EFFECT OF PROSTAGLANDIN SYNTHETASE INHIBITORS ON RENIN AND ALDOSTERONE IN MAN ON A NORMAL OR LOW SODIUM DIET

By

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ABSTRACT

The effect of two inhibitors of prostaglandin synthetase activity (acetyl-salicylic acid (ASA) and diclofenac sodium (DCFS)) on plasma renin activity (PRA) and plasma aldosterone (PA) was studied in normal subjects kept on a diet with constant sodium and potassium intake or on a low-salt diet for 7 days. In 9 subjects, 2 days of treatment with ASA (3 g/day) was followed by a significant decrease of PA in the supine position (after overnight rest); there was no significant decrease of PRA. However, both PA and PRA with the subjects in the upright position were significantly reduced after ASA. In 9 subjects treated with DCFS (200 mg for 2 days), both PA and PRA with the subjects in the supine and upright positions decreased significantly. Similar results were obtained from 4 subjects on a low-sodium diet (15-30 mEq./day) treated with DCFS (150 mg/day for 3 days). In contrast, no significant changes in PA or PRA with the subjects in the supine or upright position were observed in 4 subjects on a very low sodium diet (<15 mEq./day) treated with DCFS (150 mg/day for 3 days). Covariance analysis (with PRA as independent variable and PA as dependent variable) of the data obtained in the upright position before and

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after treatment with ASA or DCFS showed that the decrease in PA remained significant after adjustment for the PRA effect. These results suggest a direct effect of prostaglandins on PA. The data obtained from subjects on a low sodium diet indicate that sodium deprivation may counteract the effects of DCFS on PRA and PA.

The effects of prostaglandin infusion on renin and aldosterone secretion recorded in man have been inconsistent (Carr 1973; Carlson et al. 1969; Fichman et al. 1972) and the role of the various prostaglandins has not yet been clarified (Golub et al. 1976).

Prostaglandin synthetase inhibitors show promise as a tool for the study of the regulatory role of prostaglandins on renin and aldosterone secretion (Romero et al. 1976).

Since it is unlikely that any drug would act as a pure prostaglandin synthetase inhibitor it was deemed reasonable to compare the effects of different drugs with inhibitory properties and to assume that any “common” effect would be due to these properties. We have studied the effects of acetyl-salicylic acid (ASA) and of diclofenac sodium (DCFS) (Ku et al. 1975) on plasma renin activity (PRA) and on plasma aldosterone (PA) in normal subjects with either normal or low sodium intake, both in recumbency and after postural stimulation.

**SUBJECTS AND METHODS**

**Subjects**

In the first study, 18 healthy volunteers, 7 men and 11 women, ranging in age from 16 to 50 years, were studied while on a regular hospital diet, providing 150 mEq. of sodium and 80 mEq. of potassium daily. A blood sample was drawn at 9 a.m. with the subject in bed after a night rest; the subject then spent the morning standing or walking; a second blood sample was obtained at 12 a.m.

The procedure was repeated after treatment with acetyl-salicylic acid (ASA) 6 g in 2 days, in 9 subjects, or with diclofenac sodium (DCFS) 200 mg in 2 days, in 9 other subjects.

In the second study, normal volunteers, 5 men and 3 women, ranging in age from 25 to 50 years, were studied while on a low sodium intake. Four subjects received a basic diet with an additional measured amount of salt to increase the sodium content to 20–30 mEq. daily. The study was begun after 7 days on a low sodium diet according to the scheme described above: blood sampling was performed in the supine position and after 3 h in the upright position, before and after 3 days of treatment with DCFS (150 mg/day on days 8, 9, 10 of the low-salt regimen). It was deemed to increase the total dose of DCFS (from 200 mg in 2 days to 450 mg in 3 days) because it has been reported that a low-sodium intake is associated with an enhanced activity of prostaglandin synthetase (Payakkapan et al. 1975) and possibly, therefore, with a higher resistance to inhibition of this enzyme system.

