Exchangeable Sodium in Angiotensinogenic and Nonangiotensinogenic Renovascular Hypertension

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SUMMARY Previous studies have suggested that angiotensin II and sodium can act as alternative mechanisms in maintaining high blood pressure in chronic renovascular hypertension. In the present study, exchangeable sodium was measured in rats in which angiotensin II had been confirmed or excluded as the main cause of the hypertension. To determine the degree of participation of angiotensin II in the maintenance of the high blood pressure, we studied the mean blood pressure response to an angiotensin antagonist (1-Sar-8-Ala-angiotensin II) and to a converting enzyme inhibitor (SQ20,881). Rats with a decrease in blood pressure of less than 20 mm Hg, in response to both inhibitors, were classified as nonresponders; those with a decrease of 20 mm Hg or more, as responders. Fifty percent of the rats with two-kidney hypertension were nonresponders, and they had lower blood pressure and plasma renin activity than the responders. Further, these two-kidney hypertensive, nonresponder rats had normal exchangeable sodium. The two-kidney hypertensive responders, on the other hand, had significantly higher exchangeable sodium than both the two-kidney, hypertensive nonresponders and the two-kidney control rats. These results suggest that angiotensin II and exchangeable sodium do not play a major role in the maintenance of the high blood pressure in the two-kidney hypertensive nonresponders. However, there appears to be an abnormal relationship between renin and exchangeable sodium in the two-kidney hypertensive responders that could contribute to the maintenance of the hypertension.

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KEY WORDS • renovascular hypertension • sodium • angiotensin II • blood pressure • angiotensin antagonist • converting enzyme inhibitor

ANGIOTENSIN and sodium appear to play differing roles in the pathogenesis of the maintenance of high blood pressure in one- as compared to two-kidney hypertensive rats (one renal artery constricted and the contralateral kidney removed or untouched, respectively). In one-kidney hypertensive rats, sodium retention appears to be the main pathogenetic factor in the chronic phase of the hypertension. In fact, Tobian et al.\(^1\) have reported an increase in exchangeable sodium, and Swales et al.\(^2\) reported a positive cumulative sodium balance in this model of hypertension in rats. In the two-kidney model, angiotensin II has been considered the most important pathogenetic factor.\(^3\) However, in previous work, we have found that in rats with moderate, chronic, two-kidney hypertension, the maintenance of high blood pressure does not always depend on the pressor effect of angiotensin II, since many rats do not respond to the administration of an angiotensin antagonist\(^4\) or a converting enzyme inhibitor (CEI) with a decrease in blood pressure.\(^5\) Furthermore, Möhring et al.\(^6\) have reported a positive cumulative sodium balance and a positive correlation between the increase in blood pressure and sodium retention in rats with moderate two-kidney hypertension.

It could be that the high blood pressure in the two-kidney model is maintained by a dual mechanism: one related to sodium metabolism when the hypertension is moderate, and the other to the renin-angiotensin system when the hypertension is more severe. To further advance this hypothesis, in the present study we measured exchangeable sodium in rats with...
chronic renovascular hypertension in which angiotensin II had been previously confirmed or excluded as the main cause of the hypertension.

Methods

Male Sprague-Dawley rats, weighing between 150 and 200 g and fed Purina rat chow (0.42% sodium content) and tap water ad libitum, were used in this study. Hypertension was induced by placing a U-shaped silver clip, with an internal gap of 0.23 or 0.25 mm, around the left renal artery; the contralateral kidney was left untouched (two-kidney hypertensive rats) or removed (one-kidney hypertensive rats). Sham-clipped, two-kidney rats were used as controls for the two-kidney hypertensive rats. Sham-clipped rats, in which either 50% or 70% of the renal mass was removed by excising the right kidney or by excising the right kidney and both poles of the left kidney, respectively, were used as controls for the one-kidney hypertensive rats. To avoid hemorrhage when the poles of the kidney were sectioned, a piece of absorbent hemostat (oxidized regenerated cellulose: Surgicel) was placed on each incision. All surgical procedures were done under ether anesthesia.

