Basal activity of the natriuretic factor extracted from the rat kidney as a function of the diet and its role in the regulation of the acute sodium balance

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Summary

1. The effect of the sodium content of the diet on the natriuretic activity of an extract from the kidneys was studied in non-expanded and volume-expanded rats.

2. The kidney tissue was homogenized and the supernatant fractionated by gel filtration on Sephadex G-25. A single low-molecular-weight fraction eluted after the salt possessed the natriuretic activity and was tested on a rat bioassay.

3. The natriuretic activity of the fraction obtained from the kidneys of non-expanded rats was related to the sodium intake.

4. After an acute extracellular volume expansion, the natriuretic activity obtained from the fraction extracted from the kidneys was much greater than before expansion and was related to the dietary intake of sodium.

Key words: extracellular volume, kidney, natriuresis, natriuretic factor, sodium excretion.

Introduction

Sodium excretion is regulated by factors other than aldosterone and the filtered sodium load (de Wardener, Mills, Clapham & Hayter, 1961; Levinsky & Lalone, 1963; Bricker, 1967; Rector, Van Giesen, Kil & Selding, 1968). Several workers have put forward the view that an unknown substance which causes natriuresis through an inhibition of sodium reabsorption may play a role in the control of the sodium balance. Most of the studies have been performed in uraemic (Bourgoignie, Hwang, Espinel, Klahr & Bricker, 1972; Bourgoignie, Hwang, Ipakchi & Bricker, 1974) and normal man and animals (Sealey, Kirshmann & Laragh, 1969; Favre, Bourgoignie, Hwang, Schmidt & Bricker, 1975; Clarkson, Raw & de Wardener, 1976; Favre, 1978) with a chronic expansion of the extracellular fluid volume. A natriuretic factor has been consistently found in the urine of dogs and man in amounts appropriate to the need to excrete sodium by the kidneys (Favre et al., 1975; Clarkson et al., 1976; Favre, 1978). Several authors have fractionated the urine through a Sephadex G-25 column obtaining a low-molecular-weight fraction which increases the sodium excretion when injected into the rat (Bourgoignie et al., 1974; Clarkson et al., 1976). A detailed review of the subject has recently appeared in the literature (de Wardener, 1977).

Our previous studies have been in man and dogs with normal renal function who were on high salt intakes, with or without mineralocorticoid treatment (Favre et al., 1975; Favre, 1978). In both species, a sodium transport inhibitor of low molecular weight was demonstrated in the urine extracts in amounts which were related to the degree of both sodium excretion and sodium retention. The present study was designed to investigate in the rat the effect of acute extracellular volume expansion and sodium intake on
the amount of this inhibitor which could be extracted from kidney tissue. The work was done (a) to confirm the previous observation by Gonick & Saldanha (1975) and by Hillgard, Lu & Gonick (1976) that natriuretic material can be extracted from the kidney tissue after such a stimulus, and (b) to estimate the influence of the sodium content of the diet on the degree of activity of the natriuretic factor in non-expanded and acutely volume-expanded animals.

Methods

Experiments were performed on 37 normal Wistar rats weighing between 300 and 350 g. They were divided into six groups. The basic protocol was identical for all the groups. Rats were confined in an individual metabolic cage for a 5 day period during which time they received 25 g of a synthetic sodium-deficient food containing 2.2 mmol of sodium/100 g of dry powder. In addition, 0, 3 or 6 mmol of sodium chloride/day was added to the drinking water. Thus three different paired groups were investigated. Groups A, B and C were not volume-expanded at any time. Groups A, B and C were studied after acute expansion with sodium chloride solution (159 mmol/l: saline) expansion. Groups A and A were allowed to drink 25 ml of double-distilled water, groups B and B 25 ml of fluid containing 3 mmol of sodium, and groups C and C 25 ml of fluid containing 6 mmol of sodium. Rats that drank less than 25 ml/day were excluded from the study.

Cumulative sodium balance was measured during the 5 day period in the metabolic cage. On day 5, urine was collected for 24 h and creatinine and sodium were measured in the blood and urine in order to determine the glomerular filtration rate and the fractional excretion of sodium in five rats for each of the groups A, B and C. Clearance studies were performed on day 6.

Eighteen hours before these clearance studies, the urinary dead space was diminished by ligation of the dome of the bladder under light ether anaesthesia; the rats were kept without food from then on.

