THE EFFECTS OF ANTIDIURETIC HORMONE AND STATE OF POTASSIUM BALANCE ON THE RENIN-ANGIOTENSIN SYSTEM IN RATS WITH DIABETES INSIPIDUS

BY EMMA FERNÁNDEZ-REPOLLET, MANUEL-MARTÍNEZ MALDONADO AND SUSAN OPAVA-STITZER

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SUMMARY

1. The influence of ADH and the state of potassium balance on the renin-angiotensin system was studied in rats with hereditary diabetes insipidus (DI rats).
2. Plasma renin concentration in DI rats was higher than in control Long-Evans rats.
3. Spontaneous reversal of the hypokalaemia normally found in DI rats did not reduce plasma renin concentration (p.r.c.), suggesting that potassium deficiency does not contribute significantly to the elevation of p.r.c. in DI rats. Similarly, a low potassium diet failed to further increase p.r.c. in DI rats.
4. In contrast, the p.r.c. of DI rats was significantly diminished by a high potassium intake both in the presence and absence of ADH. A highly significant inverse correlation was found between p.r.c. and urinary potassium excretion in both ADH-treated and untreated DI rats on low, normal and high potassium diets.
5. Plasma renin concentration was significantly lower in ADH-treated than in untreated DI rats on a high potassium intake, suggesting that the inhibitory effects of ADH and potassium are additive.
6. ADH consistently reduced p.r.c. in DI rats independent of the state of potassium balance.
7. ADH and potassium may inhibit renin secretion via different mechanisms of action.

INTRODUCTION

Several studies have shown that rats with hereditary hypothalamic diabetes insipidus (DI rats) have a higher than normal plasma renin activity (p.r.a.) (Gross, Dauda, Kazda, Kyncl, Möhring & Orth, 1972; Gutman & Benzakein, 1971, 1972; Kyncl, Miksche, Khayyal, Möhring & Gross, 1970; Oliver, Balment & Henderson, 1974), and angiotensin II concentration (Balment, Jones, Henderson & Oliver, 1976; Möhring, Möhring, Dauda & Haack, 1974) but the mechanism responsible for the enhanced p.r.a. in these rats has not been established. Since DI rats suffer from an absence of antidiuretic hormone (ADH) (Valtin & Schroeder, 1964) and potassium deficiency (Möhring et al. 1974), and since both ADH (Buñag, Page & McCubbin, 1967;
Hesse & Nielsen, 1977; Vander, 1968; Vandongen, 1975) and potassium (Abbrecht & Vander, 1970; Dluhy, Underwood & Williams, 1970; Flamenbaum, Kleinman, McNeil, Hamburger & Kotchen, 1975; Sealey, Clark, Bull & Laragh, 1970; Vander, 1970) are known to exert an inhibitory effect on renin release, one or both of these factors might be involved in the elevation of p.r.a.

Potassium administration (both acute and chronic) has been shown to decrease p.r.a., and potassium deprivation to increase it in normal and hypertensive human subjects (Dluhy et al. 1970; Brunner, Baer, Sealey, Ledingham & Laragh, 1970), rats (Douglas, Hansen & Catt, 1978; Sealey et al. 1970) and dogs (Abbrecht et al. 1970; Flamenbaum et al. 1975; Vander, 1970). It has been suggested that potassium may indirectly inhibit renin release by altering tubular sodium reabsorption (Flamenbaum et al. 1975; Vander, 1970).

The inhibitory effect of ADH on renin secretion is also well documented. Intravenous infusion of ADH suppressed renin release in the dog (Buñag et al. 1967) and man (Hesse & Nielsen, 1977), and infusion of ADH into the renal artery inhibited renin secretion only of the infused kidney (Vander, 1968). Vandongen (1975) observed that intrarenal infusion of ADH prevented isoprenaline-stimulated renin release in the isolated rat kidney, and suggested a direct effect of ADH on the renin secretory mechanism. Thus, elevated p.r.a. in DI rats might result from the absence of a tonic inhibitory effect of ADH on renin secretion. The lack of ADH in these rats, however, has been shown to cause periods of mild dehydration (Gross et al. 1972; Valtin, 1967), low arterial pressure (Gross et al. 1972) and reduced glomerular filtration rate (Gellai & Valtin, 1979), factors which could also contribute to elevated p.r.a. (Vander, 1967).

