Sodium transport during the natriuresis of volume expansion; a study using peripheral blood leucocytes

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Summary

1. Leucocyte sodium transport was investigated as a possible assay for the small molecular weight natriuretic material isolated from the urine of normal subjects who had undergone volume expansion by saline infusion.

2. This fraction (fraction four or FIV), inhibitory to sodium transport in several other assays, was also found to inhibit leucocyte sodium transport.

3. FIV isolated from the urine of five normal subjects undergoing the natriuresis of mineralocorticoid escape was also found to be inhibitory to leucocyte sodium transport.

4. Leucocytes isolated from the blood of the same subjects during mineralocorticoid escape showed decreased sodium transport.

Key words: leucocyte, sodium transport, natriuretic factor, mineralocorticoid escape.

Introduction

It has been hypothesized that a natriuretic hormone may at least partly be responsible for the natriuresis which accompanies isotonic volume expansion [1]. Evidence for this includes the finding of natriuretic or sodium transport inhibitory activity in small molecular weight fractions of urine [2, 3, 4] and plasma [5, 6, 7] obtained from both man and animals undergoing natriuresis induced by volume expansion. The inhibitory effect on active sodium transport is presumably the mechanism whereby a natriuretic 'hormone' would work in the nephron. However, this hormone has yet to be fully characterized.

A small molecular weight material similar in characteristics to material found during the natriuresis of experimentally induced acute volume expansion has also been isolated from the urine of dogs undergoing the natriuresis of mineralocorticoid escape [3]. In dogs, mineralocorticoid escape is also associated with the appearance of natriuretic [8] and sodium transport inhibitory activity [9] in the serum and it has therefore been suggested that a natriuretic hormone may be involved. In man, however, there have been no previous studies which demonstrate inhibition of sodium transport during mineralocorticoid escape, nor has inhibitory activity in serum or urine been detected.

In general, the methods used for detection of the natriuretic fractions isolated from human urine or serum during acute volume expansion or mineralocorticoid escape [11] have involved assays with animals or animal tissue. Extrapolation in regard to human physiology therefore must be somewhat tentative.

The aims of this study were firstly to determine whether sodium transport in a human tissue, peripheral blood leucocytes, may be used as an alternative assay system for the small molecular weight fraction of urine obtained from acutely volume expanded normal subjects. Several reports suggest that sodium transport in peripheral blood cells may be inhibited by the natriuretic 'factor'. It might be expected that uraemia would be associated with an increase in a natriuretic hormone and a small molecular weight urinary natriuretic material with characteristics similar to that found in experimentally induced volume expansion has been isolated from patients with chronic uraemia [10]. This fraction has been found to be inhibitory to sodium transport in erythrocytes [11]. Moreover, both erythrocyte

Secondly, urine from subjects undergoing mineralocorticoid escape was fractionated and tested for sodium transport inhibitory activity using the leucocyte assay. Sodium transport was also investigated in leucocytes isolated from the blood of the same subjects taken whilst they were undergoing the natriuresis of escape.

Methods

Volume expansion by saline infusion

Eight healthy laboratory personnel (six male, two female) aged 23-33 were given an intravenous infusion of saline (0.9% NaCl), 20 ml/kg body weight, administered whilst supine over a period of 1 h. Twenty-four hour urine collections were made both before the infusion and immediately afterwards. The urine was stored at -20°C until required for isolation of the test material. The subjects were on an ad libitum sodium intake.

Isolation of the natriuretic fraction of urine

Fractionation of the urine was carried out by the method of Clarkson, Raw & de Wardener [2]. The sodium concentration of the urine was initially adjusted to 200 mmol/l, since the sodium concentration of urine affects the degree of extraction of the natriuretic material [2]. Urine equivalent to 2 h of the 24 h urine collection was freeze-dried and subsequently resuspended in 20 ml of distilled water. The insoluble residue was removed by centrifugation (4°C, 200 g) and the supernatant applied to a column (90 cm x 2.5 cm) of Sephadex G-25 (fine grade). Elution was carried out at 4°C by gravity, with ammonium acetate, 10 mmol/l, pH 6.8, as eluent. The fraction appearing immediately after the salts, fraction four or FIV (elution volume 660 ml), was collected and freeze-dried. In order to minimize loss and to remove ammonium and acetate ions, the extract was resuspended in distilled water, divided into aliquots equivalent to 0.5 h of the original urine collection and freeze-dried for a second time. The extracts were stored at -20°C in the presence of a desiccating material (silica gel).