On the day of the experiment, a polyethylene catheter (PE25, Portex, Hythe, Kent, U.K.), was inserted into the jugular vein under light ether anaesthesia. The rats were then confined to a restrictive cage. When the rats started to move, a priming dose of 0.7 ml of inulin solution (5 mmol/l) was injected intravenously. A solution of glucose (13.8 mmol/l) containing inulin (2.5 mmol/l) was infused with a syringe pump at a rate of 0.15 ml/min. After a 60 min equilibration, three 20 min collections were made and blood samples were taken at the middle of each period from the tip of the tail for measurements of sodium and inulin. At the end of the last collection, both kidneys were removed under ether anaesthesia and placed in 20 ml of double-distilled water adjusted to pH 3.4 with acetic acid. The solution was heated for 20 min at 65°C, then the material was homogenized and centrifuged at 300 rev/min for 25 min at 4°C. The supernatant was applied to a 2.5 cm x 95 cm column packed with Sephadex G-25 and eluted at 4°C with a ammonium acetate solution (0.01 mol/l) adjusted to pH 6.8 by using a Perpex S pump delivering 1-2 ml/min (Bourgoignie, Klahr & Bricker, 1971; Bourgoignie et al., 1974). The effluent solution which appeared immediately after the salt peak was collected (10 tubes of 12 ml each, the first tube being the one which contained less than 1 mmol of sodium/l). This effluent has been shown to contain the inhibitor of sodium transport in the urine from patients with chronic uraemia (Bourgoignie et al., 1972; Bourgoignie et al., 1974) and in urine of normal dogs (Favre et al., 1975). The effluent was freeze-dried, reconstituted in 1.5 ml of double-distilled water and stored at -25°C until the fraction was tested by bioassay. Rats of groups A, B and C underwent the same preparation as described for the first series, but on the day of the experiment they were volume-expanded for 60 min with saline containing inulin (2.5 mmol/l) at a perfusion rate of 0.5 ml/min. After a 10% increase in the body weight had been reached, saline perfusion was maintained at 0.1 ml/min during three 20 min urine collection periods. Then the kidneys were removed and treated in the way described above.

The activity of the fraction was measured in a rat by bioassay. The fraction was adjusted to pH 7.35 with NaOH (0.1 mol/l) and to sodium and potassium concentrations of 140 and 4 mmol/l respectively. The assay was performed by using normal Wistar rats weighing between 100 and 120 g. The day before the experiment, the urinary dead space was reduced by ligation of the dome of the bladder. Animals were anaesthetized with ether and a PE25 catheter was inserted into the jugular vein. After surgery, rats were kept in a restrictive cage. Sixty minutes after the rats started to move, they were infused with a priming solution containing inulin (2.5 mmol/l), sodium chloride (19.9 mmol/l) and glucose (13.8 mmol/l) over 20 min by means of a syringe pump at a rate of 0.15 ml/min.
After 25 min of equilibration, four 10 min urine collection periods (control period) were performed, and a sample of blood was drawn from the tip of the tail in the second control period. Then 1 ml of the prepared fraction, corresponding to the amount of the fraction extracted from 67% of the original kidney tissue, was slowly injected intravenously (10 min). Urine samples collected during the 10 min injection of the fraction and during the 10 min after were thrown away in order to avoid non-specific changes in the natriuresis which may have resulted from a possible change in the fluid delivery during the manipulation of the syringe. The experimental period consisted of six 10 min urine collection periods. Blood samples were taken during the second and the fourth experimental urine collection periods. The results were expressed as percentage change in the average of the fractional excretion (FE) and absolute excretion of sodium between the four control periods and the mean of the six experimental periods, according to the formula:

\[
\text{Change in } \text{FE}(\text{Na}) = \left[ \text{FE}(\text{Na})_b - \text{FE}(\text{Na})_a \right] \times 100
\]

where \( \text{FE}(\text{Na})_a \) is fractional excretion of sodium after injection of the natriuretic factor and \( \text{FE}(\text{Na})_b \) is fractional excretion of sodium before injection of the natriuretic factor. The same formula was applied for calculating percentage changes in absolute excretion of sodium and inulin clearance.

