Plasma Natriuretic Activity in Oedematous States

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Summary

Accumulating evidence suggests that extracellular volume (ECV) may be governed in part by a natriuretic hormone. To study its possible role in oedema formation plasma fractions IV from patients with the nephrotic syndrome, with cirrhosis of the liver, and with idiopathic oedema were studied for their effects on frog skin Na-transport and on rat renal Na-excretion. Plasma fractions IV from ECV-expanded healthy subjects and patients with aldosteronism significantly inhibited PD and SCC and in the rat increased urinary flow, C_{H2O}, and U_{NaV}. Neither antinatrieretic nor natriuretic activities were observed in patients with the nephrotic syndrome or liver cirrhosis. In patients with idiopathic oedema recurrent episodes of fluid retention up to 9% of b.wt. followed by spontaneous natriuresis were well correlated with antinatrieretic plasma activity. The results suggest that ECV in healthy subjects may be governed in part by a natriuretic hormone which is absent in ECV-expansion due to oedema. However, this mechanism may operate appropriately, though at an elevated threshold, in patients with idiopathic oedema.

Introduction

Positive sodium balance represents the fundamental disorder in the pathogenesis of oedema. According to our present knowledge renal regulation of sodium homeostasis is mediated by intrarenal physical factors as well as aldosterone activity, although both mechanisms may not entirely account for renal sodium handling observed in certain clinical conditions, e.g. in primary aldosteronism ('escape phenomenon'). Also the natriuresis following acute expansion of the extracellular fluid volume may occur despite reduction in glomerular filtration rate, against changes in transtubular physical forces, or in the presence of excessive amounts of mineralocorticoid activity. Therefore, a natriuretic hormone has been postulated to regulate renal sodium reabsorption (De Wardener et al, 1961; Lichardus & Pearce, 1966; Bricker et al, 1968; Buckalew et al, 1970; Kaloyanides & Azer, 1971; Sonnenberg et al, 1972; Kramer & Gonick, 1974; Kramer et al, 1974). Previous studies from this laboratory have shown that acute expansion of
the extracellular volume with isotonic saline is accompanied by the appearance of a natriuretic and antinatriferic activity in plasma from healthy subjects (Kramer et al, 1974). To evaluate the possible role of this humoral activity in ECV-expansion due to oedema formation in the present study antinatriferic and natriuretic activities of plasma from patients with the nephrotic syndrome, patients with cirrhosis of the liver, and patients with idiopathic oedema were investigated.

MATERIAL AND METHODS

Healthy human subjects were infused intravenously with one litre of isotonic Ringer solution within 30 minutes followed by two litres during the next two hours. Venous blood was drawn in these subjects before and at the end of each study and was obtained from six patients with the nephrotic syndrome, from six patients with cirrhosis of the liver and ascites, and from three patients with idiopathic oedema during episodes of fluid retention and of spontaneous fluid excretion. All blood samples were immediately placed in ice and centrifuged at 4°C for 10 minutes. Plasma was frozen stored at -18°C. Plasma samples (10 ml) were then fractionated by column chromatography using Sephadex® G-25. Single fractions of 2.0 ml were collected in plastic tubes by an automatic fraction collector and assayed for UV280-absorbance, ninhydrin reaction, and concentrations of sodium and calcium. The natriuretic fractions following the elution of calcium were then pooled to fraction IV and subsequently lyophilised (Kramer et al, 1974). The resulting powder was stored at -18°C.

For measurements of potential difference (PD) and short-circuit-current (SCC) the abdominal skin of R. temporaria was mounted into a lucite double chamber each with two half chambers. Total exposed skin surface was 5.76 cm². When PD and SCC had stabilised lyophilised plasma fractions were added to Ringer solution bathing the inside surface of the skin. At this time mean values for PD were 46 ± 2.5 mV and for SCC were 208 ± 9.5 μA (n=66). For bioassay of natriuretic plasma activity female Sprague-Dawley rats weighing approximately 250 g with previously free access to food and water were employed; 0.5 ml of lyophilised and 10-fold concentrated plasma fractions were injected intravenously, followed by at least four 15-minute urine collection periods. Statistical analysis of results was performed using double tail Student-t test. Data are presented as mean ± S.E.

RESULTS

Effects of Plasma Fractions IV from Healthy Subjects Before and After Acute ECV-expansion on PD and SCC

After the addition of post-expansion plasma fraction IV to Ringer solution bathing the inside surface of the skin an immediate fall in PD and SCC was
observed. Maximum inhibition was reached after 60 minutes and was completely reversible when fraction IV was replaced by fresh Ringer solution. In contrast, no inhibition of PD and SCC was noted with fraction IV of plasma obtained before expansion of the ECV (Table I). As shown in Figure 1A this inhibition by natriuretic fraction IV appears to be dose-related at the concentrations studied. Furthermore, the commonly observed enhancement of sodium transport induced by vasopressin is almost completely blunted in the presence of natriuretic plasma fraction IV (Figure 1B).