Assay of the natriuretic material

FIV equivalent to 0.5 h of the post infusion 24 h urine collections was dissolved in 4 ml of a physiological medium (TC199; Wellcome Laboratories Ltd.) modified by the addition of Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer (Hopkin and Williams; final concentration 20 mmol/l) and foetal calf serum (5%, Difco Laboratories). The final pH was adjusted to 7.4-7.5. The dissolved extract was passed through a Millipore filter (0.22 μm pore diameter) and the modified medium added to a final volume of 25 ml. The sodium and potassium concentrations were adjusted to 136 mmol and 6 mmol/l respectively. The addition of the protein source (foetal calf serum) was found necessary in order to maintain the viability of the leucocytes (as measured by Trypan Blue exclusion) throughout the incubation period.

Leucocytes were isolated from 60 ml of peripheral venous blood obtained from healthy laboratory personnel by the method of Baron & Ahmed [14]. The isolated leucocytes were resuspended in the incubation medium containing the urine extract. The tubes were attached to a Matburn rotatory plate and the suspension was incubated at 37°C for 90 min. As a control leucocytes were incubated without any extract for 90 min. The cells were then isolated by centrifugation (3 min, 160 g) and a sample of the supernatant taken for estimation of ammonia, zinc and urea. Ammonia has an inhibitory effect on sodium transport in toad bladder [15] and zinc has been shown to stimulate leucocyte sodium transport in vitro [16]. Urea may affect sodium transport since it has been implicated in the natriuresis of volume expansion by urine reinfusion [17]. Ammonia was estimated by the method of Gips & Wibbens-Alberts [18] and in 31 samples of medium to which the extract had been added ranged from 231 to 673 μmol/l with a mean value of 523 ± 29 (SEM) μmol/l. Experiments in which leucocytes were incubated in a similar range of concentrations of ammonium bicarbonate demonstrated no inhibition of the sodium efflux rate constant. Zinc, measured by atomic absorption spectrometry (Instrumentation Laboratory, model 243) in the same samples demonstrated a mean value of 6.1 ± 0.9 (SEM) μmol/l and urea, estimated by the method of Marsh, Fingerhut & Miller [19] was present only in trace amounts. The pH, measured on 13 separate occasions using a blood gas analyser (Radiometer, Copenhagen) was between 7.4 and 7.5 in all experiments, both before and after the incubation period.

Sodium efflux rate constants

The leucocytes were resuspended in 5 ml of medium 199 and 740 kBq (20 μCi) of 22NaCl (The Radiochemical Centre, Amersham) added.
After 20 min incubation to allow for sufficient uptake and equilibration with the isotope, the total, ouabain-sensitive and ouabain-insensitive rate constants were estimated according to the method of Hilton & Patrick [20].

**Steady state and intracellular electrolytes**

In order to determine whether the leucocytes were in a state of equilibrium after incubation for 90 min, leucocyte sodium influx, intracellular sodium content and the total sodium efflux rate constant were simultaneously estimated in nine control experiments and in five in which FIV equivalent to 0.5 h of post saline infusion urine had been added to the incubation medium. Sodium efflux rate constants were estimated as described above. Leucocyte sodium influx was calculated over a period of 8 min according to the method of Hilton & Patrick [20]. In order that the leucocyte sodium content could be estimated on the same sample, the cells were washed in ice-cold magnesium chloride (100 mmol/l) to remove contaminating extracellular $^{22}$Na and non-radioactive sodium. After drying and weighing, sodium was leached out of the cell sample by treatment with nitric acid (0-1 mol/l). The sodium concentration of the acid solution was then determined by flame photometry and the intracellular sodium content calculated on the basis of cell dry weight.

**Mineralocorticoid escape**

$9\alpha$-Fluorocortisol was administered to six healthy male medical staff (4 mg/day in four divided doses) over a period of 7 days or until the subject underwent a natriuresis and so escaped from the sodium retaining effect of the mineralocorticoid. All subjects gave informed consent to the study. 24 h urine collections were made throughout the investigation and renal sodium excretion estimated daily. Potassium supplements were given as a wax based slow release preparation to compensate for renal losses. Blood pressure was monitored at least twice daily and did not increase in the five subjects who escaped, but in a sixth subject who did not escape and who became slightly oedematous, the blood pressure rose from a pre-study value of 110/80 mmHg to 130/90 mmHg on the sixth day of intake and the mineralocorticoid was withdrawn.

