Stimulation of Renin by Acute Selective Chloride Depletion in the Rat

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SUMMARY To determine whether acute chloride depletion per se stimulates renin, we produced selective chloride depletion without sodium depletion in rats by peritoneal dialysis (PD) against 0.15 m NaHCO₃ or 0.15 m NaNO₃. Control rats were dialyzed against 0.15 m NaCl. Plasma renin activity (PRA) was measured before (PRAi) and 105 minutes after (PRAf) PD. Plasma volume was expanded after PD by infusion of salt-free albumin and was measured immediately after PRAf by [¹²⁵I]albumin. In experiment 1, rats were prepared on a normal diet. PRAi (7.0 ± 1.0 ng/ml per hr, mean ± SEM) was increased (P < 0.05) over PRAi (4.7 ± 0.7 ng/ml per hr) in Cl-depleted but not in control rats (PRAi = 3.5 ± 0.7, PRAf = 6.1 ± 0.7, P = NS). In experiment 2, to produce greater chloride depletion, all rats were prepared for 2 weeks on a low salt diet. PRAi (47 ± 5 ng/ml per hr) was increased as compared to PRAi (24 ± 2 ng/ml per hr, P < 0.005) in the Cl-depleted group but not in the control group (PRAi = 24 ± 3, PRAf = 27 ± 6 ng/ml per hr, P = NS). Serum potassium and final plasma volume were slightly but not significantly lower than controls in these Cl-depleted rats. To exclude an additive effect of these two stimuli for renin, in experiment 2a we infused chloride-depleted rats with three times as much albumin as controls and with KHCO₃, 100 mEq/liter. Despite volume expansion and potassium loading, PRAi (41 ± 6 ng/ml per hr) was significantly elevated as compared to PRAi (25 ± 4 ng/ml per hr, P < 0.01). Since acute metabolic alkalosis also was present in all Cl-depleted renin-stimulated rats, an additional group (2b) was dialyzed against 0.15 m NaNO₃; final plasma arterial pH (7.43) was not different from controls (7.42). Nevertheless, PRAi levels again were higher (36 ± 6 ng/ml per hr, P < 0.05) over PRAi (24 ± 2 ng/ml per hr) in Cl-depleted but not in control rats (PRAi = 0.3 ± 0.7, PRAi = 0.1 ± 0.7, P = NS). Serum potassium and final plasma volume were slightly but not significantly lower than controls in these Cl-depleted rats. To exclude an additive effect of these two stimuli for renin, in experiment 2a we infused chloride-depleted rats with three times as much albumin as controls and with KHCO₃, 100 mEq/liter. Despite volume expansion and potassium loading, PRAi (41 ± 6 ng/ml per hr) was significantly elevated as compared to PRAi (25 ± 4 ng/ml per hr, P < 0.01). Since acute metabolic alkalosis also was present in all Cl-depleted renin-stimulated rats, an additional group (2b) was dialyzed against 0.15 m NaNO₃; final plasma arterial pH (7.43) was not different from controls (7.42). Nevertheless, PRAi levels again were higher (36 ± 6 ng/ml per hr, P < 0.05) as compared to PRAi (23 ± 4 ng/ml per hr). In all experiments, arterial blood pressure, glomerular filtration rate, and filtered sodium load were not different. Free water reabsorption was lower in Cl-depleted than in control rats. We conclude that acute selective chloride depletion per se is a potent stimulus for renin release. Circ Res 44: 815-821, 1979

RENIN RELEASE is controlled primarily by two intrarenal receptors, a renal vascular receptor and a renal tubular receptor at the macula densa (Davis and Freeman, 1976). Renal arterial infusion of hypertonic sodium chloride or potassium chloride inhibits renin secretion by the intact dog kidney but not by the nonfiltering kidney (Shade et al., 1972), indicating that renin inhibition in these circumstances is dependent on glomerular filtration. There is recent evidence both for man (Tuck et al., 1974) and for dogs (Opava-Stitzer and Martinez-Maldonado, 1976) that renin inhibition by volume expansion with isotonic saline is dependent primarily on a macula densa mechanism responsive to sodium chloride transport. We have demonstrated (Kotchen et al., 1976, 1978; Kirchner et al., 1979) in the rat that suppression of renin by both acute and chronic sodium chloride loading is dependent on the renal effects of the administered chloride rather than on those of sodium. Since active chloride transport is the primary absorptive process in the thick ascending limb of the loop of Henle (TAL) (Rocha and Kokko, 1973; Burg and Green, 1973), changes in the absorption of chloride would likely also alter secondarily transport of sodium and possibly of other cations at that site. We have proposed that the signal for renin release via the macula densa mechanism relates to absorptive chloride transport in the TAL.