**TABLE I. Effects of plasma fractions IV from patients with liver cirrhosis and ascites from patients with the nephrotic syndrome on potential difference (PD) and short-circuit current (SCC) of isolated frog skin as compared to the effects of fractions IV from control subjects before and after ECV-expansion**

<table>
<thead>
<tr>
<th></th>
<th>20 min.</th>
<th></th>
<th>% change at 40 min.</th>
<th></th>
<th>60 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD</td>
<td>SCC</td>
<td>PD</td>
<td>SCC</td>
<td>PD</td>
</tr>
<tr>
<td>healthy subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>2.8</td>
<td>2.2</td>
<td>4.7</td>
<td>0.7</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>(2.9)**</td>
<td>(2.3)</td>
<td>(4.1)</td>
<td>(2.4)</td>
<td>(5.7)</td>
</tr>
<tr>
<td>ECV-expansion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nephrotic syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-16.3*</td>
<td>-10.3*</td>
<td>-20.7*</td>
<td>-16.7*</td>
<td>-21.7*</td>
</tr>
<tr>
<td></td>
<td>(5.4)</td>
<td>(1.5)</td>
<td>(4.2)</td>
<td>(2.4)</td>
<td>(3.7)</td>
</tr>
<tr>
<td>liver cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with ascites (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.3</td>
<td>3.5</td>
<td>4.2</td>
<td>6.2</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td>(4.8)</td>
<td>(5.1)</td>
<td>(3.3)</td>
<td>(5.7)</td>
<td>(4.0)</td>
</tr>
</tbody>
</table>

**S.E.
* p < 0.01

Effects of Plasma Fractions IV from Patients with the Nephrotic Syndrome and from Patients with Cirrhosis of the Liver on PD and SCC

As compared to the effects of natriuretic plasma from healthy subjects plasma fraction IV from patients with the nephrotic syndrome revealed no inhibitory activity on frog skin sodium transport (Table I). Sixty minutes after the addition of fraction IV to Ringer solution mean changes of PD of 9.0 ± 4.1% and of SCC of 3.1 ± 6.5% were not significantly different from the effects of control plasma.
Similarly, no antinatriferic effect was observed when fraction IV of plasma from patients with cirrhosis of the liver and ascites was added to Ringer solution (Table I). In contrast to plasma from these patients with secondary aldosteronism, plasma fraction IV from patients with primary aldosteronism revealed a significant antinatriferic activity. It further increased when patients with aldosteronoma received an additional salt load (Figure 2), which was rapidly excreted despite a rise in aldosterone excretion as observed in some of these patients.

![Graphs showing effects of plasma fraction IV on PD and SCC](image)

Figure 1. A: Dose-dependent decrease in PD and SCC of isolated frog skin by plasma fraction IV from ECV-expanded healthy subjects.
B: Effects of vasopressin on PD and SCC of isolated frog skin in the presence and absence of plasma fraction IV from ECV-expanded healthy subjects.

**Bioassay of Natriuretic Plasma Activity**

In the rat, no changes in GFR were observed after i.v. injection of 0.5 ml of lyophilised and 10-fold concentrated buffer-solution or control and post-expansion plasma fractions IV corresponding to 5.0 ml of original plasma activity. Figure 3 summarises the changes in urinary flow rate, free-water clearance, and sodium and potassium excretion during the first collection period after injection of 0.5 ml of plasma fractions IV from healthy subjects before and after acute ECV-expansion and from cirrhotic patients with ascites. While control fraction IV did not significantly alter urinary water and electrolyte excretion, fraction IV of natriuretic plasma markedly enhanced urinary flow rate, free-water clearance, and sodium excretion. Fraction IV of plasma from cirrhotic patients revealed a similar lack of natriuretic activity as did control fraction IV. There was no significant difference in mean potassium excretion between control and natriuretic fraction IV.
Figure 2. Representative time course of changes in PD and SCC of isolated frog skin induced by fraction IV of plasma from patients with aldosteronoma before and after salt load.

Figure 3. Mean changes in urinary flow rate (V), free-water clearance (CH2O), and sodium (UNaV) and potassium (UKV) excretion in the rat 15 minutes after i.v. injection of fraction IV of control and natriuretic plasma from healthy subjects and of plasma from patients with cirrhosis of the liver and ascites.
Effects of Plasma Fraction IV from Patients with Idiopathic Oedema on PD and SCC

Three of six female patients with idiopathic oedema showed recurrent episodes of sodium retention followed by spontaneous sodium excretion at time intervals of 10 to 14 days, when daily sodium intake was 130 mEq. Maximal changes in body weight of up to 9% were observed (Table II). Oedema formation was independent of menstrual cycle, of oestrogen and progesterone of renin and aldosterone activity. Plasma fraction IV obtained during periods of sodium retention revealed either no or only minor antinatriferic activity. At the onset of diuresis and natriuresis, however, a significant inhibition of frog skin sodium transport by plasma fraction IV was observed as shown in Figure 4 (Table II).