The blood samples in both studies were processed according to the methods outlined below.
Methods

Blood was collected into iced tubes containing EDTA-Na and promptly centrifuged at 4°C for 10 min. Plasma was then frozen and kept at −20°C until assay.

Plasma renin activity (PRA) was determined by the method of Haber et al. (1969), with minor modifications. The coefficient of variation on a normal plasma pool was 5.8% within-assay and 15% inter-assay.

Plasma aldosterone (PA) was determined by radioimmunoassay (Dow Chem.) of the dichloromethane plasma extracts. The coefficient of variation on a normal plasma pool was 8.9% within-assay and 16% inter-assay.

Plasma cortisol (PC) was measured by the method of Murphy (1967). Urinary sodium and potassium were determined by flame photometry. The possibility of an interference of these drugs on the analytical determination of PRA and of PA was investigated as follows: Plasma samples were tested for renin activity according to Skinner (1967), with and without addition of ASA (100 μg/ml) or of DCFS (3 μg/ml) to the reaction mixture. The concentrations are about two times larger than the one likely to be attained in the in vivo experiments (Pütter & Bauer 1970; Communication by Ciba-Geigy).

No apparent change in enzymatic activity was associated with the addition of the drugs.

A possible interference of the drugs in the radioimmunological determination of generated angiotensin I and of aldosterone was tested by comparing three standard curves for each method; one of the curves was obtained in the usual manner while to every sample in each of the other curves ASA or DFCS was added in phosphate buffer to reach a final concentration of 100 μg/ml of ASA, or of 3 μg/ml of DFCS.

The resulting curves were found to be superimposable: therefore these drugs do not seem to interfere in these radioimmunological assays.

These drugs furthermore, do not interfere with the binding of aldosterone to plasma protein, as tested by the method of Daughaday et al. (1961).

RESULTS

The mean results for the 18 subjects who had a normal sodium content diet in the first study are showed in Table 1 a, and those for the subjects with a restricted sodium intake in Table 1 b. In both tables the PRA value is expressed in ng/ml/h and the PA value in ng/100 ml.

First study (normal study) (Table 1 a)

In the first study, the increase in values in the control group after postural stimulus was significant both for PRA and for PA. It was found that there was a significant correlation between the PRA values in the two positions (r = 0.716; P < 0.001) but not for PA. On the other hand, the absolute change in PA (∆PA) was significantly correlated with the absolute change in PRA (∆PRA) (r = 0.552; P < 0.05), while PA did not show a significant correlation to PRA either in recumbency or in the standing position. When ASA was administered to 9 of these subjects there was a significant diminution of PA
Table 1a.
Mean (± sd) values for PA and PRA in subjects with a normal sodium content diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Supine</th>
<th></th>
<th>Standing</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
<td>After treatment</td>
<td>$t_1$</td>
<td>$t_2$</td>
</tr>
<tr>
<td>Control</td>
<td>PRA</td>
<td>0.39 ± 0.35</td>
<td></td>
<td>1.28 ± 0.78</td>
<td></td>
<td>6.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PA</td>
<td>8.14 ± 2.81</td>
<td></td>
<td>24.69 ± 10.13</td>
<td></td>
<td>6.55</td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>PRA</td>
<td>0.31 ± 0.23</td>
<td>0.26 ± 0.27</td>
<td>1.16 ± 0.72</td>
<td>0.54 ± 0.36</td>
<td>3.64</td>
<td>4.36</td>
</tr>
<tr>
<td>n = 9</td>
<td>PA</td>
<td>7.37 ± 2.47</td>
<td>4.02 ± 2.46</td>
<td>24.64 ± 7.84</td>
<td>14.47 ± 4.48</td>
<td>3.40</td>
<td>6.29</td>
</tr>
<tr>
<td>DCFS</td>
<td>PRA</td>
<td>0.48 ± 0.44</td>
<td>0.18 ± 0.08</td>
<td>1.39 ± 0.88</td>
<td>0.67 ± 0.58</td>
<td>3.71</td>
<td>4.37</td>
</tr>
<tr>
<td>n = 9</td>
<td>PA</td>
<td>8.91 ± 3.05</td>
<td>6.48 ± 3.28</td>
<td>24.73 ± 12.52</td>
<td>11.72 ± 4.86</td>
<td>3.29</td>
<td>3.58</td>
</tr>
</tbody>
</table>