**Assay of urinary natriuretic material**

FIV was isolated from the urine of the subjects collected on the first day of mineralocorticoid dosage and on the last day, by the method described above. Assay of FIV equivalent to 0.5 h of the 24 h urine collection was then carried out by incubation with leucocytes and subsequent determination of the sodium efflux rate constants.

**Leucocyte sodium transport during mineralocorticoid escape**

Leucocyte sodium efflux rate constants were estimated in peripheral blood leucocytes from each subject before the administration of mineralocorticoids and when the natriuresis of escape occurred. In the subject who did not demonstrate a complete escape, the second estimation of sodium efflux rate constants was carried out on the last day of drug intake. In addition, sera from three of the subjects taken during escape were preincubated with normal leucocytes for 90 min and sodium efflux rate constants then estimated as described above. As a control for these experiments, normal leucocytes were incubated in the serum of normal subjects who had not undergone volume expansion.

**Leucocyte viability after incubation**

The values obtained for the sodium efflux rate constant in the leucocytes of normal subjects after incubation in tissue-culture medium were not significantly different from those obtained without prior incubation (total sodium efflux rate constant 3.82 ± 0.08 (SEM) h$^{-1}$ (n = 27) compared with 3.79 ± 0.07 (SEM) h$^{-1}$ (n = 26) with no incubation period (P = not significant)) and viability, as assessed by exclusion of Trypan Blue was more than 95% in all cases. Statistical analysis was carried out using Student's method of analysis for paired and unpaired data. Values are expressed as the mean ± SEM.

**Results**

**Effect of FIV on leucocyte sodium transport**

The incubation of normal leucocytes with the preinfusion urine extract FIV equivalent to 0.5 h of the 24 h urine collection did not bring about a detectable change in the rate constant for sodium efflux (total sodium efflux rate constant 3.89 ± 0.16 h$^{-1}$ (n = 8) compared with 3.82 ± 0.08 h$^{-1}$ (n = 27) for controls), whereas incubation with an equivalent amount of FIV from the urine of the same subjects after saline infusion resulted in a significant fall in the total sodium efflux rate.
constant when compared with the preinfusion values \(2.67 \pm 0.11 \ (n = 8)\); \(P < 0.001\), Fig. 1; Table 1]. This fall in the total sodium efflux rate constant was the result of a significant fall in both the ouabain-sensitive and ouabain-insensitive components of the rate constant (Table 1).

There was no detectable correlation between values for the total sodium efflux rate constant and the levels of urea, ammonia or zinc in the incubation media.

**Steady state**

Leucocyte sodium influx and efflux, estimated after 90 min incubation without extract, were not significantly different from one another, suggesting that the cells were in a state of equilibrium. Similarly, sodium influx and efflux were found to balance in leucocytes incubated with extract, although the intracellular sodium content was significantly raised when compared with that of cells incubated without extract (Table 2).

**Mineralocorticoid escape**

Five of the six subjects who received 9α-fluorocortisol demonstrated escape from the sodium-retaining effect of the drug and underwent a natriuresis within 7 days. In the five subjects who escaped, 24 h sodium excretion fell from a pre-9α-fluorocortisol value of 192 ± 28 mmol/l to 55 ± 18 mmol/l on day 2 of drug intake and by day 5 had risen to 136 ± 57 mmol/l. In the sixth subject, who did not escape, 24 h sodium excretion fell to 17 mmol/l on day 2 but by day 7 was only 16 mmol/l.

**Assay of FIV**

FIV equivalent to 0.5 h of the 24 h urine collection obtained from all six subjects on the first day of 9α-fluorocortisol intake did not affect the rate of sodium efflux in normal leucocytes after 90 min incubation (total sodium efflux rate constant \(3.85 \pm 0.22 \text{ h}^{-1} \ (n = 6)\) compared with \(3.82 \pm 0.08 \text{ h}^{-1} \ (n = 27)\) for controls with no extract; \(P = \text{not significant}\), whereas FIV from the urine of the subjects who escaped effected an overall inhibition of the rate of sodium efflux from normal leucocytes compared with the values obtained with the preinfusion extracts \(2.90 \pm\)

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**Table 1. Sodium efflux rate constants estimated in leucocytes of control subjects after incubation in FIV obtained from the urine of 8 subjects (a) before infusion of saline and (b) after saline infusion**