Figure 4. Representative changes in urinary volume, sodium excretion, body weight and antinatriferic activity of plasma fraction IV from a patient with idiopathic oedema on a sodium intake of 130 mEq day.
TABLE II. Antinatriuretic effects of plasma fractions IV from patients with idiopathic oedema during periods of spontaneous fluid retention and excretion (Na-intake: 130 mEq/day)

<table>
<thead>
<tr>
<th>Patient</th>
<th>% change in body weight</th>
<th>Urinary sodium excretion (mEq/24 h)</th>
<th>% change in PD</th>
<th>SCC</th>
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<tbody>
<tr>
<td>D.S. ♂️, 32 yr</td>
<td>9.2</td>
<td>88</td>
<td>-7</td>
<td>-17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>246</td>
<td>-44</td>
<td>-43</td>
</tr>
<tr>
<td>H.S. ♂️, 41 yr</td>
<td>6.3</td>
<td>44</td>
<td>+10</td>
<td>-12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>340</td>
<td>-68</td>
<td>-40</td>
</tr>
<tr>
<td>C.U. ♂️, 41 yr</td>
<td>8.8</td>
<td>10</td>
<td>-31</td>
<td>-21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>438</td>
<td>-72</td>
<td>-44</td>
</tr>
</tbody>
</table>

To summarise the results of the present study antinatriuretic activities demonstrated in plasma from volume-expanded control subjects and patients with oedema of varying origin were plotted against their respective 24-hr urinary sodium excretion. While no definite correlation with respect to changes in SCC is observed, a significant correlation between daily urinary excretion of sodium and the decrease in PD of the isolated frog skin induced by plasma fractions IV can be demonstrated as shown in Figure 5.

Figure 5. Correlation between daily urinary sodium excretion and changes in PD of the isolated frog skin induced by plasma fraction IV from control subjects with oedema of various origin.
DISCUSSION

Previous studies from this laboratory have demonstrated that a natriuretic fraction IV can be extracted from human plasma obtained following acute expansion of the extracellular fluid volume which inhibits SCC and PD of the isolated frog skin, which is saluretic in vivo (Kramer et al, 1974), and inhibits renal Na-K-ATPase in vitro (Kramer et al, 1969). This natriuretic fraction is soluble in water, alcohol, and trichloroacetic acid. Its estimated molecular weight, inactivation by chymotrypsin, and chromatographic pattern using thin layer chromatography suggest that it might be a polypeptide of low molecular weight. Thus extracellular fluid volume in healthy subjects may be governed in part by a natriuretic hormone (Krück, 1969; Sealey et al, 1969; Clarkson & de Wardener, 1972; Kramer et al, 1972; Kramer et al, 1974), which cannot be found in plasma from patients with the nephrotic syndrome or patients with cirrhosis of the liver (Kramer, 1975). In addition, Krück (1969) has previously demonstrated that veronal buffer-extracts of urine from patients with congestive heart failure lack the natriuretic activity present in urine from hydrated healthy subjects. It is of interest to note that water immersion of healthy subjects up to the neck is accompanied by a significant natriuresis (Epstein et al, 1972), which may be related to an increase in intrathoracic volume (Arborelius et al, 1972) with stimulation of low pressure receptors (Gauer et al, 1970). Such observations might explain why in patients with the nephrotic syndrome or cirrhosis of the liver with ascites formation of oedema, i.e. fluid expansion confined to the interstitial space, is not associated with the natriuresis commonly expected with expansion of both the interstitial and intravascular fluid volume. This interpretation might also apply to the positive demonstration of plasma natriuretic activity in patients with primary aldosteronism, who — despite excessive aldosterone activity — respond to additional salt ingestion with a marked diuresis and natriuresis (personal observation). Finally, the mechanism of spontaneous natriuresis following recurrent periods of fluid retention in patients with idiopathic oedema cannot be explained by factors commonly known to modulate renal sodium excretion (Oelkers et al, 1975). Yet, the present findings may suggest that a humoral natriuretic factor may operate appropriately, though at an elevated threshold, in these patients. The relative importance of this mechanism in the regulation of body fluid balance and its respective role in oedema formation, however, must await further investigation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Miss Angela Bäcker and the secretarial assistance of Mrs Gerti Gibbels.
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