Effect of Anteroventral Third Ventricle Lesions on Vascular Sodium-Pump Activity in Two-Kidney Goldblatt Hypertension

EMEL SONGU-MIZE, PH.D., STEVE L. BEALER, PH.D., AND R. WILLIAM CALDWELL, PH.D.

SUMMARY We studied the effects of anteroventral third ventricle (AV3V) lesions on the vascular Na⁺-pump activity and blood pressure of rats prepared by the two-kidney Goldblatt procedure. Blood pressures and Na⁺-pump activity of the isolated tail arteries, measured as ouabain-sensitive ⁸⁶Rb⁺-uptake, were determined in rats with renal artery clips, rats with AV3V lesions, and rats with AV3V lesions. Rats with renal artery clips developed higher blood pressures (40%) and higher vascular Na⁺-pump activity (20%-35%) than rats with no renal clips. Placement of AV3V lesions prior to the placement of renal clips prevented the increase in blood pressure and the increase in vascular Na⁺-pump activity. Plasma potassium and creatinine concentrations, nonspecific ⁸⁶Rb⁺-uptake, and hematocrit were not different among these groups. Plasma sodium concentration was elevated in the AV3V lesioned control group. These experiments suggest a possible role of this CNS region in the regulation of vascular Na⁺-pump function during hypertensive states.

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KEY WORDS • two-kidney, one clip Goldblatt hypertension • Na⁺-pump activity • Rb⁺-uptake • AV3V lesion • tail artery • renal artery clips

THE biochemical correlate of the electrogenic Na⁺-pump, Na⁺, K⁺-ATPase, is responsible for the maintenance of the cellular membrane potential and thus contributes to vascular smooth muscle tone.¹⁻³ Changes in vascular Na⁺-pump activity and monovalent cation transport have been reported in animal models and several forms of human hypertension.⁴⁻⁵ Several models of hypertension with expanded fluid volumes are stated to exhibit a lowered vascular Na⁺-pump activity.⁴ DOCA-salt,⁶ reduced renal-mass,⁷ one-kidney, one wrapped⁸ and one-kidney, one clip Goldblatt hypertension⁴ are among these. However, other investigators have found increased vascular Na⁺-pump activity in some types of hypertension associated with volume expansion. For example, elevated vascular Na⁺-pump activity has been reported in rats with DOCA-salt,⁹ one- and two-kidney one clip Goldblatt¹⁰ and rats of the Dahl salt-sensitive strain.¹¹,¹²

We have recently reported that in an advanced stage of DOCA-salt hypertension, vascular Na⁺-pump activity is depressed in rats.¹³ The advanced malignant stage of DOCA-salt hypertension has been shown by others to be accompanied by volume loss, rather than volume expansion.¹⁴ Furthermore, we have demonstrated that lesions in the anteroventral portion of the third brain ventricle (AV3V region) prevent this suppression of Na⁺-pump activity, as well as preventing DOCA-salt hypertension.¹³ We suggested that there is CNS involvement in the regulation of vascular Na⁺-pump activity in DOCA-salt hypertension.¹³

Because two-kidney, one clip Goldblatt hypertension, like the DOCA-salt model, is prevented by AV3V lesions,¹⁵ we have determined the effect of prior AV3V lesions on vascular Na⁺-pump activity and development of hypertension in this model. We found that vascular Na⁺-pump function was elevated in hypertensive rats and that prior destruction of the AV3V region prevented the development of hypertension as well as the increase in vascular Na⁺-pump activity.
Methods

Male Sprague-Dawley rats weighing 170–210 g, purchased from Harlan Company, Madison, Wisconsin, were used throughout these experiments. Systolic blood pressures and vascular Na⁺-pump activities were measured in all groups of rats studied.

Experiment 1
Two groups of rats were studied: 1) two-kidney, Goldblatt hypertensive rats with left renal artery clips of 0.2 mm i.d.; and 2) sham-operated controls with no renal artery clips.