Systolic blood pressure was measured weekly by the tail-cuff method in unanesthetized rats. Body weight was also measured weekly and rats that did not show normal growth when compared to the control group were excluded from the study. Eight to 14 weeks after clipping, or sham-clipping, the blood pressure response to the angiotensin antagonist, 1-Sar-8-Ala-angiotensin II, and converting enzyme inhibitor, SQ20,881, was studied in each rat. For this purpose, PE 10 catheters were chronically implanted into the abdominal aorta and inferior vena cava, through the femoral artery, and the femoral vein. Both catheters were led subcutaneously to the scapular region of the rat, as previously described. Two to 3 days later, direct mean blood pressure (BP) was recorded by a pressure transducer (Micron MP 15) and four-channel recorder (Brush 440). During the experiment, unanesthetized rats were kept in cages and were partially restricted by a harness attached to a spring on their back. After the blood pressure had stabilized, 100 ng/rat of angiotensin I (1-Asp-5-Ile-Ang I), angiotensin II (Hypertensin, CIBA), and 1-norepinephrine (Levophed, Winthrop) were administered as separate bolus injections, via the venous catheter. All injections were given in a volume of 0.1 ml, followed by 0.2 ml of 5% dextrose. Subsequently, the angiotensin antagonist was infused for 1 hour through the venous catheter at 4 µg/min/rat, in a 5% dextrose solution, using a Harvard pump at 7.9 µl/min. The BP was continuously recorded during the infusion. One to 4 hours later, when the blood pressure had returned to control levels, 4 mg/kg of the CEI was injected, as a bolus, through the venous catheter, and the BP was continuously recorded for the next 60 minutes. Rats showing a decrease in BP of 20 mm Hg or more either to the angiotensin antagonist or to the CEI were classified as responders; those with an increase, no change, or a decrease of less than 20 mm Hg were classified as non-responders. Response to the pressor substances was again obtained at the end of the infusion of the angiotensin antagonist and within 30 minutes after injection of CEI.

Blood (0.5 ml) was drawn from each rat for hematocrit and plasma renin activity (PRA) determination before and after both inhibitors were administered. The same amount of blood that was drawn was immediately replaced with blood obtained from nephrectomized donor rats. Rats in which the hematocrit was lower than 40% were excluded from the study, since this could indicate internal bleeding caused by surgical handling. This bleeding could alter renin release and the response to both inhibitors. In addition, all the rats in which the body weight decreased 10% or more during the 2 days following surgery were excluded from this study. A significant decrease in body weight could indicate that the rat was not eating; this could produce a negative sodium balance which, in turn, could alter the exchangeable sodium and/or the response to both peptides. The hematocrit was determined by centrifuging the blood samples at 2000 rpm at 4°C for 20 minutes. The PRA was determined by using a modification of the radioimmunoassay method of Haber et al. as previously described. The PRA was expressed as ng of generated angiotensin I, per ml of plasma, per hour of incubation.

Exchangeable Sodium

Exchangeable sodium was measured by the method of Miller and Wilson as modified by Tobian et al. For this, 48 to 72 hours after the infusion of the angiotensin antagonist and CEI, the rats were nephrectomized through a flank incision, and injected with 0.2 to 0.4 μEq of 42Na+ (42NaCl) through the venous catheter. The 42Na+ was given in a volume of 0.2 ml of 5% dextrose and followed by another 0.2 ml of dextrose to flush the catheter. The rats were then placed in metabolic cages for 24 hours without food. This fixed period of time was used for equilibration of the radioactive sodium. The metabolic cages were used for the collection of feces. Note that no urine collection was done, since the rats were nephrectomized before the injection of 42Na+. At the end of this equilibration period, the animals were bled through the arterial catheter. The same amount of 42Na+ that had been injected into each rat was added to each of four volumetric flasks, which were then filled with water and used as a standard. Since the half-life of 42Na+ is only 15 hours, 1 ml of each standard and 1 ml of serum of each rat were counted at the same time, and the average counts per minute (cpm) of the four standards were used to calculate the number of counts that had been injected in each rat. Because serum samples and standards were counted simultaneously, it was not necessary to correct for radioactive decay. The activity of serum samples was usually more than 10 times that of the background activity, and counting errors were less than ± 1%. The
fecal material of each rat was collected and its radioactivity measured. The cpm of the feces was discounted from the total counts injected in each rat. The \(^{22}\)Na\(^+\) concentration per ml of serum was measured using a flame photometer with lithium as an internal standard (Model 143, Instrumentation Laboratory Lexington, MA). The exchangeable sodium per rat was calculated using the following formula recommended by Miller and Wilson\(^6\):

\[
\text{cpm injected} - \text{cpm in feces}} \times (^{22}\text{Na}^+ \text{mEq/ml serum}) = \text{cpm/\text{ml serum}}
\]