$t_1 = t$ statistic (Student's for paired data) for comparison of supine data, from before to after treatment.
$t_2 = t$ statistic for comparison of standing data, from before to after treatment.
$t_3 = t$ statistic for comparison of before-treatment data, from supine to standing.
$t_4 = t$ statistic for comparison of after-treatment data, from supine to standing.

All comparisons except from $t_1$ PRA for ASA group, were statistically significant. ($a = 0.05$).
Mean values (and SEM) of plasma renin activity (PRA) (●) and of plasma aldosterone (PA) (○) in supine (△) and upright position (▲) before (continuous line) and after (broken line) treatment with acetyl-salicylic acid (ASA) or diclofenac sodium (DCFS) on normal sodium intake (upper diagrams) or on low or very low sodium intake (lower diagrams).

but not of PRA in the supine position. In the upright position there was significant lowering of both PRA and of PA (Fig. 1). Both the increase in PRA and in PA after assuming the upright position was also statistically significant after ASA treatment. This was also the case before drug treatment i.e. the correlation between the values in the recumbent and those in the upright position was significant for PRA ($r = 0.774; P < 0.01$) but not for PA ($r = 0.474; N.S.$).

The absolute change in PRA ($\Delta$PRA) from the recumbent to the upright position was significantly decreased after treatment (from $m = 0.87$, $sd = 0.621$, to $m = 0.28$, $sd = 0.231$ $t_{dep} = 3.244; P < 0.02$) as was the absolute change in PA ($\Delta$PA) (from $m = 17.27$, $sd = 8.223$ to $m = 10.455$, $sd = 3.963$ $t_{dep} = 2.442; P < 0.05$).

In a second series of 9 subjects the administration of DCFS brought about a significant diminution of both PA and PRA in the supine position. In the upright position, both PRA and PA were significantly reduced after treatment with DCFS (Fig. 1).
Correlation between $\Delta$PA and $\Delta$PRA before treatment.

In 5 patients plasma cortisol concentration was determined as the mean of the level at 9 and 12 a.m. before and after treatment with DCFS. The mean value before treatment was 65.1 ng/ml (sd 34.48) and after treatment 52.7 ng/ml (sd 21.60) ($t_{dep} = 1.37$; N. S.).

The change in position from the supine to the upright one was associated, like that before treatment, with a significant rise in PA with a significant correlation “supine-upright” for PRA but not for PA. The absolute change in PRA and in PA, from the recumbent to the upright position, was significantly reduced after treatment.

Correlation between $\Delta$PA and $\Delta$PRA after treatment with acetyl-salicylic acid or diclofenac sodium.
Since ASA and DCFS appeared to exert identical effects in this study, a statistical analysis was also performed on the whole series of results obtained with either drug so as to take advantage of the larger set of data. Since it might be assumed that ΔPA is causally related to ΔPRA, we investigated the statistical correlation between the pairs of values of ΔPA and of ΔPRA, before and after treatment. The correlation was significant before treatment \(r = 0.552, P < 0.05\) but not after treatment \(r = 0.09, \text{N.S.}\) (Figs. 2 and 3).

Analysis of covariance with PA as dependent variable and PRA as independent variable showed that the diminution of PA after treatment remained significant after adjustment for PRA effects, both in the supine \((F_{1,33} = 9.14, P < 0.01)\) and in the upright position \((F_{1,33} = 8.35, P < 0.01)\). Using ΔPRA and ΔPA from the supine to the upright position as independent and dependent variables, the diminution of ΔPA after treatment was not statistically significant, though a trend was evident \((F_{1,33} = 3.21, P < 0.1)\).