Experiment 2
Four groups of rats were studied. One group of rats received AV3V lesions prior to placement of renal artery clips (Group LG). The second group also received AV3V lesions, but no renal artery clips (Group LC). The third group underwent sham-lesion surgery and received silver clips (Group SG). The fourth group underwent sham-lesion surgery but received no clips (Group SC).

Experimental Protocol
Systolic blood pressure measurements were made in the conscious state, once prior to any treatment, and then once weekly throughout the experiment by tail cuff plethysmography.

Sodium-pump activity of the isolated tail arteries was assessed 6 to 7 weeks after the initiation of the Goldblatt procedures by measuring ⁸⁶Rb⁺-uptake with methods similar to those described previously. Specific uptake of ⁸⁶Rb⁺ (nmol/mg wet weight per 15 minutes) was determined as the difference between uptake in the absence and presence of 1.0 mM ouabain. ⁸⁶Rb⁺-uptake in the presence of ouabain, termed nonspecific uptake, reflects the distribution of ⁸⁶Rb⁺ in extracellular spaces and passive penetration into the cells. Results are expressed relative to wet weight, as the wet weight to dry weight ratio of the vascular tissue was found to be the same in each group.

For the placement of the AV3V lesions, rats were subjected to a DC current of 2.5 mA for 15 seconds in the AV3V region of the brain, or underwent identical surgical procedures except that no current was passed. This procedure has been previously described in detail. The extent and the location of the lesions were verified histologically upon completion of the experiments and data were discarded from rats that did not receive bilateral periependymal damage in the AV3V region. Only the rats in Experiment 2 received AV3V lesions.

Rats weighing 190 to 210 g underwent surgery for the placement of a silver clip (0.2 mm i.d.) on the left renal artery. The right kidney was left untouched. Rats of Experiment 2 were allowed to recover from the brain surgery before the placement of the renal artery clips. This modification of the two-kidney Goldblatt hypertension procedure was adopted from Brooks et al. and the silver clips were prepared in our laboratory as described by those authors. Control rats underwent sham-surgical procedures but did not receive renal artery clips.

Flame photometry (Model 343, Instrumentation Laboratories, Watertown, Massachusetts) was used to measure the plasma sodium and potassium concentrations. Hematocrit and plasma creatinine measurements were also made.

Data are reported as mean ± se. Data were compared using Student’s t test (Experiment 1) and one-way analysis of variance and Newman-Keuls a posteriori test (Experiment 2) to evaluate the differences between multiple means.

Results
Initial, pretreatment systolic blood pressure values were similar in all groups. The combined mean was 115 ± 2 mm Hg, n = 50.

Experiment 1
Figure 1 shows the systolic blood pressure of rats after placement of renal artery clips or sham-surgery (control group). There were significant differences between the blood pressures of the rats in the Goldblatt group and the control group beginning with the 2nd week after this procedure. Blood pressures of rats in the Goldblatt group rose to 150 mm Hg in the 2nd week, and to 175 mm Hg by the end of the 5th week and remained stable at 175–185 mm Hg through the
TABLE 1. Sodium Pump Activity, Systolic Blood Pressure, Plasma Sodium, Potassium, and Hematocrit Measurements in Two-Kidney, One Clip Goldblatt Hypertension

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific #Rb⁺-uptake (nmol/mg/15 min)</th>
<th>Nonspecific #Rb⁺-uptake (nmol/mg/15 min)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>[Na⁺] (mEq/liter)</th>
<th>[K⁺] (mEq/liter)</th>
<th>Hematocrit (%)</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.326±0.018</td>
<td>0.073±0.005</td>
<td>138±5</td>
<td>135.4±4.3</td>
<td>3.20±0.11</td>
<td>41±1</td>
<td>8</td>
</tr>
<tr>
<td>Goldblatt</td>
<td>0.443±0.030*</td>
<td>0.077±0.003</td>
<td>178±8†</td>
<td>135.8±1.8</td>
<td>3.12±0.07</td>
<td>42±1</td>
<td>15</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to control.
†p < 0.001 compared to control.

