Effect of chronic and acute changes in sodium balance on the urinary excretion of prostaglandins \( E_2 \) and \( F_{2a} \) in normal man

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Summary

1. The effects of changes in sodium balance on renal prostaglandins have been hitherto studied mainly in experimental animals and the results have been controversial. In this study the 24 h urinary excretion of prostaglandins \( E_2 \) and \( F_{2a} \) was measured by radioimmunoassay in seven normal subjects under basal conditions and after 5 days of a diet containing <20 mmol of sodium/day. Subsequently a sodium chloride (150 mmol/l: saline) load (300 mmol of sodium over 4 h) was infused and prostaglandins were again measured in hourly urine collections. Plasma renin activity and aldosterone were also measured under basal conditions, after the low sodium diet and at 2 and 4 h of the saline infusion.

2. Dietary sodium restriction was associated with a marked increase in prostaglandin \( E_2 \) excretion (from 769.7 ± 201.6 SEM to 1761.3 ± 304.9 ng/24 h, \( P < 0.0005 \)). Prostaglandin \( F_{2a} \) also increased from 1187.0 ± 390.1 to 1435.6 ± 344.6 ng/24 h, but this was not statistically significant. The prostaglandin \( E_2 \)/prostaglandin \( F_{2a} \) ratio increased from 0.83 ± 0.2 to 1.52 ± 0.34 (\( P < 0.01 \)). Plasma renin activity and aldosterone rose significantly (\( P < 0.05 \) and <0.0025 respectively).

3. During the saline load prostaglandin \( E_2 \) decreased after 2 h from 142.4 ± 29.9 to 86.7 ± 22.9 ng/h (\( P < 0.05 \)) and to 36.9 ± 5.96 ng/h after 4 h. Prostaglandin \( F_{2a} \) decreased at a slower rate, from 98.4 ± 18.7 to 37.5 ± 8.8 ng/h at 4 h (\( P < 0.02 \)). At 4 h the prostaglandin \( E_2 \)/prostaglandin \( F_{2a} \) ratio returned to control values (0.90 ± 0.17). Plasma renin activity and aldosterone decreased significantly after 2 h (\( P < 0.02 \) and <0.0025 respectively) and reached control values after 4 h.

4. The present study demonstrates that chronic and acute changes in sodium balance induce changes in the excretion of prostaglandin \( E_2 \) parallel to changes in plasma renin activity and aldosterone. The similar but quantitatively smaller changes in prostaglandin \( F_{2a} \) and the inversion of the ratio between the two prostaglandins during sodium deprivation suggest that at least two factors are involved: increased delivery of substrate for prostaglandin synthase and decreased activity of the prostaglandin \( E_2 \) 9-ketoreductase. Prostaglandins probably play an important role in the adaptation of the kidney to changes in sodium balance.

Key words: prostaglandins \( E \), prostaglandins \( F \), sodium.

Introduction

The interrelationships between renal prostaglandins and sodium (Na) balance have raised considerable interest in the past. The data available are contradictory in many respects. Systemic administration of prostaglandin \( E \) induces natriuresis in animals and in man (Lee, Kanegiesser, O'Toole & Westura, 1971; Fulgraff & Brandenbusch, 1974). Prostaglandins have been shown to influence Na transport in nephron segments \textit{in vitro} (Fine & Trizna, 1977; Iino & Imai, 1978). Studies of Na excretion after prostaglandin synthase inhibition in animals and...
man have shown antinatriuresis (Donker, Arisz, Brentjens, Van der Hem & Hollemans, 1976; Düssing, Opitz & Kramer, 1977), natriuresis (Kirschbaum & Stein, 1976) or no effect at all (Patak, Mookerjee, Bentzel, Hysert, Babej & Lee, 1975). The natriuresis induced by diuretics which inhibit Na transport in the loop of Henle has been thought to be mediated by prostaglandins, as it may be blunted by previous prostaglandin synthesis inhibition (Patak et al., 1975).

