latter group \((p < 0.001)\) (Figure 1). Figure 3 depicts plasma levels of catecholamines and catecholamine metabolites at 4 weeks. In the hypertensive group, plasma NE and DOPEG were 195 ± 9 pg/ml and 478 ± 22 pg/ml, respectively, which were significantly lower than the corresponding values (303 ± 34 and 655 ± 39, respectively, \(p < 0.01\)) in the control group. On the other hand, the metabolites DOMA and NMN were significantly higher \((p < 0.02\) for DOMA and \(p < 0.001\) for NMN) in the hypertensive than the control group.

Table 2 shows values of catecholamines and their metabolites in blood obtained via an indwelling arterial catheter at 4 weeks. In the 1K1C group, the cardiac content of DOMA was 44 ± 3 fmol/mg protein and 65 ± 4 fmol/mg protein in the control group \((p < 0.01)\). In the 1K1C group, the cardiac content of DOMA and NMN was increased \((p < 0.01)\), whereas NE \((p < 0.01)\) and DOPEG \((p < 0.05)\) were reduced. At 4 weeks, the heart weight/body weight ratio was 3.5 ± 0.2 in the 1K1C group and 2.8 ± 0.2 in the control group \((p < 0.05)\).

**Discussion**

Our studies showed that the plasma concentration of NE is decreased at 3 days in 1K1C hypertensive rats, whereas the plasma levels of catecholamine metabolites did not change during the developmental phase of hypertension. This finding is in contrast to that reported by Dargie et al., who found an increase in the plasma levels of NE in rats at 7, 14, and 28 days of 1K1C hypertension and no change at 24 hours. However, in those studies, blood was collected from the trunk of decapitated rats, and their values are manyfold higher than those of the present study and the study of other investigators.

**Table 2. Levels of Catecholamines and Catecholamine Metabolites in Blood Obtained via an Indwelling Arterial Catheter at 4 Weeks**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>NE (pg/ml)</th>
<th>E (pg/ml)</th>
<th>DOMA (pg/ml)</th>
<th>DOPEG (pg/ml)</th>
<th>NMN (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1K1C ((n = 7))</td>
<td>131 ± 15</td>
<td>45 ± 8</td>
<td>2260 ± 187</td>
<td>516 ± 29</td>
<td>1482 ± 120</td>
</tr>
<tr>
<td>Control ((n = 8))</td>
<td>217 ± 33</td>
<td>48 ± 6</td>
<td>1387 ± 165</td>
<td>638 ± 35</td>
<td>1140 ± 104</td>
</tr>
<tr>
<td>(p) value</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 1.

**Table 3. Cardiac Content of Adrenergic Receptors, Catecholamines and Their Metabolites at Four Weeks of 1 Kg Rats and Controls**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1K1C ((n = 7))</th>
<th>Controls ((n = 7))</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-receptors</td>
<td>(^{125})I-HYP ((\text{fmol/mg protein}))</td>
<td>57 ± 5</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>(\alpha)-receptors</td>
<td>(^3)H WB4101 ((\text{fmol/mg protein}))</td>
<td>44 ± 3</td>
<td>65 ± 4</td>
</tr>
<tr>
<td>E ((\text{ng/mg protein}))</td>
<td>0.1 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>NE ((\text{ng/mg protein}))</td>
<td>3.6 ± 0.5</td>
<td>8.0 ± 1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DOMA ((\text{ng/mg protein}))</td>
<td>3.1 ± 0.14</td>
<td>2.2 ± 0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DOPEG ((\text{ng/mg protein}))</td>
<td>3.7 ± 0.34</td>
<td>5.4 ± 0.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NMN ((\text{ng/mg protein}))</td>
<td>1.0 ± 0.1</td>
<td>0.48 ± 0.04</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 1.
A growing body of experimental evidence indicates that the pathophysiologic alterations in clip hypertension vary depending upon many factors that include the presence or absence of the contralateral kidney, sodium balance, absence of adrenal glands, and the stage of hypertension. In both the 1K1C and 2K1C models, there is an initial angiotensin-dependent phase which evolves to an angiotensin-independent phase within 8 to 12 weeks in the 2K1C model and within a few days in the 1K1C model. The early rise in plasma renin concentration and the positive sodium balance and plasma volume expansion that follow seem to be the primary events accounting for the development of hypertension in 1K1C model. The reduced plasma NE at 3 days in 1K1C hypertensive rats, observed in our series, may be secondary to a reduction in sympathetic activity induced by acute plasma volume expansion and hypertension. Nevertheless, since angiotensin has been shown to stimulate central sympathetic vasomotor neurons and the adrenal medulla, the cardiovascular effects of ANG II could be adrenergically mediated. Such a possibility, however, was not substantiated in our study. Petty and Reid found a reduction in the content of NE in specific nuclei of the brain stem and hypothalamus at 72 hours but not at 7 days or 4 weeks in the 1K1C model. These changes were interpreted as secondary effects from compensatory mechanisms. Therefore, it seems likely that the elevation of blood pressure during the acute stage of 1K1C hypertension is related to the renin-angiotensin system.