TABLE 2. Effect of AV3V Lesions on Nonspecific Rubidium Uptake, Plasma Sodium, Potassium, Creatinine and Hematocrit Values in Two-Kidney, One Clip Goldblatt Hypertension

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nonspecific #Rb⁺-uptake (nmol/mg/15 min)</th>
<th>[Na⁺] (mEq/liter)</th>
<th>[K⁺] (mEq/liter)</th>
<th>Creatinine (mg/100 ml)</th>
<th>Hematocrit (%)</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>0.059±0.003</td>
<td>143.1±0.5</td>
<td>3.59±0.10</td>
<td>0.3±0.01</td>
<td>43±1</td>
<td>12</td>
</tr>
<tr>
<td>SG</td>
<td>0.059±0.006</td>
<td>143.2±1.1</td>
<td>3.60±0.15</td>
<td>0.4±0.02</td>
<td>41±1</td>
<td>9</td>
</tr>
<tr>
<td>LC</td>
<td>0.047±0.004</td>
<td>146.3±0.4</td>
<td>3.85±0.16</td>
<td>0.3±0.01</td>
<td>40±1</td>
<td>8</td>
</tr>
<tr>
<td>LG</td>
<td>0.064±0.008</td>
<td>141.9±2.5</td>
<td>3.58±0.06</td>
<td>0.2±0.02</td>
<td>41±1</td>
<td>6</td>
</tr>
</tbody>
</table>

SC = sham-operated control rats, no clips; SG = sham-operated rats with clips; LC = AV3V-lesioned control rats, no clips; LG = AV3V-lesioned rats with clips.

7th week; pressures of the control group remained unchanged over this period. #Rb-uptake in the tail arteries of these rats was measured between the 6th and the 7th weeks (table 1). A significant increase (35%) was observed in the ouabain-sensitive (specific) #Rb⁺-uptake in the tail arteries of the Goldblatt group compared to the control group. There were no significant differences in the plasma potassium or sodium concentrations, hematocrit values or nonspecific #Rb⁺-uptake between Goldblatt hypertensive rats and the control group (table 1).

**Experiment 2**

Rats that received renal artery clips following sham lesions (Group SG) developed significantly higher systolic blood pressures than sham-lesion rats without clips (Group SC). AV3V lesions when introduced prior to the placement of the arterial clips (Group LG) prevented the increase in blood pressure normally caused by renal artery clipping. In addition, rats with AV3V lesions without renal artery clips (Group LC) had blood pressures similar to those of Group SC rats, indicating that AV3V lesions alone do not alter blood pressure (fig. 2).

Vascular Na⁺-pump activity in Group SG was increased by 20% (p < 0.01) compared to Group SC. Na⁺-pump activity in Group LG was the same as Group SC indicating that the AV3V lesion prevented the elevation of Na⁺-pump activity associated with Goldblatt hypertension. AV3V lesions did not alter basal vascular Na⁺-pump activity, as values for Groups SC and LG did not differ from those of Group SC rats (fig. 2).

**FIGURE 2.** Effect of anteroventral third ventricle (AV3V) lesions in rats with and without renal artery clips (Goldblatt treatment). Measurements were made 6 to 7 weeks after the initiation of the Goldblatt treatment. Abbreviations: SC = Sham-operated control rats, no clips; SG = Sham operated rats with clips; LC = AV3V-lesioned control rats, no clips; LG = AV3V-lesioned rats with clips. Results are analyzed with one-way ANOVA. *Significantly different from SC, LC, and LG, p < 0.01.
SC and LC were not different. Ouabain insensitive, or
non-specific, \(^{86}\)Rb" uptake, hematocrit and plasma so-
dium, potassium and creatinine concentrations were
the same in all groups (table 2).