This hypothesis would predict that chloride depletion, if it resulted in reduced chloride transport in the TAL, would stimulate renin secretion. In many clinical and experimental circumstances, however, chloride depletion is accompanied by volume depletion, which might stimulate renin via the renal vascular receptor. We have employed our recently described (Khanh and Luke, 1976) model of selective chloride depletion, without sodium or volume depletion, to determine whether chloride depletion per se stimulates renin release in the rat. Chloride depletion produced by peritoneal dialysis (PD) against NaHCO₃ or NaNO₃, with maintenance of volume by intravenous administration of albumin, stimulated renin release. However, renin did not change in rats dialyzed against NaCl, despite the absence of differences in arterial blood pressure or pH, plasma volume, glomerular filtration rate (GFR), filtered sodium load, or sodium balance between the Cl-depleted and control groups.
Methods

Male Sprague-Dawley rats weighing 220-330 g were studied. Selective chloride depletion was produced by PD against NaHCO₃ or NaNO₃, and control rats were dialyzed against NaCl. One control and one experimental rat were studied simultaneously. After induction of anesthesia with Inactin (Promonta), 100 mg/kg body weight, ip, a tracheostomy was performed, and catheters (PE 50) were placed in the left external jugular vein for infusion of fluid and in the right femoral artery to monitor blood pressure (Statham pressure transducer P23dc and Gibson polygraph model MSP) and collect arterial blood samples. Inulin, 5% in 0.15 m NaHCO₃, was infused throughout the experiment at a rate of 0.5 ml/100 g body weight per hr. After a 30-minute equilibration period, chloride depletion was produced, as previously described (Khanh and Luke, 1976), by a single exchange PD against 20 ml of a solution containing glucose (15 g/liter), NaHCO₃ or NaNO₃ (150 mmol/liter), and KHCO₃ (4 mmol/liter). Dwell time was 30 minutes and the returned dialysate was collected for measurement of volume and of sodium, potassium, and chloride content. Control rats were dialyzed against a solution containing glucose (15 g/liter), NaCl (150 mmol/liter), and KHCO₃ (4 mmol/liter). After PD, 6% salt-free bovine plasma albumin (Sigma Chemical Co.) dissolved in distilled water was infused over 15 minutes in all groups to ensure maintenance, at least, of plasma volume. After a 30-minute equilibration period, a bladder catheter was inserted into a suprapubic cystostomy, and urine was collected under mineral oil in preweighed containers during two 30-minute periods (Ci and C₂) for determination of inulin clearance, sodium, potassium, chloride, and osmolality. Plasma inulin was measured at the beginning and at the end of each clearance period. Plasma renin activity (PRA) was measured before dialysis (PRA₁) and at the end of the second clearance period (PRA₂). Immediately after obtaining blood for PRA₂, we infused [¹³¹I]-albumin to measure plasma volume (Belcher and Harriss, 1957). Ten minutes later, arterial blood was obtained for measurements of plasma electrolyte concentrations, arterial pH and Pco₂, plasma osmolality, and plasma volume. Arterial hematocrit also was measured immediately before PD and at the end of the experiment (hematocrit 1 and hematocrit 2) to estimate fractional change in plasma volume. Total volume of blood drawn in each group, including that for measurement of PRA₂, was 0.8 ml.