In order to evaluate the contribution of ADH and potassium to the regulation of renin release, plasma renin concentration (p.r.c.) was measured in DI rats maintained on a potassium-free, normal potassium or high potassium diet with and without ADH treatment.

Although DI rats have been reported to suffer from potassium deficiency (Mohring et al. 1974), we have shown (Fernández-Repollet, Martínez-Maldonado, & Opavski-Stitzer, 1980) that this hypokalaemia is a transient condition which can be corrected by placing the rats in individual metabolism cages for several days. This spontaneous potassium repletion is apparently related to alterations in water balance which occur when the rats have non-competitive access to drinking water. Plasma renin concentration was also measured in DI rats before and after spontaneous correction of hypokalaemia in order to evaluate the role of this mild potassium deficiency in the elevation of plasma renin levels.

METHODS

Experimental animals

Experiments were performed in male and female Long-Evans hooded rats of the Brattleboro strain homozygous for the hypothalamic diabetes insipidus trait (DI rats) and in normal Long-Evans hooded rats. Body weights ranged from 150 to 300 g. Experimental and control groups were matched for sex and weight and there was no significant difference in body weight between any experimental group and its respective control group.

Balance studies

Prior to any balance study the rats were placed in individual metabolism cages (ACME Research Products AC-5062, Chicago) for an equilibration period of 6 days to ensure normokalaemia (with
the exception of the experiment in which p.r.c. was measured before and after spontaneous reversal of hypokalaemia).

Balance studies consisted of daily measurement of body weight, food and water intake. Twenty-four hr urine samples were collected under mineral oil for the determination of volume, osmolality, and sodium and potassium concentration.

**Bleeding techniques**

Blood samples for measurement of p.r.c. and plasma electrolyte concentrations were always obtained between 7.00 and 7.30 a.m., by heart puncture under light ether anaesthesia. For this purpose, rats were placed in a bell jar containing ether-impregnated gauze. The rats were sufficiently anaesthetized in less than 1 min. 0.5 ml. blood was withdrawn from the heart using a 1 ml. cold plastic syringe 23 gauge needle. Half of this sample was rapidly transferred to a plastic centrifuge tube, on ice, containing 10 μl. Na₂EDTA (50 mg/ml.). This sample was used for renin measurement. The remaining blood was allowed to coagulate and used for measurement of serum [Na⁺] and [K⁺].

**Plasma renin concentration before and after spontaneous reversal of hypokalaemia**

Following a 60 hr equilibration period, twelve DI rats and twelve Long-Evans rats were studied for three consecutive days in metabolism cages as described under balance studies. On day 4 individual blood samples were obtained for determination of p.r.c. and plasma sodium and potassium concentration. Six DI rats and six Long-Evans rats were studied for eight additional days, at the end of which p.r.c. and serum sodium and potassium concentrations were measured as previously.

**Effects of varying potassium intake on p.r.c. of DI rats in the presence and absence of ADH**

The basic protocol consisted of a 2 week control period, a 3 week period of adaptation to the experimental diet and/or ADH treatment, and a 2 week experimental period. During the control period the rats were fed a normal diet and were untreated. In the first week a 4 day balance study was carried out and the rats were bled for measurement of p.r.c. and serum [Na⁺] and [K⁺] on day 5. In the second week the rats were again bled following a 2 day balance study. Following the control period the rats were subjected immediately to one of the following protocols:

1. (a) Six rats were maintained on a normal diet (Purina laboratory chow; K: 0.92 %, Na: 0.45 %) for the remainder of the experiment. (b) Six rats were placed on a high potassium diet (Purina laboratory chow + 15 % (w/w) KCl) for the duration of the experiment.