The effects of chronic and acute changes in Na balance on renal prostaglandins synthesis have been difficult to evaluate, mainly because of technical problems in prostaglandins assay. Most studies have dealt with animal models and the results have been conflicting (Zusman, Spector, Cadwell, Speroff, Schneider & Mulrow, 1973; Papanicolaou, Safer, Hornych, Fontaliran, Weiss, Bariety & Milliez, 1975; Tobian & O'Donnell, 1976; Weber, Larsson & Scherer, 1977; Davila, Davila, Oliw & Anggard, 1978; Lifschitz, Patak, Radem & Stein, 1978). We therefore decided to study the effects of changes in Na balance on the urinary excretion of prostaglandin E₂ and prostaglandin F₂α (PGF₂α and PGF₂α respectively) that reflect the renal synthesis of these prostaglandins (Frolich, Wilson, Sweetman, Smigel, Nils, Carr, Waston & Oates, 1975; Dunn & Hood, 1977).

**Material and methods**

Seven normal subjects were studied while hospitalized at Meir Hospital. They were four males and three females, aged 28–40 years (mean 31 years). The absence of renal disease was established by a normal urine sediment and creatinine clearance. Blood pressure was within the normal range (≤140/90 mmHg). The experimental protocol was as follows. On day 1 the subjects were given a free diet and a 24 h urine collection was obtained for measurement of basal excretion of prostaglandins and Na (baseline). On day 2, blood for plasma renin activity and aldosterone were obtained at 08.00 hours while the subjects were supine. The subjects were then put on an isocaloric diet containing less than 20 mmol of Na and 70 mmol of potassium for 5 days. On day 6 a 24 h urine collection was obtained for measurement of prostaglandins and Na (low Na). On day 7, the subjects remained recumbent. At 06.00 hours the 24 h urine collection was completed and urine was then collected until 08.00 hours. This 2 h collection served as baseline (0 h) for the subsequent study. At 08.00 hours blood was obtained for (supine) plasma renin activity and aldosterone measurements (low Na and 0 h values). Sodium chloride (150 mmol/l) was then infused at a constant rate for 4 h (500 ml/h). Urine collections were obtained at hourly intervals for prostaglandins and Na determination. Blood samples were obtained at 2 and 4 h for plasma renin activity and aldosterone measurements. The subjects stood briefly to void spontaneously. Blood for plasma renin activity was placed in ice-cooled tubes containing EDTA (ethylene diamine tetra-acetic acid), 1 mg/ml of blood, immediately centrifuged at 4°C and kept at −20°C until assayed. Blood for aldosterone was placed in tubes containing heparin as anticoagulant and plasma was kept at −20°C until assayed. Urine was collected under refrigeration and samples for prostaglandins determination were kept at −20°C until assayed.

Na was measured by flame photometry. Plasma renin activity and aldosterone were measured by radioimmunoassay (Haber, Koerner, Page, Kliman & Purnode, 1969; Malvano, Gandolfi, Giannessi, Giannotti & Grosso, 1976).