In the initial studies (experiment 1), rats were maintained on normal rat chow until the day of the experiment. Nine control rats weighed 260 ± 10 g (mean ± SEM), and nine experimental rats weighed 253 ± 7 g (P = NS). To produce a more profound chloride depletion, we performed an additional series of studies (experiment 2) in rats prepared for 2 weeks by a low salt diet (ICN Pharmaceuticals, Inc.), which contained less than 2 µEq/g of sodium and of chloride, as determined by nitric acid extraction in our laboratory. Eight control rats weighed 249 ± 4 g, and eight experimental rats weighed 244 ± 7 g (P = NS) at the time of study. In both experiments 1 and 2, to ensure that plasma volume was at least as great in the experimental as in the control rats, we infused salt-free albumin (Sigma Chemical Co.), 0.75 and 0.5 ml/100 g body weight, in the experimental and control groups, respectively. Based on previous experiments (Khanh and Luke, 1976), these volumes of albumin were infused after PD to restore the hematocrit to at least its predialysis value. In experiment 2, final plasma potassium and plasma volume were not statistically different in the experimental as compared to the control rats; however, in each case the value was lower in the experimental group. To exclude the possibility that these factors (i.e., hypokalemia or a decreased plasma volume) were not, singly or together, contributing to stimulation of PRA in the experimental chloride-depleted rats, we studied an additional 13 rats (group 2a, weighing 289 ± 8 g), and the volume of infused salt-free albumin was increased to 1.5 ml/100 g body weight (i.e., three times control); serum potassium was maintained by increasing the potassium concentration in the PD fluid to 25 mEq/liter and by adding KHCO₃, 100 mEq/liter, to the maintenance sodium bicarbonate-inulin infusion.

Acute metabolic alkalosis was present in all rats dialyzed against NaHCO₃. To exclude this acid-base change as a cause of renin stimulation in the experimental groups, we dialyzed an additional seven rats (group 2b, weighing 242 ± 6 g) against a solution containing glucose, 15 g/liter; NaNO₃, 150 mmol/liter; and KHCO₃, 4 mmol/liter. After PD, 6% salt-free bovine plasma albumin was infused (0.75 ml/kg) over 15 minutes as in experiments 1 and 2 to maintain plasma volume. Inulin, 5% in 0.15 NaNO₃, was infused throughout the experiment at a rate of 0.5 ml/100 g body weight per hr (similar to experiments 1 and 2).

A summary of the diets, dialysates, and infusions used in the various groups is given in Table 1. Sodium and potassium concentrations in plasma, urine, and dialysate fluid were determined by a flame photometer with an internal lithium standard (Instrumentation Laboratory, Inc.), and plasma, urine, and dialysate chloride were measured by a chloridometer (Buchler Instruments Division, Nuclear-Chicago). Arterial pH and Pco₂ were measured by an IL blood-gas analyzer, and plasma bicarbonate was derived by the Siggaard-Andersen nomogram. Urinary and plasma osmolality were measured by a Fiske osmometer (Fiske Associates, Inc.). Plasma volume was estimated by the method of Belcher and Harriss (1957), with appropriate correction for plasma trapping. PRA was measured in quadruplicate by the radioimmunoassay proce-
The enzyme responsible for the conversion of angiotensin I to angiotensin II is chloride-dependent (Erdos, 1976), and we have shown previously that differences in serum chloride concentrations do not affect the measurement of PRA (Kotchen et al., 1978).

Calculations

Electrolyte balance for PD was determined by subtracting the amount returned in the dialysate from that instilled. The change in plasma volume from before PD to the end of the experiment was calculated from hematocrit 1 and 2 according to the following formula (Galla et al., 1977):

$$\Delta PV (\%) = \left[ \frac{(hct_1/hct_2 - 1)}{1 - hct_1} \right] \times 100$$

In a recent study in rats by Maddox et al. (1977), change in plasma volume calculated from change in hematocrit corresponds closely with the change measured by $[^{131}I]$albumin. Free water reabsorption ($T_{\text{H}_{2}O}$) was calculated according to the formula:

$$T_{\text{H}_{2}O} = C_{\text{osm}} - V,$$

where $C_{\text{osm}} = (U_{\text{osm}} \times V)/P_{\text{osm}}$, $V$ = urinary output in ml/min, and $U_{\text{osm}}$ and $P_{\text{osm}}$ are urinary and plasma osmolalities, respectively, in mOsmol/kg plasma water.

Comparison within groups was made by a paired Student’s $t$-test, and between groups by an unpaired $t$-test.

Results

In both experiments 1 and 2, dialysis against NaHCO₃ produced a negative chloride balance (Table 2) and hypochloremic metabolic alkalosis (Table 3) as compared to a positive chloride balance and normal acid-base status in the control groups dialyzed against NaCl. Sodium balance for PD was less positive in the experimental group (Table 2), and the higher potassium concentration in the dialysate in group 2a was associated with a more positive potassium balance and a negative water balance as compared to group 2 controls. In both experiments 1 and 2, plasma sodium and potassium concentrations did not differ between experimental and control groups, and the additional potassium administration in group 2a resulted in the same mean plasma potassium concentration as in group 2 controls (Table 3). Dialysis against NaN₃ produced no change in acid-base status as compared to control (Table 3).