During the established phase (4 weeks) of 1K1C hypertension in the present study, plasma levels of NE and DOPEG were significantly decreased, whereas the concentration of DOMA and NMN were increased (Figure 3). Results were identical in blood specimens obtained by puncture of the lateral tail vein or via indwelling arterial catheters from undisturbed and freely moving rats. However, Dargie et al. and De Quattro et al. reported increased plasma levels of NE during the established phase of 1K1C hypertension of the rat. Again, methodological differences could account for the divergence of results.

Our experiments showed that the content of cardiac catecholamines and catecholamine metabolites at 3 days was similar in both groups, whereas at 4 weeks the content of NE and DOPEG was lower and that of DOMA and NMN was higher in 1K1C hypertensive rats than in their controls. Although data on cardiac catecholamine metabolites are not available in the literature, a decreased cardiac NE content also has been reported in the DOCA-salt hypertensive rat, the renal hypertensive rat, and the sinoaortic-denervated hypertensive rabbit. The decreased levels in plasma and cardiac NE and DOPEG observed at 4 weeks of 1K1C hypertension should indicate decreased synthesis, decreased uptake, and decreased intraneuronal catecholamine metabolism during the established phase of hypertension. Such an interpretation is supported by experimental evidence indicating that DOPEG is mainly an intraneuronal metabolite and its tissue amounts reflect NE neuronal uptake and metabolism. On the other hand DOMA is mainly an extraneuronal metabolite, whereas, NMN is exclusively produced in extraneuronal sites. The tissue concentration of NE reflects the NE accumulated and stored in sympathetic nerve terminals (storage vesicles) and not the physiologically active portion of NE, which reaches postsynaptic receptors and accounts for cellular function. That part of NE cannot be measured. However, since the portion of NE that reaches receptor sites is rapidly metabolized, tissue and plasma levels of NMN should be a measure of NE at extraneuronal sites, including postsynaptic receptors. In the present study, the observed increased levels of DOMA and NMN in 1K1C group with established hypertension suggests enhanced catecholamine metabolism at extraneuronal sites.

Our adrenergic receptor binding studies demonstrated a significant decrease in the number in both α- and β-receptors in the cardiac membranes of 1K1C hypertensive rats at 4 weeks of hypertension, but no change at 3 days, whereas binding affinities were not different in either stage of hypertension. A reduced number in adrenergic receptors as well as reduced isoproterenol stimulated adenylyl cyclase have been previously reported in cardiac membranes of 1K1C and DOCA-salt hypertensive animals. The observed significant decrease in the density of cardiac adrenergic receptors at 4 weeks of 1K1C hypertension could be the result of chronic exposure of the receptors to increased quantities of catecholamines. Such an interpretation is supported by a large number of studies demonstrating an inverse relationship between receptor density and extent of receptor occupancy by agonists. It is likely that at 4 weeks of 1K1C hypertensive model increased amounts of norepinephrine are shifted to extraneuronal tissue including adrenergic receptor sites accounting for the observed decreased density of adrenergic receptors and increased levels of NMN and DOMA (products of extraneuronal metabolism). In addition, such an increased amount of NE at receptor sites could account for or contribute to the maintenance of blood pressure elevation in this hypertensive animal model.

The observed change in cardiac receptors, however, may be part of a generalized decrease in plasma membrane components due to cardiac hypertrophy, which was found in the hearts of 1K1C rats at 4 weeks. Although in the present study activities of plasma membrane enzymes were not determined, Woodcock et al. failed to detect any difference in enzyme activities in hypertrophied hearts of 1K1C rats after 3 weeks of hypertension. Alternatively, the measured decrease in cardiac adrenergic receptors could be mediated by increased sodium, since monovalent cations may modulate receptor characteristics. However, such a possibility is unlikely since cardiac receptors did not change at 3 days in 1K1C rats, in which an increased sodium retention has been reported. Furthermore, sodium administration has been associated with an increased rather than decreased density of adrenergic receptors in the kidney of salt-sensitive Dahl rats.
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References


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