2. (a) Six rats were maintained on a normal diet of Purina chow and treated daily with ADH (100 μu./100 g body wt., s.c.; Pitressin tannate in oil, Parke-Davis). (b) Six rats were maintained on the same normal diet and treated daily with a volume of sesame oil equivalent to the volume of ADH suspension (0.1 ml./100 g body wt.). (c) Six rats were placed on a high potassium diet (laboratory chow + 15 % (w/w) KCl) and treated daily with ADH as previously described.

3. (a) Six rats were maintained on a normal diet (potassium-free diet, General Biochemicals, +10 % corn-starch, +12 % (w/w) KCL) for the remainder of the experiment. The corn-starch was added to prevent diarrhoea. (b) Six rats were placed on a potassium-free diet (+10 % corn-starch), without KCl supplement, for the remainder of the experiment.

4. (a) Six rats were maintained on a normal diet (as in 3(a)) and treated daily with ADH as previously described. (b) Six rats were placed on a potassium-free diet (as in 3(b)) and treated with ADH as previously described.

Following 3 weeks on a given dietary regimen and/or treatment with ADH a 4 day balance study was carried out with bleeding for measurement of p.r.c. and serum [Na⁺] and [K⁺] on day 5. One week later the rats were again bled following a 2 day balance study.

**Analyses**

**Plasma renin concentration**

Plasma renin concentration was measured by determination of the amount of angiotensin I which is generated during incubation at 37 °C with an excess amount of renin substrate and inhibitors of converting enzyme and angiotensinases (Churchill, Churchill & McDonald, 1973; Haber, Koerner, Page, Kliman & Purnode, 1969). Substrate was prepared from nephrectomized heterozygous rats according to the method of Schaechtelin et al. (Schaechtelin, Regoli & Peters, 1967; Schaechtelin, Chometti, Regoli & Peters, 1966) as modified by Churchill et al. (1973). 200 μl. substrate preparation (pH 7.0) was added to each of three 20 μl. aliquots of each plasma sample. One of the three
sample–substrate mixtures was maintained at 4 °C; the others were incubated at 37 °C for 15 and 30 min respectively. Linearity of angiotensin I generation over time of incubation was taken as evidence of the presence of substrate in excess. P.r.c. was expressed as ng of angiotensin I generated per ml. of plasma per hr (ng AI/ml. per hr). The p.r.c. of the 4 °C control sample was subtracted from that of each incubated sample. The 15 and 30 min values were averaged.

Angiotensin I concentration was measured by radioimmunoassay using materials provided by New England Nuclear Corp., Boston, MA (Haber et al. 1969). [125I]Angiotensin I was counted in a Picker refrigerated beta/gamma counter, Model no. 110.

**Flame photometry**

Sodium and potassium concentration in urine and serum were measured using a Radiometer (Model FLM2E) flame photometer.

**Table 1. Plasma renin concentration (p.r.c.) and serum [K+] of normokalaemic and hypokalaemic DI rats and Long-Evans rats**

<table>
<thead>
<tr>
<th></th>
<th>P.r.c.</th>
<th>Serum [K+]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng AI/ml. per hr)</td>
<td>(mM)</td>
</tr>
<tr>
<td>Hypokalaemic DI</td>
<td>99 ± 15*</td>
<td>3.7 ± 0.2*</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-Evans</td>
<td>68 ± 7</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normokalaemic DI</td>
<td>97 ± 8*</td>
<td>4.3 ± 0.2†</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-Evans</td>
<td>70 ± 6</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± s.e. of means. Values in hypokalaemic rats were obtained after 3 days in metabolism cages and compared to values obtained simultaneously in Long-Evans rats. Values in normokalaemic DI rats and their control Long-Evans rats were obtained after 9 days in metabolism cages.

* Significantly different from Long-Evans rats studied at the same time (P < 0.05).
† Significantly different from values in hypokalaemic DI rats (P < 0.05).

**Osmolality**

Urine osmolality was determined by freezing-point depression using an Advanced Instruments Osmometer (Model 6731 L).

**Statistical methods**

Data were analysed using paired t and Student's t tests, utilizing the statistics programs supplied by Hewlett Packard for their programmable calculator (Model 9100B). The least squares method was used for the calculation of linear regression coefficients. Fisher Z transformation was applied to assess the difference in linear regression coefficients. Regression lines were compared using analysis of covariance.