**Experimental Groups**

The following groups were studied: 1) Sham-clipped, two-kidney controls (15 rats); 2) Two-kidney, borderline, hypertensive nonresponders (18 rats). In these rats the BP before the angiotensin antagonist (initial BP) was lower than 140 mm Hg; 3) Two-kidney hypertensive nonresponders (14 rats). In these rats, the initial BP was 140 mm Hg or higher and the BP either increased, did not change, or decreased less than 20 mm Hg in response to the angiotensin antagonist and CEI; 4) Two-kidney hypertensive responders (15 rats). The initial BP of these rats was also 140 mm Hg or higher and the BP decreased 20 mm Hg or more in response to angiotensin antagonist or CEI. Four rats included in this group were classified as malignant hypertensive according to the criteria previously established by Mohring et al.; 5) Sham-clipped, one-kidney controls (14 rats); 6) Controls with 70% of the renal mass removed (12 rats); 7) One-kidney hypertensive nonresponders (12 rats).

All results are expressed as a mean ± SE. The significance between individual groups was determined by analysis of variance, and the significance of the differences among all groups of rats was determined by pair t test.

**Results**

The infusion of 4 \(\mu\)g/min/rat of the angiotensin antagonist completely blocked the pressor effect of angiotensin I and angiotensin II, but did not change the pressor effect of 1-norepinephrine. The bolus injection of 4 mg/kg of body weight of CEI completely blocked the pressor effect of angiotensin I, but did not affect the pressor response of angiotensin II or 1-norepinephrine.

**Blood Pressure Response to the Angiotensin Antagonist and CEI**

Table 1 summarizes the data regarding BP before (initial BP) and after both inhibitors for the seven groups of rats. The initial BP was significantly lower \((p < 0.001)\) in two-kidney hypertensive, nonresponder rats (Group 3) than in two-kidney hypertensive responders (Group 4).

In two-kidney hypertensive rats (Groups 2, 3 and 4), BP response to the angiotensin antagonist significantly correlated with BP response after CEI (fig. 1). Changes in BP during the angiotensin antagonist infusion and after administration of the CEI significantly correlated with the BP before treatment \((r = 0.77, p < 0.01; \text{and } r = 0.72, p < 0.01, \text{respectively})\).

**Exchangeable Sodium**

Figure 2 shows the individual values and the average mean values ± se of the exchangeable sodium in two-kidney (left panel) and in one-kidney rats (right panel). The exchangeable sodium of the two-kidney, borderline, hypertensive nonresponders (Group 2) and the two-kidney hypertensive nonresponders (Group 3) was not significantly different from the exchangeable sodium of the sham-clipped, two-kidney controls (Group 1). In the two-kidney hypertensive responders (Group 4), the exchangeable sodium was significantly higher \((p < 0.01)\) than in the sham-clipped, two-kidney control rats (Group 1).

Although the exchangeable sodium was higher in the sham-clipped, one-kidney controls (Group 5) than in the two-kidney controls (Group 1), the difference was not statistically significant \((p > 0.05)\). In the control rats with 70% of the renal mass removed (Group 6) and in the one-kidney hypertensive rats (Group 7), the exchangeable sodium was significantly higher than in the sham-clipped, two-kidney control rats (Group 1) \((p < 0.01)\). The highest mean value of exchangeable sodium was found in one-kidney hypertensive rats (Group 7). The average exchangeable sodium of the two-kidney hypertensive responders (Group 4) and of the one-kidney hypertensive rats (Group 7) was not significantly different. The exchangeable sodium of the one-kidney hypertensive rats was significantly higher than in the sham-clipped, one-kidney controls (Group 5) and in the controls with 70% of the renal mass removed (Group 6) \((p < 0.01 \text{ and } p < 0.05, \text{respectively})\). Exchangeable sodium significantly correlated with the initial BP in two-kidney hypertensive...
TABLE 1. Mean Blood Pressure (BP) Before and During the Angiotensin Antagonist (Ang. Ant.) Saralasin and Before and After the Converting Enzyme Inhibitor (CEI) SQ20,881