Second study (restricted sodium) (Table 1 b)

In the second study, in subjects with a moderately restricted sodium intake (15–30 mEq. daily) it was found that both PRA and PA appeared to be higher than in the subjects on a normal sodium diet before treatment, both in the supine and in the upright position (PRA in supine position: \(t = 3.39, P < 0.005\); PRA in upright position \(t = 1.75, P < 0.1\). PA in supine position: \(t = 2.83, P < 0.02\); PA in upright position: \(t = 2.32, P < 0.05\)) (Fig. 1).

The urinary excretion of sodium and potassium did not show significant changes after treatment with DCFS; however, urinary sodium had a tendency to increase after treatment, and potassium a slight tendency to decrease.

The diet with the lowest sodium content (10–15 mEq. daily) was associated with still higher values of PRA and PA in comparison with normal sodium intake (PRA in supine position: \(t = 5.41, P < 0.001\); PRA in the upright position: \(t = 13.27, P < 0.001\). PA in supine position: \(t = 7.31, P < 0.001\); PA in upright position: \(t = 6.84, P < 0.001\)) (Fig. 1).

In this group, too, urinary sodium and potassium did not undergo significant changes after treatment with DCFS, with only a slight tendency of the urinary sodium to increase.

A highly significant negative correlation seemed to exist between sodium excretion and PA (in upright position) in the whole series of patients on a low and on a very low-sodium diet before and after treatment with DCFS \((r = 0.797, P < 0.001)\) (Fig. 4).

Moreover PA in supine position appeared to be negatively correlated with sodium excretion, though less significantly \((r = 0.569, P < 0.05)\).

After treatment with DCFS only minor and inconsistent change in PRA and PA were observed (Fig. 1).
Table 1b.
Mean (± sd) values for PA, PRA, urinary Na (mmol/24 h) and urinary K (mmol/24 h), before and after treatment with DCFS in subjects on a low or very low sodium diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
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<tr>
<td>DCFS, sodium</td>
<td>PRA</td>
<td>mean</td>
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</tr>
<tr>
<td>15–30 mmol/24 h</td>
<td></td>
<td>sd</td>
<td>0.71</td>
</tr>
<tr>
<td>n = 4</td>
<td>PA</td>
<td>mean</td>
<td>15.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sd</td>
<td>10.06</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>mean ± sd</td>
<td>19.0 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>mean ± sd</td>
<td>34.9 ± 14.4</td>
</tr>
<tr>
<td>DCFS, sodium</td>
<td>PRA</td>
<td>mean</td>
<td>1.55</td>
</tr>
<tr>
<td>&lt; 15 mmol/24 h</td>
<td></td>
<td>sd</td>
<td>0.59</td>
</tr>
<tr>
<td>n = 4</td>
<td>PA</td>
<td>mean</td>
<td>30.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sd</td>
<td>12.62</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>mean ± sd</td>
<td>4.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>mean ± sd</td>
<td>33.2 ± 11.4</td>
</tr>
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</table>
DISCUSSION

The results of direct infusion of prostaglandins on renin secretion in man are contradictory (Carr 1973; Carlson et al. 1969; Fichman et al. 1973). However, consistent results have been obtained with substances acting \textit{in vivo} on prostaglandin synthetase. According to Oates \textit{et al.} (1975), the administration of ethyl arachidonate, a precursor of prostaglandins, increases renin, while indomethacin reduces it. Indomethacin administration decreases renin in man, in both the supine and in the upright position (Rumpf \textit{et al.} 1975; Frölich \textit{et al.} 1976\textit{a}), after furosemide (Frölich \textit{et al.} 1976\textit{a}) and isoprenaline infusion (Frölich \textit{et al.} 1976\textit{b}).