**RESULTS**

**P.r.c. before and after spontaneous reversal of hypokalaemia**

As shown in Table 1, there was no difference in p.r.c. of DI rats after 3 days and after 9 days in metabolism cages. After 3 days DI rats were hypokalaemic compared to Long-Evans rats but were normokalaemic after 9 days in metabolism cages. The p.r.c. of DI rats was higher than that of Long-Evans rats after both 3 days and 9 days in metabolism cages, P < 0.05.
<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Urine flow (ml./24 hr per 100 g body wt.)</th>
<th>Urine osmolality (m-osmole/kg H₂O)</th>
<th>Urinary Na excretion (µequiv/100 g body wt. per 24 hr)</th>
<th>Urinary K excretion (µequiv/100 g body wt. per 24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal</td>
<td>61.0 ± 6.2</td>
<td>232 ± 17</td>
<td>945 ± 34</td>
<td>1638 ± 19</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>Normal</td>
<td>61.0 ± 7.1</td>
<td>210 ± 28</td>
<td>1009 ± 42</td>
<td>1717 ± 56</td>
</tr>
<tr>
<td>Experimental</td>
<td>Normal</td>
<td>78.1 ± 2.2</td>
<td>216 ± 9</td>
<td>1031 ± 37</td>
<td>1814 ± 61</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>High [K]</td>
<td>92.2 ± 15.1*†</td>
<td>288 ± 18*†</td>
<td>1340 ± 85*†</td>
<td>7562 ± 582*†</td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>78.0 ± 8.0</td>
<td>211 ± 8</td>
<td>1096 ± 59</td>
<td>1716 ± 112</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>Normal</td>
<td>56.6 ± 0.7*</td>
<td>1836 ± 158*</td>
<td>965 ± 49*</td>
<td>1507 ± 75*</td>
</tr>
<tr>
<td>( + ADH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>Normal</td>
<td>76.1 ± 6.2</td>
<td>208 ± 11</td>
<td>1030 ± 48</td>
<td>1700 ± 66</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>High [K]</td>
<td>23.0 ± 3.1*†</td>
<td>1233 ± 84*†</td>
<td>858 ± 61*</td>
<td>8192 ± 723*†</td>
</tr>
</tbody>
</table>

Values are means ± s.e. of means.

* Significantly different from values obtained in the same rats in the control period (0.001 < P < 0.05).
† Significantly different from values in the control group during the same period (0.001 < P < 0.05).
Effect of varying potassium intake on p.r.c. in the presence and absence of ADH

High K\textsuperscript{+} diet in the absence of ADH

As shown in Table 2 there was a significant increase in urine flow and urine osmolality in DI rats on a high K\textsuperscript{+} diet when compared to values obtained during a control period in the same rats or to values in DI rats maintained concurrently on a normal diet. After 3 weeks on a high potassium diet both urinary sodium excretion and urinary potassium excretion of DI rats increased significantly. A significant difference was also observed when the urinary sodium and potassium excretion of DI rats on a high potassium diet were compared to those of the group maintained on a normal diet ($P < 0.01$). Potassium intake of DI rats on the high potassium diet rose from $1903 \pm 128$ to $15,687 \pm 722 \mu$equiv/24 hr per 100 g body wt. ($P < 0.01$) but no significant change was observed in sodium intake. Serum potassium concentration was $4.9 \pm 0.1$ mm in DI rats on a high potassium diet, significantly higher than the value of $4.0 \pm 0.3$ observed during the control period in the same rats ($P < 0.05$). No significant change was observed in plasma sodium concentration.

The effect of a high potassium diet on p.r.c. of untreated DI rats is shown in the upper left quadrant of Table 4. It is evident from the results that there was a significant decline in p.r.c. on a high potassium diet compared to that in the same rats on a normal diet. The p.r.c. was also significantly lower than that of the control DI rats maintained concurrently on a normal diet.