<table>
<thead>
<tr>
<th>Group</th>
<th>Two-kidney rats</th>
<th>One-kidney rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Ang. Ant.</td>
<td>During Ang. Ant.</td>
</tr>
<tr>
<td>1 - Sham-clipped</td>
<td>113 ± 1.6 (15)</td>
<td>116 ± 2.2</td>
</tr>
<tr>
<td>2 - Borderline hyper.</td>
<td>123 ± 1.7 (18)</td>
<td>122 ± 2.1</td>
</tr>
<tr>
<td>3 - Hyper, nonresponder</td>
<td>156 ± 2.4† (14)</td>
<td>152 ± 2.2†</td>
</tr>
<tr>
<td>4 - Hyper, responder</td>
<td>185 ± 4.6† (15)</td>
<td>138 ± 5.5†</td>
</tr>
<tr>
<td>5 - Sham-clipped</td>
<td>108 ± 2.2 (14)</td>
<td>110 ± 2.8</td>
</tr>
<tr>
<td>6 - 70% Renal mass removed</td>
<td>115 ± 3.5 (12)</td>
<td>112 ± 4.5</td>
</tr>
<tr>
<td>7 - Hypertensive</td>
<td>193 ± 5.8† (12)</td>
<td>189 ± 5.8†</td>
</tr>
</tbody>
</table>

Values are means ± se; numbers in parentheses indicate the number of rats in each group. p values refer to the differences between before and during Ang. Ant. and before and after CEI. Differences from the corresponding Control (Group 1 or 5) are represented by: *p ≤ 0.05; †p ≤ 0.01.

No significant correlation was found between initial BP and exchangeable sodium in one-kidney hypertensive rats (Group 7) (r = −0.44).

Discussion

The present study was designed to investigate whether or not exchangeable sodium is increased in rats with renovascular hypertension in which the maintenance of high blood pressure is not dependent on the vasoconstrictor effect of angiotensin II. The degree of angiotensin participation in the maintenance of high blood pressure was studied in each rat by blocking the renin-angiotensin system with angiotensin antagonist (1-Sar-8-Ala-angiotensin II) and with CEI (SQ20,881). By using these two peptides in tandem, it is possible to define more precisely the participation of angiotensin II in the maintenance of high blood pressure. The decrease in blood pressure produced by angiotensin antagonist significantly correlates with the decrease produced by CEI (r = 0.91). However, the CEI was slightly more effective than the angiotensin antagonist in decreasing blood pressure in the normotensive rats and in the nonresponder rats (table 1).

In all seven groups, PRA significantly increased after administration of the angiotensin antagonist and CEI (table 2). This increase in PRA could be explained by the inhibition of the angiotensin negative feedback that normally participates in the control of renin release. The increase after the angiotensin antagonist was less than after the CEI. The agonistic effect of the angiotensin antagonist may explain this smaller increase.

In the two-kidney hypertensive rats (Groups 2, 3, and 4), the decrease in blood pressure after the angiotensin antagonist and CEI significantly correlated with the BP before treatment. Thus, we have confirmed previous studies from our laboratory which found that, in this model, there is maximal participation of the renin-angiotensin system in maintaining elevated blood pressure in severe or malignant hypertension but little or no participation in moderate hypertension. The decrease in blood pressure...
These increased water intake, and loss of body weight. had BP greater than 200 mm Hg, very high PRA, in-

classified as having malignant hypertension, since they

our nonresponders (Groups 2 and 3); whereas they

contrary to what we expected, since Mihring et al.

(\text{table 1}).

observed between the decrease in BP produced by both

inhibitors, and to the great variability normally

found in the \text{PRA} in rats.\textsuperscript{16} Furthermore, the correla-
tion between \text{PRA} and initial BP was also low (r =

0.56), suggesting the limited participation of renin in

this type of hypertension.