Inulin was determined by the colorimetric method of Zender & Falbriard (1966). Creatinine was measured by the method of Knoll & Stamm (1970). Sodium was measured by a flame spectrophotometer and pH by a pH-meter with a combined glass electrode. The titrations of the solution and the fractions were made with an automatic titrator.

The variation for sets of measurements was expressed as SEM. Statistical analysis was performed with Student's \( t \)-test (paired \( t \)-test and unpaired \( t \)-test). Changes were considered significant when the \( P \) value was less than 0.05.

Results

Data for cumulative sodium balance for the 5 day period and physiological results on day 5 are presented in Table 1. All the rats given a sodium-deficient diet (group A) were in negative sodium balance. Rats receiving 3 mmol of sodium/day (15 mmol for 5 days, group B) exhibited a positive sodium balance, which differed significantly from the one observed in group A (B vs A, \( P < 0.0005 \)). Animals given a 6 mmol of sodium/day diet (30 mmol of sodium for 5 days, group C) had a more positive cumulative sodium balance than those from group B (C vs B, \( P < 0.025 \)).

A 24 h urine collection on day 5 showed a mean creatinine clearance identical for groups B and C; the values obtained for group A were slightly lower.

The lowest values of absolute and fractional excretion of sodium were observed in group A. Both absolute and fractional excretions of sodium increased significantly in relation to the dietary sodium intake (groups B and C).

Results of changes in glomerular filtration rate, urinary sodium excretion and fractional excretion of sodium are given in Table 2. There were no statistical differences in urinary sodium excretion, fractional excretion of sodium and glomerular filtration rate estimated by inulin clearances between the three non-expanded groups.

Acute expansion with saline (groups \( \text{A}_{\text{Be}}, \text{B}_{\text{Be}} \) and \( \text{C}_{\text{Be}} \)) caused a large increase in urinary sodium excretion and fractional excretion of sodium compared with the values observed in the paired non-expanded rats (Table 2). The increases in urinary sodium excretion and fractional excretion

<table>
<thead>
<tr>
<th>Group</th>
<th>GFR (ml/min)</th>
<th>Urinary sodium excretion (( \mu )mol/min)</th>
<th>Fractional excretion of sodium (%)</th>
<th>5-day cumulative Na balance (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.79 ± 0.07</td>
<td>0.06 ± 0.017</td>
<td>0.06 ± 0.02</td>
<td>-0.937 ± 0.11</td>
</tr>
<tr>
<td>B</td>
<td>0.974 ± 0.043</td>
<td>1.77 ± 0.33</td>
<td>1.36 ± 0.27</td>
<td>6.57 ± 1.18</td>
</tr>
<tr>
<td>C</td>
<td>0.99 ± 0.042</td>
<td>3.76 ± 0.17</td>
<td>2.72 ± 0.16</td>
<td>15.63 ± 1.51</td>
</tr>
</tbody>
</table>
Means results ± SE are shown. Glomerular filtration rate (GFR), urinary sodium excretion and fractional excretion of sodium were determined after the animals had been perfused with glucose solution for 1 h for groups A, B and C, and after the rats were expanded with a saline solution for groups A_E, B_E and C_E.

<table>
<thead>
<tr>
<th>Group</th>
<th>GFR (ml/min)</th>
<th>Urinary sodium excretion (μmol/min)</th>
<th>Fractional excretion of sodium (%)</th>
<th>Group</th>
<th>GFR (ml/min)</th>
<th>Urinary sodium excretion (μmol/min)</th>
<th>Fractional excretion of sodium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.34 ± 0.19</td>
<td>0.253 ± 0.06</td>
<td>0.076 ± 0.018</td>
<td>A_E</td>
<td>3.16 ± 0.33</td>
<td>2.14 ± 1.88</td>
<td>5.04 ± 0.26</td>
</tr>
<tr>
<td>B</td>
<td>2.0 ± 0.18</td>
<td>0.54 ± 0.32</td>
<td>0.21 ± 0.13</td>
<td>B_E</td>
<td>2.15 ± 0.43</td>
<td>2.98 ± 0.9</td>
<td>5.97 ± 0.6</td>
</tr>
<tr>
<td>C</td>
<td>2.97 ± 0.34</td>
<td>0.35 ± 0.15</td>
<td>0.08 ± 0.035</td>
<td>C_E</td>
<td>2.94 ± 0.38</td>
<td>2.73 ± 3.54</td>
<td>6.71 ± 0.34</td>
</tr>
</tbody>
</table>