High K\textsuperscript{+} diet in the presence of ADH

As shown in Table 2 urine flow decreased significantly in DI rats on both normal and high potassium diets during ADH treatment. As expected, urine osmolality increased significantly in both groups of rats although urine osmolality of DI rats on a high potassium diet was significantly lower than that of DI rats on a normal diet.

ADH administration significantly decreased the urinary sodium excretion ($P < 0.01$) in rats maintained on a normal diet as well as in rats maintained on a high potassium diet ($P < 0.01$). ADH-treated DI rats on a high potassium diet showed significant increases in urinary potassium excretion, as shown in Table 2, and potassium intake was increased from $1721 \pm 69$ to $13,755 \pm 1,110 \mu$equiv/24 hr per 100 g body wt. ($P < 0.01$). In contrast, urinary potassium excretion (Table 2) and potassium intake of DI rats on a normal diet were significantly decreased after ADH administration. Potassium intake fell from $1760 \pm 67$ to $1568 \pm 50 \mu$equiv/24 hr per 100 g body wt. ($P < 0.01$).

Serum potassium concentration increased significantly after ADH treatment in DI rats maintained on both normal ($3.7 \pm 0.1$ vs. $4.0 \pm 0.1$ mm, $P < 0.05$) and high ($3.7 \pm 0.1$ vs. $5.0 \pm 0.1$ mm, $P < 0.01$) potassium diets; however, the increase was significantly greater in the rats fed a high potassium diet ($P < 0.01$). No significant change in serum sodium concentration was observed in either group of rats.

The effects of ADH on the p.r.c. of DI rats fed normal and high potassium diets are shown in the lower left quadrant of Table 4. Although ADH administration significantly diminished the p.r.c. in both groups of rats, it was significantly lower in rats treated with both ADH and a high K\textsuperscript{+} diet than in those treated with ADH alone (normal diet). P.r.c. during combined ADH and K\textsuperscript{+} treatment was also lower...
### Table 3. Urine flow, urine osmolality and urinary sodium and potassium excretion in untreated and ADH-treated DI rats on normal† and potassium-free diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Urine flow (ml./24 hr per 100 g body wt.)</th>
<th>Urine osmolality (m-osmole/kg H₂O)</th>
<th>Urinary Na excretion (μequiv/100 g body wt per 24 hr)</th>
<th>Urinary K excretion (μequiv/100 g body wt per 24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal</td>
<td>56 ± 4</td>
<td>189 ± 7</td>
<td>989 ± 35</td>
<td>884 ± 67</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>Normal</td>
<td>56 ± 4</td>
<td>172 ± 5</td>
<td>908 ± 44</td>
<td>804 ± 44</td>
</tr>
<tr>
<td>Experimental</td>
<td>Normal</td>
<td>54 ± 3</td>
<td>199 ± 13</td>
<td>1007 ± 22</td>
<td>972 ± 66</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>K-free</td>
<td>35 ± 2*†</td>
<td>154 ± 13*†</td>
<td>798 ± 12*†</td>
<td>24 ± 2*†</td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>56 ± 4</td>
<td>167 ± 7</td>
<td>883 ± 29</td>
<td>925 ± 43</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>Normal</td>
<td>3±0 ± 0·2*</td>
<td>2290 ± 187*</td>
<td>621 ± 44*</td>
<td>685 ± 47*</td>
</tr>
<tr>
<td>(+ ADH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>Normal</td>
<td>62 ± 4</td>
<td>148 ± 10</td>
<td>964 ± 57</td>
<td>959 ± 47</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>K-free</td>
<td>6·0 ± 1·4*</td>
<td>1416 ± 118*†</td>
<td>703 ± 58*</td>
<td>18 ± 3*†</td>
</tr>
<tr>
<td>(+ ADH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E. of means.

* Significantly different from values obtained in the same rats in the control period (0·001 < P < 0·005).

† Significantly different from values in the control group during the same period (0·001 < P < 0·005).