<table>
<thead>
<tr>
<th>(a) Pre-infusion FIV</th>
<th>(b) Post-infusion FIV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>1</td>
<td>3.65</td>
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<tr>
<td>2</td>
<td>3.63</td>
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<tr>
<td>3</td>
<td>3.44</td>
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<tr>
<td>5</td>
<td>4.41</td>
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<tr>
<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>4.06</td>
</tr>
<tr>
<td>8</td>
<td>4.12</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>3.89</td>
</tr>
<tr>
<td><strong>± SEM</strong></td>
<td>0.16</td>
</tr>
</tbody>
</table>

Compared with pre-infusion

\(P < 0.001\) \(P < 0.01\) \(P < 0.002\)
Table 2. Measurements of sodium influx, sodium content, sodium-efflux rate constants and sodium efflux in normal leucocytes after incubation with urine fraction FIV (post-saline infusion)

Values are compared with the results from nine experiments in which no extract was added during the period of incubation. N.S., not significant.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total sodium-efflux rate constant (h⁻¹)</th>
<th>Intracellular sodium content (mmol/kg cell dry wt.)</th>
<th>Influx (mmol h⁻¹ kg⁻¹ cell dry wt.)</th>
<th>Efflux (mmol h⁻¹ kg⁻¹ cell dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-02</td>
<td>98</td>
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<td>296</td>
</tr>
<tr>
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<td>115</td>
<td>222</td>
<td>250</td>
</tr>
<tr>
<td>3</td>
<td>2.76</td>
<td>116</td>
<td>290</td>
<td>320</td>
</tr>
<tr>
<td>4</td>
<td>3-00</td>
<td>94</td>
<td>304</td>
<td>290</td>
</tr>
<tr>
<td>5</td>
<td>2.41</td>
<td>93</td>
<td>236</td>
<td>224</td>
</tr>
<tr>
<td>Mean</td>
<td>2.60 ± 0.10</td>
<td>103 ± 5</td>
<td>270 ± 17</td>
<td>276 ± 17</td>
</tr>
<tr>
<td>Control</td>
<td>3.82 ± 0.08</td>
<td>78 ± 5</td>
<td>266 ± 12</td>
<td>276 ± 19</td>
</tr>
</tbody>
</table>

P < 0.001   P < 0.001   N.S.      N.S.

Leucocyte sodium efflux rate constants during mineralocorticoid escape

The natriuresis of escape in all five subjects was associated with a fall in the leucocyte sodium efflux rate constant to values below the normal range (2.77 ± 0.14 h⁻¹ compared with 3.76 ± 0.07 h⁻¹ before 9α-fluorocortisol; P < 0.001; n = 5 pairs; Fig. 2). This was primarily due to a fall in the ouabain-sensitive component of the rate constant (2.08 ± 0.20 h⁻¹ compared with 2.87 ± 0.20 h⁻¹ before 9α-fluorocortisol, n = 5 pairs, P < 0.05) and to a secondary, but not significant fall in the ouabain-insensitive component (0.71 ± 0.13 h⁻¹ compared with 0.95 ± 0.15 h⁻¹ for controls for the ouabain-insensitive; P = not significant). The value of 3.38 h⁻¹ for the leucocyte sodium efflux rate constant of the sixth subject who did not demonstrate a complete escape, estimated on the sixth day of drug intake was lower than the value obtained before 9α-fluorocortisol (3.84 h⁻¹), but still fell within the normal range.

Inhibition of leucocyte sodium transport was observed in preliminary experiments in which sera from three subjects taken during the escape period were incubated with control leucocytes. [Total sodium efflux rate constant 2.53 ± 0.34 h⁻¹ (n = 3) compared with 3.89 ± 0.13 h⁻¹ (n = 5) obtained with control sera; P < 0.001]. The value obtained after incubation of normal leucocytes with the serum from the subject who did not demonstrate a complete escape was 3.30 h⁻¹, which fell within the normal range.