Discussion

We have demonstrated that the vascular Na"-pump
activity is increased in two-kidney, one clip hyperten-
sion. Electrolytic destruction of the AV3V region prior
to the renal artery clip placement prevented this in-
crease in Na"-pump activity, as well as preventing the
hypertension. In addition, we have recently shown that
suppressed vascular Na"-pump activity seen at the ad-
vanced stage of DOCA-salt hypertension is prevented
by the destruction of the AV3V area prior to the
DOCA-salt treatment. A number of recent studies
have shown that the integrity of the AV3V area is
essential for the development and maintenance of sev-
eral experimental forms of hypertension, including
the DOCA-salt and two-kidney, one clip model. Since
the present study demonstrates that the altered
vascular Na"-pump activity which accompanies the
two-kidney, one clip hypertension is also abolished by
the ablation of this area, as is the case of DOCA-salt
hypertension, it extends the role of this brain region in
controlling the vascular Na"-pump activity accompa-
nying hypertension to another experimental model.

Changes in vascular Na"-pump activity have been
reported in human types and several animal models. It
has been suggested by Haddy and his colleagues that
hypertension characterized by volume expansion is as-
associated with lowered vascular Na"-pump activity.
For example, animals with DOCA-salt, reduced
renal mass, one-kidney, one wrapped and one-kid-
ney, one clip Goldblatt hypertension exhibit re-
duced vascular Na"-pump activity. Those authors sug-
uggested that a ouabain-like humoral substance might be
responsible for Na"-pump suppression in these mod-
els. However, other investigators have recently report-
ed increased vascular Na"-pump activity in some types
of hypertension characterized by expanded fluid vol-
ume. For example, Overbeck’s laboratory has found that
DOCA-salt one- and two-kidney, one clip Goldblatt
and salt-sensitive Dahl rats display increased pump activity. With regard to DOCA-salt hyperten-
sion, a reason for the discrepancy may be differences
in the stage of hypertension when vascular Na"-pump
activity was measured. This form of hypertension is
characterized by a fluid volume expansion at the early
or benign stage, and a volume loss at the late or mali-
gnant stage. In fact we have recently reported sup-
pressed vascular Na"-pump activity at the late phase of
DOCA-salt hypertension, but we have also noted that
the pump activity is increased at the early stages of this
form of hypertension (unpublished observations). We
have additionally demonstrated that plasma taken from
the rats at the advanced stage of DOCA-salt hyperten-
sion inhibits Na"-pump activity in vascular tissue iso-
lated from untreated rats, suggesting the existence of a
plasma factor.

During the development of two-kidney, one clip
Goldblatt hypertension in its benign stage, fluid vol-
ume expansion has been shown to occur. In the pres-
ent study, we have produced a benign form of two-
kidney, one clip Goldblatt hypertension: the systolic
blood pressure did not exceed 190 mm Hg and plasma
sodium, potassium, and creatinine levels were normal.
We have shown that vascular Na"-pump activity is
increased in contrast to the DOCA rats of late hyper-
tensive stage. Malignant hypertension in two-kidney
Goldblatt hypertension is associated with loss of sodi-
um, thus reduction of extracellular fluid volume, and
kidney damage resembling the malignant stages of
DOCA-salt hypertension. We therefore predict that
vascular Na"-pump would be suppressed at this stage
of Goldblatt hypertension.

Although simultaneous measurements of fluid vol-
ume and Na"-pump activity have not been made in
these studies, the existence of a direct correlation be-
tween these two variables appears probable. Thus, if
inhibition of the vascular Na"-pump is due to a digitalis-
like substance, vascular volume should be consid-
ered as a possible determinant of the release of this
substance. Moreover, the AV3V region may be the locus
of the central relay area controlling release of the fac-
tor. Our findings, that AV3V lesions prevent changes
in Na"-pump activity associated with hypertension in
both DOCA-salt and two-kidney Goldblatt rats, sup-
port this proposition.

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