‡ This normal diet consisted of the K⁺-free diet plus 1·2% (w/w) KCl.
than that in untreated DI rats on a high K⁺ diet ($P < 0.01$). In addition, the decline in p.r.c. observed when ADH treatment was combined with a high potassium diet ($\Delta = -77 \pm 14$) was significantly greater than that observed due to ADH treatment alone ($-27 \pm 10, P < 0.01$) or due to a high potassium diet alone ($-30 \pm 8, P < 0.01$). There was no difference in p.r.c. before and after oil injections in DI rats, (100 $\pm$ 17 vs. 89 $\pm$ 8 ng AI/ml. per hr).

**TABLE 4. Plasma renin concentration (p.r.c.) in untreated and ADH-treated DI rats on normal, high potassium and potassium-free diets**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>P.r.c. (ngAI/ml. per hr)</th>
<th>Diet</th>
<th>P.r.c. (ngAI/ml. per hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Control</td>
<td>Normal 101 $\pm$ 11</td>
<td>Normal† 80 $\pm$ 9</td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>Normal</td>
<td>109 $\pm$ 15</td>
<td>Normal† 97 $\pm$ 16</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>Normal</td>
<td>74 $\pm$ 7</td>
<td>Normal† 95 $\pm$ 19</td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>High [K]</td>
<td>44 $\pm$ 6*†</td>
<td>K-free 94 $\pm$ 9</td>
<td></td>
</tr>
<tr>
<td>ADH-treated</td>
<td>Control</td>
<td>Normal 79 $\pm$ 7</td>
<td>Normal† 97 $\pm$ 11</td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>Normal</td>
<td>52 $\pm$ 4*†</td>
<td>Normal† 48 $\pm$ 6*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(+ADH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>Normal</td>
<td>111 $\pm$ 16</td>
<td>Normal† 106 $\pm$ 10</td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>High [K]</td>
<td>34 $\pm$ 3*†</td>
<td>K-free 71 $\pm$ 13*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(+ADH)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means $\pm$ s.e. of means.
* Significantly different from values obtained in the same rats in the control period ($0.001 < P < 0.05$)
† Significantly different from values in the control group during the same period ($0.001 < P < 0.05$)
‡ It should be noted that this normal diet consisted of the K-free diet plus 1-2% (w/w) KCl and thus was different from the control normal diet for high potassium intake studies shown on the left.

**Potassium-free diet in the absence of ADH**

As shown in Table 3, after 3 weeks on a potassium-free diet, urine flow and urine osmolality were significantly decreased in DI rats when compared to the control group or to values obtained in the same rats during a control period ($P < 0.01$). Urinary sodium excretion was also significantly lower than the respective control measurement in the same rats ($P < 0.01$), and significantly lower than that of the control group ($P < 0.05$). Sodium intake was also decreased by a potassium-free diet from 1044 $\pm$ 74 to 848 $\pm$ 25 $\mu$equiv/24 hr per 100 g body wt. ($P < 0.01$).

Urinary potassium excretion was dramatically decreased on the potassium-free diet compared to values in the same rats on a normal diet as well as to rats in the control group ($P < 0.001$).

As expected, DI rats maintained on a potassium-free diet were hypernatraemic ($148 \pm 1$ mm) and hypokalaemic ($2.6 \pm 0.2$ mm) compared to values in the same rats fed a normal diet ($141 \pm 1$ and $3.6 \pm 0.1$ mm) and to the control group ($141 \pm 3$ and $3.8 \pm 0.1$ mm) ($P < 0.01$).

The p.r.c. of rats on a potassium-free diet was not significantly different from that of the same rats during a control period nor from the p.r.c. of the control group, as shown in the upper right quadrant of Table 4.
Potassium-free diet in the presence of ADH

The influence of ADH on urine flow, urine osmolality and urinary sodium and potassium excretion of DI rats maintained on normal and potassium-free diets is also presented in Table 3. As expected, ADH treatment caused a significant decrease in urine flow and a significant increase in urine osmolality of DI rats fed both normal and potassium-free diets ($P < 0.01$). It should be noted, however, that after ADH administration urine osmolality of DI rats on a potassium-free diet was significantly lower than that of DI rats on a normal diet ($P < 0.01$).