In the one-kidney model, no correlation was

observed between the decrease in BP produced by both

inhibitors and the initial BP or the initial \text{PRA}.

Furthermore, the decrease in BP after the administra-
tion of both peptides was always less than 20 mm Hg
(table 1).

The reason for measuring exchangeable sodium

after the degree of participation of angiotensin II in

the maintenance of the blood pressure had been deter-

mined was to ascertain if angiotensin and sodium are

alternative mechanisms in the maintenance of high

blood pressure in renovascular hypertension.\textsuperscript{17}

However, our results in the two-kidney hypertensive

rats do not lend support to this hypothesis, since the

rats with angiotensinogenic hypertension (responders)
have high exchangeable sodium while the rats with

non-angiotensinogenic hypertension (nonresponders)
have normal exchangeable sodium. These results were

contrary to what we expected, since Möhring et al.\textsuperscript{4}
have reported a positive sodium balance that cor-

related with the increase in blood pressure in the two-

kidney rats with moderate hypertension, equivalent to

our nonresponders (Groups 2 and 3); whereas they

reported a negative sodium balance in rats with more

severe or malignant hypertension, equivalent to our

responders (Group 4). In our Group 4, four rats were

classified as having malignant hypertension, since they

had BP greater than 200 mm Hg, very high \text{PRA},

increased water intake, and loss of body weight.\textsuperscript{6} These

rats also had high exchangeable sodium (fig. 2). Our

study differs from Möhring’s in two respects: 1) we
determined whether or not the hypertension was

angiotensinogenic in origin; and 2) we measured ex-

changeable sodium instead of sodium balance. It is

possible that by grouping the rats on the basis of their

response to the angiotensin antagonist and CEI, we

have formed different groups than when the rats are
categorized according to the severity of the hyperten-
sion. Although no good explanation was forthcoming,

the high exchangeable sodium in the two-kidney,
hypertensive, responder rats could be explained by the

possibility that they have secondary aldosteronism,

which is supported by the fact that these rats have very

high \text{PRA}. Although plasma aldosterone was not

measured, it is logical to assume that it was elevated

since the renin-angiotensin system controls

aldosterone released by the adrenal glands in the rat,
as in other species.\textsuperscript{18, 19} These results in the two-

kidney, hypertensive, responder rats (Group 4) agree

with those reported by Doyle et al.\textsuperscript{20} that showed

high exchangeable sodium in two-kidney hypertensive

rats with severe hypertension and high \text{PRA}. Both our

results and those of Doyle et al. conflict with those of

Tobian et al.,\textsuperscript{1} who reported normal exchangeable

sodium in rats with two-kidney hypertension. In

Tobian’s study, the rats were not grouped according to

their angiotensin dependency, severity of hyperten-
sion, or \text{PRA}; and the intermixing of nonresponder

and responder rats could explain these varying results.