Fig. 1. Effects of an active fraction obtained from the whole renal tissue of a volume-expanded rat (left panel) and a non-expanded rat (right panel) on urinary sodium excretion, fractional excretion of sodium and glomerular filtration rate in the assay. The broken line represents the mean base-line control value observed before infusion of the fraction.

of sodium were significantly greater in rats previously fed on a diet with sodium (groups B_E and C_E) than in those maintained on a diet without additional sodium (group A_E) (P < 0.005). Inulin clearances were similar for the three volume-expanded groups.

Fig. 1 depicts the effects in a rat of fractions derived from the kidney tissue obtained from a rat belonging to a volume-expanded group (B_E) and from a rat of a non-expanded group (C). Values are shown for urinary sodium excretion, fractional excretion of sodium and glomerular filtration rate.

Injection of a fraction from a non-expanded rat did not alter the glomerular filtration rate, the urinary sodium excretion and the fractional excretion of sodium. In contrast, when the fraction was provided by an expanded rat, urinary sodium excretion and fractional excretion of sodium increased significantly during 60 min without any consistent change in glomerular filtration rate.

Table 3 summarizes the changes in glomerular filtration rate, urinary sodium excretion and fractional excretion of sodium observed in the assays performed with a natriuretic fraction derived from the kidneys from the three non-expanded groups. Changes in fractional excretion of sodium calculated in the rat assays increased progressively in proportion to the sodium content of the diet which defined the three groups A, B and C. The differences between the groups are not significant and these results are not clearly supported by a parallel increase in urinary sodium excretion. However, a correlation does exist between the fractional excretions of sodium from the 24 h urine collections on day 5 in the metabolic cage and the changes in fractional excretion of sodium in the rat assay after injection of the natriuretic factor derived from the kidney, for the same animal, when these three groups are considered together (r = 0.64, P < 0.0025).

By contrast, fractions provided by the expanded groups A_E, B_E and C_E (Table 3) significantly increased the urinary sodium excretion as well as fractional excretion of sodium in the rat assay.

When the activity of the fractions of the same paired groups (expanded vs non-expanded groups) was compared, a significant difference in the urinary sodium excretion and fractional excretion of sodium existed: A_E vs A, P < 0.025 and P < 0.01 for urinary sodium excretion and fractional excretion of sodium respectively; B_E vs B, P < 0.01 and P < 0.0025; C_E vs C, P < 0.0005 and P < 0.0025.

There was also a significant difference after acute saline load in the activity of the natriuretic
**TABLE 3. Effect of the kidney fractions of non-expanded rats and volume-expanded rats in the rat assay, on glomerular filtration rate, urinary sodium excretion and fractional excretion of sodium**

Mean values ± SE are shown for glomerular filtration rate (GFR), urinary sodium excretion and fractional excretion of sodium for the collection periods obtained over the six experimental periods after infusion of the fractions; the mean values were compared with those obtained during the four control periods. Statistical significances (P) of the differences between the control periods and the experimental periods (absolute data) were analysed by the paired t-test for each group. In addition percentage changes are given for each experiment. N.S., Not significant. Groups are defined in the text.

<table>
<thead>
<tr>
<th>Group (no. of rats)</th>
<th>Control periods</th>
<th>Experimental periods</th>
<th>Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GFR (ml/min)</td>
<td>Urinary sodium excretion (µmol/min)</td>
<td>Fractional excretion of sodium (%)</td>
</tr>
<tr>
<td>A (n = 10)</td>
<td>1.39 ± 0.15</td>
<td>1.29 ± 0.31</td>
<td>0.807 ± 0.23</td>
</tr>
<tr>
<td>B (n = 7)</td>
<td>1.18 ± 0.28</td>
<td>1.17 ± 0.43</td>
<td>1.17 ± 0.43</td>
</tr>
<tr>
<td>C (n = 5)</td>
<td>1.84 ± 0.26</td>
<td>0.66 ± 0.33</td>
<td>0.7 ± 0.08</td>
</tr>
<tr>
<td>A₂ (n = 5)</td>
<td>1.17 ± 0.18</td>
<td>0.66 ± 0.28</td>
<td>0.63 ± 0.34</td>
</tr>
<tr>
<td>B₂ (n = 6)</td>
<td>1.58 ± 0.19</td>
<td>0.87 ± 0.39</td>
<td>0.685 ± 0.41</td>
</tr>
<tr>
<td>C₂ (n = 6)</td>
<td>1.58 ± 0.19</td>
<td>0.28 ± 0.1</td>
<td>0.12 ± 0.03</td>
</tr>
</tbody>
</table>

N.S., Not significant.
factor when previously salt-depleted rats were compared with rats fed on a diet containing sodium ($A_e - B_e + C_e$, $P < 0.0025$).