Mean blood pressure did not change significantly in any group during the experiment and did not differ among groups (Table 4). Likewise, GFR was not different between control and chloride-depleted rats (Table 4). Therefore, since plasma sodium also was not different, filtered sodium load was not different between the control and experimental groups. Despite this absence of difference, as previously observed (Luke et al., 1977), sodium excretion during the clearance period was greater in the chloride-depleted groups (Table 4). Nevertheless,
Table 3: End Plasma Electrolytes, Acid-Base Status, and Plasma Volume in Chloride-Depleted and Control Rats

<table>
<thead>
<tr>
<th>Experiment 1 (normal diet)</th>
<th>Control</th>
<th>Cl-depleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Na⁺ (mEq/liter)</td>
<td>138</td>
<td>4.6*</td>
</tr>
<tr>
<td>K⁺ (mEq/liter)</td>
<td>5.0</td>
<td>4.6*</td>
</tr>
<tr>
<td>Cl⁻ (mEq/liter)</td>
<td>100</td>
<td>81*</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/liter)</td>
<td>21</td>
<td>32†</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>35</td>
<td>45†</td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
<td>7.47†</td>
</tr>
<tr>
<td>FV (ml/100 g body wt)</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>ΔPV (%)</td>
<td>+14</td>
<td>+16</td>
</tr>
<tr>
<td>Po₂ (mOsmol/kg)</td>
<td>297</td>
<td>288</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.

* P < 0.001 as compared to control.
† P < 0.05 as compared to control.
‡ P < 0.001 as compared to control.

cumulative sodium balance remained positive in all groups because of the maintenance infusion. Plasma volumes and plasma osmolalities did not differ between chloride-depleted and control groups in either experiment 1 or experiment 2 (Table 3). Calculated change in plasma volume showed an increase within every group (P < 0.05, except for group 2b); this was not different between the chloride-depleted groups and controls (Table 3).

Despite the absence of differences in blood pressure, GFR, filtered sodium load, plasma osmolality, plasma volume, and percent increase in plasma volume between control and experimental groups, PRA increased significantly after chloride depletion in experiment 1 (Fig. 1, from 4.7 ± 0.7 to 7 ± 1.0 ng/ml per hr, mean ± SEM, P < 0.005) and in experiment 2 (Fig. 2, from 24 ± 2 to 47 ± 5 ng/ml per hr, P < 0.005), but did not change within the control groups in response to dialysis against NaCl. After dietary salt restriction (experiment 2, Fig. 2) PRA, levels (prior to PD) were significantly higher (a 5-fold increase) in all groups than those obtained after a normal salt intake in experiment 1 (compare Figures 1 and 2, P < 0.001). In experiment 1 (normal NaCl diet), PRA₂ (final plasma) levels of control and experimental groups did not differ significantly (Fig. 1). However, PRA₂ levels were significantly different between the control and Cl-depleted groups in experiment 2 (Fig. 2, P < 0.05).

Although plasma potassium and plasma volume of control and chloride-depleted groups did not differ significantly, the level of each was less in

Table 4: Glomerular Filtration Rate, Blood Pressure, Electrolyte Excretion, and Free Water Absorption in Chloride-Depleted and Control Rats

<table>
<thead>
<tr>
<th>Experiment 1 (normal diet)</th>
<th>Control</th>
<th>Cl-depleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>GFR (µl/min per 100 g)</td>
<td>816</td>
<td>808</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>UVₙa (µEq/min)</td>
<td>1.0</td>
<td>2.5‡</td>
</tr>
<tr>
<td>UV₁ (µEq/min)</td>
<td>3.0</td>
<td>0.07‡</td>
</tr>
<tr>
<td>FE₂ (％)</td>
<td>1.5</td>
<td>0.04‡</td>
</tr>
<tr>
<td>UV₂ (µEq/min)</td>
<td>2.7</td>
<td>3.9</td>
</tr>
<tr>
<td>V (µl/min)</td>
<td>12</td>
<td>188</td>
</tr>
<tr>
<td>Cₘ (µl/min)</td>
<td>70</td>
<td>74</td>
</tr>
<tr>
<td>Tₙ₋₀ (µl/min)</td>
<td>58</td>
<td>53</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. Clearance and electrolyte values represent the mean of periods C₁ and C₂.