Prostaglandins were measured by radioimmunoassay. For the extraction of samples, to a 4 ml portion of urine a small amount (2000 c.p.m.) of [³H]PGE₂ or [³H]PGF₂α (80–100 Ci/mmol, The Radiochemical Centre, Amersham, Bucks., U.K.) was added for determination of recovery. Neutral lipids were extracted from the urine with petroleum ether. The pH of the aqueous phase was then adjusted to pH 4.0 with sodium acetate buffer (1 mol/l, pH 3.8) and the prostaglandins were extracted with diethyl ether. The diethyl ether was evaporated under nitrogen and the residue dissolved in 1 ml of Tris/HCl buffer (50 mmol/l), pH 7.5, containing sodium chloride (0.1 mol/l) and 0.1% bovine serum albumin. Recovery of [³H]-labelled prostaglandins after the extraction procedure was 82.8 ± 1.6% (SEM) (n = 20) for PGE₂ and 86.5 ± 4.1% (n = 20) for PGF₂α. The concentrations of PGE₂ and PGF₂α were determined in the aqueous phase with antisera to these prostaglandins produced in rats by immunization with conjugates of prostaglandins and bovine serum albumin. The properties of anti-PGE₂ have been described by Bauminger (1976). The antiserum to PGF₂α cross-reacts significantly (30%) with prostaglandin F₁₀, but only to a negligible extent (<0.1%) with prostaglandins A₂, B₂, E₂ and 13,14-dehydro-15-keto-PGF₂α. The radioimmunoassay was done essentially as described by Bauminger, Zor & Lindner (1973). Separation of free from antibody-bound prostaglandin was accomplished by rabbit anti-(rat IgY). The radioimmunoassay curves were linear over the range 15–500 pg/assay tube for PGE₂ and 50–1000 for PGF₂α. When known amounts of PGE₂ (0.25–1 ng/ml) or...
PGF$_{2\alpha}$ (0.5-4 ng/ml) were added to urine samples the values obtained in the aqueous phase after extraction were closely correlated with the expected values (recovery of $105\pm 7.2\%$ for PGE$_2$, $n = 6$, and $100\pm 9.6\%$ for PGF$_{2\alpha}$, $n = 4$). The specificity of the assay was supported by experiments in which the amount of prostaglandins determined on extracts subjected to thin-layer chromatography was compared with that measured in crude lipid extracts. Thin-layer chromatography was performed as described by Green & Samuelsson (1964) and the zones corresponding to PGE$_2$ or PGF$_{2\alpha}$ were eluted and subjected to radioimmunoassay. Similar amounts of prostaglandins were obtained in crude extracts and in chromatographically purified fractions. The correlation coefficient was 0.931 for PGE$_2$ and 0.955 for PGF$_{2\alpha}$ and the regression line $y = 1.055x - 20.15$ for PGE$_2$ and $y = 0.997x - 0.899$ for PGF$_{2\alpha}$ ($y$ = results obtained after chromatography, $n = 6$). When varying portions of the non-chromatographed urine sample extracts (0.025-0.2 ml) were subjected to radioimmunoassay of PGE$_2$ or PGF$_{2\alpha}$ the curves obtained were parallel to the standard curves for the respective prostaglandins ($P < 0.001$ for non-parallelism by Student’s $t$-test). The intra-assay coefficient of variation was 5% for PGE$_2$ ($n = 6$) and 8.5% for PGF$_{2\alpha}$ ($n = 8$). The interassay coefficient of variation was 8.7% for PGE$_2$ ($n = 4$) and 9.6% for PGF$_{2\alpha}$ ($n = 4$). The 24 h values obtained by us are comparable with those found by others using radioimmunoassay (Dray, 1978; Zia, Zipser, Speckart & Horton, 1978).

All data are presented as means ± SEM. Results were analysed by means of the paired $t$-test. All subjects consented to the study after being given a fully detailed description of the procedure. The study was approved by the Helsinki Committee of Meir Hospital.

Results

The effects of a low Na diet are presented in Table 1 and Fig. 1. The urinary excretion of Na

![](https://example.com/fig1.png)

**Table 1. Effects of low sodium diet in normal subjects**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Low Na</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary output (ml/24 h)</td>
<td>1314 ± 176</td>
<td>1037 ± 151</td>
<td>N.S.</td>
</tr>
<tr>
<td>Urinary sodium (mmol/24 h)</td>
<td>160.4 ± 20.2</td>
<td>17.3 ± 2.4</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>67.7 ± 4.6</td>
<td>65.7 ± 4.5</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Plasma renin activity (ng h$^{-1}$ ml$^{-1}$)</td>
<td>0.71 ± 0.11</td>
<td>4.55 ± 1.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>34.9 ± 4.2</td>
<td>153.7 ± 26.7</td>
<td>&lt;0.0025</td>
</tr>
<tr>
<td>Urinary PGE$_2$ (ng/24 h)</td>
<td>769.7 ± 201.6</td>
<td>1761.3 ± 304.9</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Urinary PGF$_{2\alpha}$ (ng/24 h)</td>
<td>1187.0 ± 390.1</td>
<td>1435.6 ± 344.6</td>
<td>N.S.</td>
</tr>
<tr>
<td>PGE$<em>2$/PGF$</em>{2\alpha}$ ratio</td>
<td>0.83 ± 0.20</td>
<td>1.52 ± 0.34</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
TABLE 2. Effects of sodium chloride infusion in normal sodium-depleted subjects