It is not possible to say if the increase in ex-

changeable sodium participates in the maintenance of

the high blood pressure in the two-kidney hypertensive

responder. However, after blockage of the pressor

effect of angiotensin II by the angiotensin antagonist

or \text{CEI}, the blood pressure was still higher than in the

control group. Thus, it could be that the difference

between the BP in the control group and in the

responders after the blockage of angiotensin was

\begin{table}
\centering
\caption{Plasma Renin Activity (PRA) Before and After the Angiotensin Antagonist (Ang. Ant.) Saralasin and Converting Enzyme Inhibitor (CEI) SQ80,881}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Groups} & \multicolumn{2}{c|}{\textbf{Before Ang. Ant.}} & \multicolumn{2}{c|}{\textbf{After Ang. Ant.}} & \textbf{p Value} \\
\hline
\textbf{Before CEI} & \textbf{After CEI} & \textbf{Before CEI} & \textbf{After CEI} & \textbf{p Value} \\
\hline
\textbf{Two-kidney rats} & & & & & \\
1 - Sham-clipped & 2.3 & 0.6 & (15) & 4.7 & 1.7 & <0.05 & 2.6 & 1.0 & (11) & 29.9 & 11.8 & <0.01 \\
2 - Borderline hyper. & 4.2 & 0.7 & (18) & 20.9 & 6.6 & <0.01 & 4.2 & 1.0 & (18) & 38.3 & 6.7 & <0.001 \\
3 - Hyper. nonresponder & 7.6 & 2.1 & (14) & 39.1 & 11.2 & <0.01 & 12.1 & 3.6 & (13) & 88.6 & 16.8 & <0.001 \\
4 - Hyper. responder & 27.4 & 4.5 & (15) & 94.1 & 18.8 & <0.001 & 30.6 & 4.9 & (13) & 112.9 & 18.3 & <0.001 \\
\hline
\textbf{One-kidney rats} & & & & & \\
5 - Sham-clipped & 3.9 & 0.9 & (14) & 7.5 & 2.2 & <0.05 & 3.6 & 0.9 & (14) & 34.8 & 9.6 & <0.01 \\
6 - 70\% Renal mass removed & 2.6 & 0.8 & (12) & 20.4 & 8.0 & <0.05 & 3.6 & 1.2 & (12) & 21.4 & 7.2 & <0.01 \\
7 - Hypertensive & 5.6 & 0.9 & (12) & 27.4 & 8.2 & <0.01 & 6.8 & 1.4 & (11) & 63.1 & 11.4 & <0.001 \\
\hline
\end{tabular}
\end{table}

Values are means ± se; numbers in parentheses indicate the number of rats in each group. p values refer to the differences between before and after Ang. Ant. or CEI. Differences from the corresponding Control (Group 1 or 5) are represented by *p ≤ 0.05; †p ≤ 0.01.
caused by sodium retention. Yet, most of the increase in blood pressure was angiotensinogenic in origin. In two-kidney hypertensive, nonresponder rats the mean decrease in blood pressure after the angiotensin antagonist and CEI was only 4 and 8 mm Hg, respectively. These findings suggested that very little of the hypertension was angiotensinogenic in origin. Furthermore, since the exchangeable sodium in these rats was normal, it is difficult to explain the mechanism of their high blood pressure.

In the one-kidney hypertensive rats, the exchangeable sodium was significantly higher than in the one- and two-kidney control rats. Tobian et al.\textsuperscript{1} have reported similar results; however, they used only two-kidney hypertensive and two-kidney normotensive rats as controls. In our study, reduction of the renal mass produced an increase in exchangeable sodium that reached statistical significance when 70\% of the renal mass was removed. Thus, part of the increase in exchangeable sodium in the one-kidney hypertensive rats, may be caused by a reduction in the renal mass. However, this reduction does not, by itself, explain the total increase since the exchangeable sodium in the one-kidney hypertensive rats was even higher than in the control rats in which 70\% of the renal mass was removed. In the one-kidney hypertensive rats, the decrease in blood pressure after the angiotensin antagonist and CEI was 4 and 12 mm Hg, respectively. As with the two-kidney hypertensive, nonresponder rats, a very small part of the hypertension in this group was angiotensinogenic in origin. The exchangeable sodium in these rats was the highest of any group, which makes it inviting to postulate that the hypertension was due to sodium retention. Yet individually many of these rats have an exchangeable sodium similar to the normotensive rats from the control group with 70\% of the renal mass removed. Therefore, it would not be logical to assume that the hypertension was due only to an increase in exchangeable sodium.

An increase or decrease in exchangeable sodium does not necessarily mean that it will or will not participate in maintaining high blood pressure. It could be that sodium distribution is more important than its absolute amount. It has been reported that sodium is increased in arterioles during hypertension.\textsuperscript{21,23} It could be that this alteration in its distribution produces an increase in peripheral resistance and, consequently, in blood pressure, without altering exchangeable sodium.

In conclusion, exchangeable sodium was normal in two-kidney hypertensive rats in which the hypertension was not angiotensinogenic in origin. On the other hand, in the two-kidney hypertensive rats with angiotensinogenic hypertension, exchangeable sodium was increased, probably due to the development of secondary aldosteronism. In the one-kidney hypertensive rats, exchangeable sodium was increased. This increase could be due, at least in part, to the reduction of the renal mass. Finally, although an increase in ex-
changeable sodium could contribute to the maintenance of high blood pressure, the hypertension cannot be explained solely by this increase.

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