* Prior to PD.
† During C₂.
‡ P < 0.01 as compared to control.
§ P < 0.05 as compared to control.
‖ P < 0.001 as compared to control.
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chloride-depleted rats in experiment 2. To exclude a potential synergistic role of these factors in stimulating PRA, we studied an additional group of chloride-depleted rats (group 2a) in which plasma volume was significantly more expanded than in the first chloride-depleted group of experiment 2 ($P < 0.05$), and in which mean plasma $K^+$ was the same as that of the control group (Table 3). Despite these increases in plasma volume and potassium, significant stimulation of renin again was observed in group 2a (from $25 \pm 4$ to $41 \pm 6$ ng/ml per hr, $P < 0.01$). PRA$_2$ levels in group 2a were greater than PRA$_1$ levels ($P < 0.01$) in control rats and not different from PRA$_2$ levels in the first chloride-depleted group of experiment 2 (Fig. 2). We attribute the tendency to a lower plasma $K^+$ in the chloride-depleted groups of experiments 1 and 2 to a pH-induced cellular shift of potassium and to the greater urinary potassium excretion (Table 4) in these groups. There was also a significant stimulation of renin in group 2b (from $23 \pm 4.0$ to $36 \pm 6$ ng/ml per hr, $P < 0.05$, Fig. 2), even though acute metabolic alkalosis did not occur.* PRA$_2$ levels in this group differed from PRA$_2$ levels of controls ($P < 0.05$) but did not differ from PRA$_2$ levels in Cl-depleted rats in experiments 2 or 2a.

The relationship between osmolar clearance ($C_{\text{osm}}$) and $T^*_{\text{H,O}}$ in the clearance periods is shown in Figure 3. In the control rats on both a normal and low salt diet, a close and significant positive correlation exists between $C_{\text{osm}}$ and $T^*_{\text{H,O}}$ (for the regression line shown for all control rats, $r = 0.99$, $P < 0.001$). All of the values in the four experimental groups fall outside the 95% confidence limits for the line, suggesting less $T^*_{\text{H,O}}$, relative to osmolar clearance, in the chloride-depleted rats.

*In additional studies of rats dialyzed against NaNO$_3$ we observed an arterial pH of 7.30–7.40 between PD and the second clearance period.
Discussion

These experiments demonstrate that chloride depletion, without volume or sodium depletion and without changes in arterial blood pressure, GFR, filtered sodium load, arterial pH, or plasma bicarbonate, is a potent stimulus to renin release. Stimulation of renin occurs in rats that were previously on either a normal or a low sodium chloride intake. The absence of appropriate baroreceptor stimuli for renin release suggests that chloride depletion stimulates renin via a renal tubular (macula densa) mechanism. The importance of this control mechanism is demonstrated by the stimulation of renin by chloride depletion despite the presence of significant volume expansion, which would tend to suppress renin release.

PD against sodium bicarbonate led to two additional differences between the control and experimental groups, namely, metabolic alkalosis and a lower plasma potassium in the chloride-depleted groups. Hypokalemia per se stimulates renin (Himathongkam et al., 1975). However, the stimulation of renin in group 2a, which received additional potassium in the dialysate and the infusate, and which had the same mean plasma potassium concentration as controls, excludes hypokalemia as the cause of renin stimulation in the present experiments. Because group 2a rats also received additional albumin, these results also exclude a synergistic effect of a subtle depression in plasma volume and plasma potassium as the stimulus to renin release. In group 2a, once again, the importance of the chloride signal is emphasized by stimulation of renin despite significant volume expansion and despite potassium loading, both of which are inhibitors of renin secretion (Davis and Freeman, 1976).

Acute metabolic alkalosis is excluded as the cause of stimulation of renin by the absence of differences in arterial pH and plasma bicarbonate between the group dialyzed against sodium nitrate (group 2b) and controls. In our earlier experiments employing acute and chronic loading with various sodium salts, changes in arterial pH also did not correlate with changes in PRA, in contrast to the consistent relationship present between depressed renin and increased filtered and reabsorbed chloride (Kirchner et al., 1979). Taken together, these past and present results suggest that change in plasma pH or bicarbonate is not the mechanism altering renin secretion in both acute chloride depletion and acute chloride loading.