Values are expressed as means ± SEM. Significance of differences when compared with 0 h: * P < 0·05; ** P < 0·02; *** P < 0·01.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Urinary output (ml/h)</th>
<th>Urinary sodium (mmol/h)</th>
<th>Plasma renin activity (ng h⁻¹ ml⁻¹)</th>
<th>Aldosterone (pg/ml)</th>
<th>Urinary PGE₂ (ng/h)</th>
<th>Urinary PGF₂α (ng/h)</th>
<th>PGE₂/PGF₂α ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>56·2 ± 21·5</td>
<td>1·35 ± 0·61</td>
<td>4·55 ± 1·5</td>
<td>153·7 ± 26·7</td>
<td>142·4 ± 29·9</td>
<td>98·4 ± 18·7</td>
<td>1·52 ± 0·29</td>
</tr>
<tr>
<td>1</td>
<td>74·6 ± 13·9</td>
<td>4·52 ± 1·99</td>
<td>7·03 ± 2·41**</td>
<td>81·6 ± 18·6**</td>
<td>170·5 ± 22·2</td>
<td>83·3 ± 16·9</td>
<td>2·03 ± 0·59</td>
</tr>
<tr>
<td>2</td>
<td>75·9 ± 11·7</td>
<td>7·03 ± 2·41**</td>
<td>1·64 ± 0·75**</td>
<td>86·7 ± 22·9*</td>
<td>110·1 ± 22·2</td>
<td>68·7 ± 20·4</td>
<td>0·00 ± 0·0</td>
</tr>
<tr>
<td>3</td>
<td>77·7 ± 14·5</td>
<td>8·46 ± 2·59</td>
<td>0·97 ± 0·29</td>
<td>93·8 ± 11·9</td>
<td>52·8 ± 10·2</td>
<td>48·1 ± 14·8</td>
<td>2·00 ± 0·52</td>
</tr>
<tr>
<td>4</td>
<td>93·8 ± 11·9</td>
<td>8·56 ± 1·64</td>
<td></td>
<td></td>
<td></td>
<td>37·6 ± 8·8**</td>
<td>0·90 ± 0·17**</td>
</tr>
</tbody>
</table>

**FIG. 2. Effects of sodium chloride infusion on plasma renin activity, plasma aldosterone and urinary PGE₂ and PGF₂α. Symbols are as in Fig. 1.**

The effects of sodium chloride infusion are presented in Table 2 and Fig. 2. The urinary output increased slightly after the first hour of the infusion, but this was not statistically significant. A further increase was noted in the last hour. This increase also was not significant. The urinary excretion of Na increased progressively. Plasma renin activity and aldosterone decreased significantly after 2 h (P < 0·02 and < 0·01 respectively) and the decrease continued at 4 h. Both prostaglandins decreased progressively during the infusion. However, whereas the decrease in PGE₂ was statistically significant after 2 h (P < 0·05), the decrease of PGF₂α reached significance only at 4 h (P < 0·01). The mean PGE₂/PGF₂α ratio remained high as compared with baseline values until 3 h, despite lower levels of PGE₂ than PGF₂α at this time. At 4 h the PGE₂/PGF₂α ratio returned to control values (0·90 ± 0·17).

**Discussion**

The effects of changes in Na balance on the excretion of prostaglandins have been hitherto studied mainly in experimental animals. In the rat an inverse relationship between Na intake and renal tissue prostaglandin E was found (Tobian & O'Donnell, 1976). In rabbits no change in urinary prostaglandin E occurred during chronic changes in Na intake in one study (Lifschitz et al., 1978). However, Weber et al. (1977) described changes in the PGE₂/PGF₂α ratio during changes in Na balance in rabbits and measured different levels of activity of the enzyme PGE₂ 9-ketoreductase in renal tissue. This enzyme is responsible for the conversion of PGE₂ into PGF₂α. Davila et al. (1978) found an increase in both urinary prostaglandins during Na deprivation in the rabbit.

In man no changes in plasma levels of prostaglandins E and F have been found after chronic changes in Na intake (Zusman et al., 1973). An increase of prostaglandin E in renal
venous blood was induced by an intravenous saline load (Papanicolaou et al., 1975). However, the assessment of renal prostaglandins synthesis by measurement of their levels in plasma has been criticized (Dunn & Hood, 1977) and it is now recognized that urinary prostaglandins are much better correlated with the renal synthesis of these substances (Frolich et al., 1975; Dunn & Hood, 1977). Epstein, Lifschitz, Hoffman & Stein (1979) described an increase in urinary prostaglandin E during water immersion, a state similar in many respects to extracellular volume expansion. Additional evidence for a relationship between sodium-volume balance and renal prostaglandins in man has been demonstrated by the finding of elevated urinary prostaglandin E in patients with decompensated cirrhosis (Zia et al., 1978) and in patients with congestive heart failure (Walshe & Venuto, 1979).