ADH administration significantly decreased urinary sodium excretion of DI rats on a normal diet but not on a potassium-free diet. Sodium intake did not differ significantly in ADH-treated rats on normal or potassium-free diets compared to the period before ADH treatment. ADH administration significantly decreased urinary potassium excretion of DI rats on a normal diet with no significant change in potassium intake. Urinary potassium excretion of potassium-depleted, ADH-treated DI rats was significantly reduced compared to values in the same rats during a control period and values in the control group ($P < 0.01$).

Serum potassium concentrations fell from $3.9 \pm 0.2$ to $2.8 \pm 0.1$ mm in ADH-treated DI rats on a potassium-free diet ($P < 0.01$), while serum sodium concentration rose from $137 \pm 1$ to $142 \pm 1$ mm ($P < 0.01$). Similar changes in serum sodium and potassium concentration were observed when these parameters were compared with those of the control group ($138 \pm 1$ and $3.9 \pm 0.1$ mm) ($P < 0.01$).

As shown in the lower right quadrant of Table 4, ADH administration significantly decreased the p.r.c. of DI rats on both normal and potassium-free diets. There was no significant difference between the p.r.c. of ADH-treated rats on a normal diet and ADH-treated rats on a potassium-free diet.

DISCUSSION

In agreement with the findings of others (Gross et al. 1972; Kynèl et al. 1970; Oliver et al. 1976), p.r.c. was higher in DI than Long-Evans rats at the end of both 3 day and 9 day balance studies. No significant change in p.r.c. was observed in either DI or Long-Evans rats between the 3 day measurement and the 9 day measurement despite correction of the hypokalaemia in DI rats. Thus it seems that the mild hypokalaemia observed in DI rats does not play a significant role in the elevation of p.r.c. Other factors such as the absence of ADH or episodes of volume contraction may be important. This hypothesis is supported by experiments in which changes in volume were a more potent stimulus than changes in potassium in the regulation of renin secretion in the dog (Oettinger & Wells, 1978).

After several weeks on a high potassium diet urine flow and urine osmolality of DI rats were significantly higher when compared to control values. The high urine flow rate is presumed to be the result of the increased osmotic excretory load of potassium chloride causing retention of water in the tubular lumen. In addition, the increment of potassium in the urine fully accounted for the increase in urine osmolality which was observed. In agreement with numerous previous reports (Berliner, Kennedy & Hilton, 1950; Young, McCaa & Guyton, 1976) a high potassium
intake was associated with a significant natriuresis as well as the expected increase in urinary potassium excretion. P.r.c. in DI rats was significantly reduced on a high potassium intake (Table 4). This observation is compatible with the inhibitory effect of potassium on p.r.a. in man (Brunner et al. 1970), rat (Sealey et al. 1970; Douglas, et al. 1978) and dog (Flamenbaum et al. 1975; Vander, 1970) and may be the result of increased distal sodium load.

A high potassium diet had similar effects in the presence of ADH as in its absence. As expected urinary potassium excretion, potassium intake, and serum potassium concentration of ADH-treated DI rats were increased by a high potassium intake. The p.r.c. of ADH-treated rats on a high potassium diet was significantly lower than that of ADH-treated rats on a normal diet, an indication that the effects of these two inhibitors of renin release (ADH and potassium) are additive.