Chloride depletion also was associated with a reduced $T_{\text{H}_2\text{O}}$ relative to osmotic load, compared to controls, in which renin was not stimulated. Depression of $T_{\text{H}_2\text{O}}$ by chloride depletion confirms previous findings (Luke et al., 1977; Wallin et al., 1973). We also have previously reported a greater $T_{\text{H}_2\text{O}}$ relative to osmotic load during acute infusion of salts such as sodium chloride and sodium bromide, which suppressed renin, than during infusion of salts such as sodium bicarbonate and sodium nitrate, which did not suppress renin (Kirchner et al., 1979). In neither the present nor the previous experiments was there a difference in plasma osmolality or volume to account for differences in $T_{\text{H}_2\text{O}}$ between control and experimental groups because of differences in antidiuretic hormone release. These differences in $T_{\text{H}_2\text{O}}$, then, indirectly support but do not prove (Levinsky and Levy, 1973) the hypothesis that chloride absorption in the TAL was diminished in the chloride-depleted groups. Active chloride transport occurs at sites immediately proximal and distal to the macula densa in the TAL and early distal tubule (Rocha and Kokko, 1973; Burg and Green, 1973; Malnic and Giebisch, 1972). Moreover, there is anatomic evidence that the macula densa is part of the TAL (Kaissling et al., 1977). We postulate that chloride depletion and the concomitant decrease in filtered chloride were associated with a diminished absorptive transport of chloride (and, secondarily, of sodium) in the TAL including the macula densa, and hence stimulated renin release. Since urinary chloride excretion was greater after sodium nitrate (group 2b) than in the control rats of experiment 2 (Table 4), it is possible that nitrate interfered with chloride absorption in the distal nephron; indeed, there is previous evidence from studies on dogs for such an effect in the TAL (Kahn et al., 1975). Thus in group 2b, in addition to decreased absorptive chloride transport due to decreased chloride delivery, inhibition of chloride transport by nitrate may have contributed to the stimulation of renin.

Diuretics that inhibit active chloride transport in the loop of Henle (Burg and Stoner, 1976) stimulate renin release by this direct intrarenal effect (Vander and Carlson, 1969; Hesse and Neilsen, 1976). At the same time, these diuretics markedly impair $T_{\text{H}_2\text{O}}$ in the presence of antidiuretic hormone (Goldberg, 1973). Potassium depletion also is associated with renin stimulation by an as-yet-unestablished mechanism (Abbrecht and Vander, 1970; Sealy and Largh, 1974). In the presence of potassium depletion there is also impairment of both concentrating (Mannitius et al., 1960) and diluting capacity (Eknoyan et al., 1970) in the rat, and in both rats (Luke and Levitin, 1967) and man (Garella et al., 1970) there is evidence of chloride wasting. We recently have shown that fractional reabsorption of chloride is reduced in the loop segment in potassium-depleted rats (Luke et al., 1978). It is possible that stimulation of renin by potassium depletion is also related to impaired chloride transport in the TAL. Thus diminished chloride transport in the TAL may be the common factor contributing to the impaired TAL function and stimulation of renin produced by acute chloride depletion (in the present experiments), by the administration of loop diuretics such as furosemide and ethacrynic acid, and by potassium depletion.

Chloride delivery and transport at the macula
densa also has been shown to be an important stimulus for tubuloglomerular feedback in single-
nephron perfusion studies (Schnermann et al., 1976). In some microperfusion experiments, single-
nephron renin has been found to be increased or activated by increased sodium chloride transport
(Thurau and Mason, 1974), in apparent contrast to our hypothesis. However, the relationships between
changes in single-nephron renin content and/or activation and renin release by the kidney remain to be
clarified.

In summary, PRA is stimulated by selective chloride depletion unrelated to changes in GFR, arterial
blood pressure, arterial pH, filtered sodium load, plasma volume, and plasma potassium. Impaired
\( T_{\text{H},2} \) was observed in acutely chloride-depleted rats, offering indirect evidence for diminished chlo-
ride (and sodium) transport in the TAL. These findings are consistent with the hypothesis that acute diminution in chloride transport in the TAL-macula densa segment stimulates renin release. We
have reported previously that acute inhibition of renin release by selective chloride loading is associ-
ated with increased \( T_{\text{H},2} \) (Kirchner et al., 1979). Taken together, these observations support the hy-
pothesis that renin release via the macula densa mechanism is inversely related to the magnitude of
absorptive chloride transport in the TAL.

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