The results of this study in man demonstrate a response of renal PGE₂ to decreased Na intake and to an acute load of sodium chloride parallel to those of plasma renin activity and aldosterone. The changes in PGF₂α excretion are relatively small. The overall effect of Na deprivation is an inversion of the PGE₂/PGF₂α ratio, which returns to normal after rapid restoration of the extracellular fluid volume. These data are in agreement with the results in animal experiments (Tobian & O'Donnell, 1976; Weber et al., 1977).

It is of interest to consider the possible factors that might induce the described changes in prostaglandins excretion. Prostaglandins are known to be synthesized in renal cortical (vascular) structures, in renal medullary interstitial cells and in the collecting ducts (Smith & Graham-Bell, 1978; Muirhead, Germain, Leach, Pitcock, Stephenson, Brooks, Bronsius, Daniels & Kinman, 1972). Probably, different stimuli induce prostaglandins synthesis by different cells. Since their action is considered to be limited to sites close to those of synthesis, it is also likely that they exert different effects on different portions of the nephron. Changes in the urinary excretion of prostaglandins could therefore be related to changes in synthesis in any of the prostaglandins synthesizing structures.

The parallel changes in both prostaglandins argue in favour of changes in the delivery of substrate to, or changes in the activity of, prostaglandin synthase. Conversely, the relatively selective increase in PGE₂ excretion, resulting in the inversion of the PGE₂/PGF₂α ratio during Na depletion, suggests a decrease in the activity of PGE₂ 9-ketoreductase. It is likely that both mechanisms are important. During sodium-volume depletion angiotensin II and antidiuretic hormone increase. Both these substances are capable of increasing the deacylation of phospholipids, thus augmenting the delivery of arachidonic acid for the activity of prostaglandin synthase (Gimbrone & Alexander, 1975; Zusman & Keiser, 1977). This probably occurs in the vascular structures (angiotensin II) and in the collecting ducts (antidiuretic hormone). Here prostaglandin E probably modulates the effect of these peptide hormones (Dunn & Hood, 1977). This effect is particularly important in mitigating the effect of the powerful vasoconstrictor angiotensin II, thus preventing renal ischaemia (Dunn & Hood, 1977). In this respect, it is known that levels of angiotensin II comparable with those present during volume depletion drastically reduce renal blood flow when given to dogs treated with inhibitors of prostaglandin synthesis (McGiff, Malik & Terragno, 1976). Although the activity of PGE₂ 9-ketoreductase cannot be measured directly in man, a decrease of its activity has been demonstrated in rabbits under conditions of Na depletion and the inverse during Na load (Weber et al., 1977). A similar mechanism appears likely in man. These changes in PGE₂ 9-ketoreductase activity may take place in the medullary interstitial cells. During Na depletion this could induce an increased delivery of PGE₂ into the loop of Henle, where the majority of prostaglandin E was shown to be excreted into the tubular lumen (Frolich, Williams, Sweetman, Smigel, Carr, Hollifield, Fleisher, Nies, Frisk-Homberg & Oates, 1976). During states of increased proximal tubular reabsorption of Na, such as Na depletion, a decrease in the absolute rate of Na absorption occurs in the loop of Henle, leading to a relatively constant fractional reabsorption of Na in that segment of the nephron (Landwehr, Schnerman, Klose & Giebisch, 1968). The increased delivery of PGE₂ at this site may mediate this 'tubulo-tubular balance'. The mechanism of the changes in the activity of PGE₂ 9-ketoreductase is not clear, but a role of the kallikrein-kinin system has been suggested (McGiff, Itskovitz, Terragno & Wong, 1976). During rapid restoration of extracellular volume the opposite changes take place (decrease of angiotensin II and of antidiuretic hormone and increased activity of PGE₂ 9-ketoreductase) and the basal levels of prostaglandins are thus restored. In conclusion, the observed changes in urinary prostaglandins excretion in response to variations in Na balance suggest that these substances play an important role in the adaptation and in the regulatory response of the kidney to decreased extracellular fluid volume.
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