On a potassium-free diet urine flow and urine osmolality of DI rats were significantly reduced. The diminished urine flow rate and urine osmolality were associated with a marked reduction in the quantity of urinary potassium. As expected, potassium intake and serum potassium concentration were also reduced. Interestingly, a potassium-free diet did not increase p.r.c. in DI rats. Several possible explanations for this finding should be considered. First, p.r.c. might have been maximally stimulated before induction of potassium deficiency and thus no further increase could occur. This would not seem to be the case, however, since unpublished observations from our laboratory indicate that the p.r.c. of DI rats can be stimulated further by various factors including stress, noise and dehydration. An alternative explanation is that regulation of renin by potassium is asymmetric in the sense that the renin secretory mechanism responds to increases in plasma potassium levels above normal but not to decreases below normal. This has not been found by others. Potassium deficiency and small decreases in plasma potassium concentration have been shown to increase p.r.a. in man (Brunner et al. 1970), dog (Abbrecht & Vander, 1970) and rat (Sealey et al. 1970). Since potassium depletion of DI rats caused hypernatraemia and decreased sodium excretion it is possible that our failure to observe a rise in renin was due to a concurrent inhibition of renin release due to sodium retention. Although Sealey et al. (1970) and Abbrecht & Vander (1970) have shown that p.r.a. is increased by potassium depletion despite sodium retention, a greater degree of sodium retention in our rats could explain the difference between our data and those of others. In addition, the effect of sodium retention on p.r.a. might be expected to be greater in the hypovolaemic DI rat than in the normovolaemic animals used in other studies. Finally, our failure to observe an increase in p.r.c. after potassium depletion might simply be due to the small number of animals used and variability in renin values. As shown in Fig. 1, a highly significant inverse correlation was found between p.r.c. and urinary potassium excretion in DI rats on potassium-free, normal, and high potassium diets.

In the presence of ADH a potassium-free diet did not induce a significant change in urine flow rate of DI rats, but urine osmolality was significantly lower than that of ADH-treated DI rats on a normal diet. The difference in urine osmolality appears therefore to be due to the reduction in the amount of potassium found in the urine. Although the p.r.c. of ADH-treated DI rats on a potassium-free diet appeared to be higher than that of ADH-treated DI rats on a normal diet the difference was not
significant. Such a tendency, however, suggests that, in the presence of ADH, potassium deficiency might under some circumstances stimulate p.r.c. in DI rats, as observed in normal rats. As in untreated DI rats, a highly significant inverse correlation was found between p.r.c. and urinary potassium excretion in ADH-treated DI rats on potassium-free, normal and high potassium diets (Fig. 1).

As in other studies (Gutman & Benzakein, 1971; Kynel et al. 1970; Oliver et al. 1976; Balment et al. 1976) ADH administration significantly decreased p.r.c. of DI rats on a normal diet. The present experiments have also shown that this ADH-induced decrease in p.r.c. of DI rats occurs regardless of the dietary potassium intake. In fact, the p.r.c. of ADH-treated DI rats was lower than that of untreated DI rats on any potassium intake. This is apparent in Fig. 1 in which the relationship between p.r.c. and urinary potassium excretion is shown separately for ADH-treated (n = 23) and untreated (n = 26) DI rats. While the slopes of the two regression lines were not different (m = −0.0042 ± 0.0012 and −0.0055 ± 0.0007 respectively) the y intercepts were highly significantly different (b = 62.8 ± 4.7 and 91.9 ± 4.0, respectively; P < 0.001). These results suggest that the same relationship between p.r.c. and urinary potassium excretion exists in the presence and absence of ADH, but that the presence of ADH affects the absolute level of plasma renin.

The effects of dual inhibition by potassium and ADH were additive, p.r.c. being
lower in ADH-treated rats on a high potassium diet than in those on a normal diet. Since we have found (unpublished observation) that the dose of ADH used has maximal effects on renin secretion, it is likely that potassium and ADH inhibit renin secretion via different mechanisms of action. These studies have not dealt with the mechanisms by which ADH or potassium inhibits renin release. Several actions might be postulated, e.g. a direct action of either ADH or potassium on the granular cells or the renal baroreceptor, an indirect action of potassium through changes in sodium delivery to the macula densa, or indirect effects of ADH secondary to water and sodium retention. Although other studies have suggested direct actions of both potassium and ADH these have utilized intrarenal infusion of these substances in anaesthetized animals and are not comparable to our chronic studies in conscious animals.

Our data also indicate that the potassium deficiency of DI rats does not play a significant role in the elevation of p.r.c. in these rats, since spontaneous reversal of the hypokalaemia did not reduce p.r.c. These results, taken together with the fact that ADH was able to reduce p.r.c. independently of the state of potassium balance, strongly suggest that the absence of ADH either directly or indirectly is the principal factor involved in the elevated p.r.c. characteristic of DI rats.

REFERENCES