**Discussion**

This study demonstrates that a substance of low molecular weight, which possesses a natriuretic activity when injected into a rat bioassay system, can be extracted from the kidney tissue in the rat after acute extracellular volume expansion with saline.

This finding confirms the observation by Gonick & Saldanha (1975) and Hillgard *et al.* (1976), who also used a Sephadex G-25 column and collected an active fraction after the peak containing the sodium and other solutes. However, the buffer used by these workers was acetic acid, whereas we used ammonium acetate as we did in our previous studies; Favre *et al.*, 1975; Favre, 1978. It is impossible to decide whether or not the substances isolated in the work by Gonick and in our own study are identical, although they have several common properties. The time courses of the natriuretic response induced by the active fraction in these two studies are similar when the changes in urinary sodium excretion and fractional excretion of sodium are considered between 20 and 120 min after injection of the material into a rat assay (Fig. 1). However, the material we have isolated does not cause an increased urine flow, which confirms our previous findings (Favre *et al.*, 1975; Favre, 1978) and the finding of Clarkson *et al.* (1976). Gonick & Saldanha (1975), on the other hand, found a diuresis as well as a natriuresis. We injected more material than Gonick and obtained twice the natriuresis that he recorded if we consider the rats from group $B_e$, which received a similar salt intake before the expansion as the animals used by Gonick. Even if we accept a similarity between the two materials, it is hard to quantify their biological activity, as the bioassay models are different.

Gonick injected the natriuretic fraction directly into the renal artery of an anaesthetized, acutely uninephrectomized rat and collected the urine immediately after the injection of the fraction. In our study, the material was injected intravenously into non-anaesthetized, intact rats and urine collected 20 min after the beginning of the injection, a procedure which could make the assay less sensitive, but which has the advantage of diminishing the number of false positive responses. However, preliminary studies have demonstrated that the natriuretic effect of the fraction was immediate after the beginning of the injection when the urine collected during the first 20 min has been examined. This time course of the natriuretic response is relatively comparable with the one observed by Clarkson *et al.* (1976) for the low-molecular-weight substance (de Wardener, 1977).

The extent of the natriuretic response to an acute load of sodium is known to depend on the sodium content of the diet given to the animals before the acute experiment (Higgins, 1971; Diaz-Buxo, Haas, Ott, Cuche, Marchand & Knox, 1976; Nizet, 1976). The present data indicate that the activity of the natriuretic factor after acute expansion is significantly higher when material is extracted from the kidneys of rats that have previously received a diet containing sodium than from salt-depleted animals. For the three non-expanded groups, it can be seen from Table 1 that natriuretic factor activity is present in the kidney in proportion to the sodium content of the diet. No activity at all is detectable in the rats fed on a diet without added sodium, but some activity is present in the kidney tissue of animals on a 6 mmol of sodium/day diet. This activity correlates with the fractional excretion of sodium observed in the metabolic cage, which reflects the sodium intake of the rats, but not with the sodium elimination measured after 18 h of fasting, which is clearly independent of the amount of sodium ingested. Dietary sodium may stimulate the production or release of the natriuretic factor, an effect which could persist at least 18 h after the last meal. Our data indicate that the amount of natriuretic material present in the kidney is related to the sodium intake, the differences between the groups being revealed when an acute saline load is superimposed. Under this latter condition, the activity of the natriuretic material is significantly lower in the group of rats fed on a diet without added sodium than in those rats ingesting 3 or 6 mmol of sodium/day, although the degree of acute expansion was similar.

In conclusion, the present data demonstrate that after acute volume expansion with saline, a material with natriuretic activity can be extracted from rat kidney, and that the amount of activity which can be extracted varies with the previous sodium content of the diet.

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