Discussion

This study demonstrates inhibition of sodium transport in leucocytes by the small molecular weight natriuretic fraction which appears in the urine of normal subjects during the natriuresis of
volume expansion. The same fraction (FIV) has potent natriuretic activity when injected into the tail vein of rats [2] and inhibits active sodium transport in frog skin [4] and toad bladder [3]. It is also active after extraction from the urine of patients with chronic uraemia, as measured by assay in the rat [10]. Sodium transport in peripheral blood leucocytes might therefore present a suitable alternative assay for the natriuretic fraction FIV. It is apparent, therefore, that leucocytes are susceptible to the sodium transport inhibitory properties of the small molecular weight natriuretic material of human urine and two similar studies in erythrocytes agree with these findings. FIV isolated from the urine of patients with chronic uraemia has been shown to inhibit sodium transport in erythrocytes [11] and, in common with the present study, inhibition of both the Na+/K+-ATPase-mediated component of sodium efflux and the glycoside-insensitive component were demonstrated. In a preliminary report of a study of the small molecular weight natriuretic substance isolated from the urine of salt loaded subjects, Clarkson, MacGregor & de Wardener [21] have demonstrated a reduction in the total sodium efflux rate constant in radio-labelled erythrocytes after incubation with the natriuretic material.

The demonstration that the natriuretic material isolated from the urine of subjects after saline infusion inhibited both the ouabain-sensitive and insensitive-components of the sodium efflux rate constants would suggest that the substance may not be a specific inhibitor of Na+/K+-ATPase. Viskoper, Wald, Schwartz & Czaczkes [22] were unable to demonstrate a direct effect of natriuretic material on Na+/K+-ATPase, but the fraction tested was of larger molecular weight than that of the present study. It has been suggested that the natriuresis of volume expansion is achieved by alterations in the permeability of the collecting duct [23]. However, leucocyte sodium influx was not increased by incubation with the natriuretic fraction, whilst cell sodium increased, indicating an effect on the active components of cell sodium transport and not on cell permeability. The ouabain-insensitive component of sodium efflux in leucocytes is poorly understood, but it has been suggested that in the erythrocyte it may represent a second sodium pump [24] and that in kidney cortical tissue it may play a role in the regulation of cell volume [25].

Although no significant sodium transport inhibitory activity could be detected in FIV equivalent to 0.5 h of the preinfusion urine collection, activity was detectable (in preliminary experiments) when the amount of extract was doubled, suggesting the presence of some natriuretic activity in FIV even in the non-volume-expanded state. This would agree with the study by Clarkson et al. [2] in which natriuretic activity was detected in FIV in both salt-loaded and salt-depleted man, but to a lesser extent in the salt-depleted state.

The demonstration of an eventual escape from the sodium-retaining effect of mineralocorticoids in five of the six subjects studied is in agreement with other reports [26, 27]. The expected effect of mineralocorticoids on sodium transport would be stimulatory. Although this has not been described in leucocytes, mineralocorticoids stimulate Na+/K+-ATPase in renal tubules [28] and appear to translocate sodium out of smooth muscle cells in vitro [29]. The observed fall of the ouabain-sensitive sodium efflux rate constant in the leucocytes of normal subjects during escape is strongly indicative of the fact that the sodium-retaining effect of circulating mineralocorticoids is counterbalanced by an increase in a circulating natriuretic substance. This is supported by the preliminary results obtained demonstrating inhibition of leucocyte sodium transport with escape sera. The nature of this substance must remain speculative, but the simultaneous appearance of the small molecular weight sodium transport inhibitory material in the urine would suggest that an increase in the hypothesized natriuretic hormone may occur. An analogous situation to that described in this study may occur in rats with hypertension induced by one kidney DOCA (deoxycorticosterone acetate)—salt volume expansion. These animals demonstrate abnormalities of the sodium pump in the tail artery [30], and it has been demonstrated that a sodium transport inhibitor of similar molecular weight to the postulated natriuretic hormone exists in the serum [31]. In the case of the one-kidney DOCA—salt rat, where the effect of mineralocorticoids would be to stimulate sodium transport and the inhibitor to depress it, the net effect is decreased transport [30], similar perhaps to the situation occurring in mineralocorticoid escape.

The depression of the ouabain-sensitive sodium efflux rate constant described during escape is remarkably similar to that found in the leucocytes of patients with essential hypertension [32, 33], an observation which may not be entirely circumstantial. We have recently described (in leucocyte/serum incubation experiments identical with those of this study) that the serum of patients with essential hypertension [33] has sodium transport inhibitory properties similar to that of the ‘escape’ serum. Taken together, the results of this study and of that in essential hypertension
would lend support to the current theory that inhibition of sodium transport in essential hypertension might be secondary to increased production of the ‘natriuretic hormone’ [34, 35].

References