The effects of prostaglandins on renin secretion might be explained by their action on blood pressure (Larsson \textit{et al.} 1974), renal sodium handling (Vander 1968), or enzyme kinetics (Kotchen & Miller 1974). However, a prostaglandin-induced renin release in renal slices (Weber \textit{et al.} 1976) and in isolated renal cells (Dew & Michelakis 1974), consistent with a direct effect of prostaglandins on renin, has recently been reported.

Prostaglandin synthetase inhibitors have been found useful in the treatment of Bartter's syndrome, where they decrease renin and aldosterone levels (Verbeckmoes \textit{et al.} 1976; Norby \textit{et al.} 1976; Bartter 1976).

Our results show that the administration of aspirin and diclofenac sodium, two non-steroidal anti-inflammatory drugs, of different molecular structure (Flower & Vane 1974), which inhibit prostaglandin synthetase activity (Vane 1971; Ku \textit{et al.} 1975) is followed by a decrease in renin and aldosterone in the
supine and in the upright position. The absolute change of renin (ΔPRA) and of aldosterone (ΔPA) induced by postural stimulation were significantly smaller after treatment, though still statistically significant. It is interesting that while a significant correlation exists between ΔPRA and ΔPA before treatment, it is eliminated by the administration of prostaglandin inhibitors (Fig. 2). The results of an analysis of covariance suggest that the decrease in renin is not the only determining factor for the diminution of aldosterone after treatment, since the changes induced by aldosterone are still highly significant even after adjustment for the changes in renin.

The decrease of aldosterone in recumbency after treatment is unlikely to be due to renin diminution because the renin system does not appear to control aldosterone in recumbency (Armbruster et al. 1975), as is also suggested by our data on the lack of correlation between renin and aldosterone before treatment. It seems likely therefore that, as proposed by Fichman et al. (1973), prostaglandins also play a non-renin mediated role in aldosterone secretion. According to Frölich et al. (1976a) and to Golub et al. (1976) there is no evidence for a direct role of prostaglandin on the human adrenal cortex in vivo. The discrepancies between our own and Frölich's data may be due in part to the different experimental model: Frölich et al. (1976b) investigated the effect of indomethacin on angiotensin II stimulated aldosterone in subjects with post-malignant hypertension, while Golub et al. (1976) conducted the same experiment in 8 normal subjects given a single dose of indomethacin 2 h before starting the test. They state that the peak response of aldosterone was decreased by indomethacin from 19.7 ng/100 ml (SE 3.2) to 11.7 (SE 2.4). However, by re-calculating the mean and the standard error of the data reported in the paper we obtain a result after treatment of 12.8 (SE 1.72). The paired "t" is 7.46. Thus Golub's data also show a highly significant inhibition of aldosterone response to angiotensin II, supporting the possibility of a role of prostaglandins in modulating aldosterone secretion.

The decrease of aldosterone in our experiment might be due in part to a decrease in ACTH release or to a diminished responsiveness of the adrenals to endogenous ACTH. The decrease of aldosterone in recumbency, where ACTH seems to act as the main controlling factor of aldosterone secretion (Armbruster et al. 1975) might support this hypothesis; our data on plasma cortisol are not sufficiently large to settle this point. Urinary sodium and potassium excretion both before and after treatment with diclofenac, in patients on low or very low-sodium diet, was found to be significantly correlated to plasma aldosterone (both in recumbency and in upright position) in such a way that the plot of sodium excretion versus plasma aldosterone closely resembles the normal diagram of sodium excretion versus daily urinary aldosterone excretion. The fact that sodium excretion after diclofenac appears to be related to plasma aldosterone in the same way as before treatment would
suggest that sodium and potassium excretion is not influenced directly by treatment with diclofenac, but through the changes in aldosterone secretion. It should be noted that the effects of prostaglandin synthetase inhibitors are still evident in subjects with a moderate sodium depletion, while they are no longer seen in severe sodium depletion. It is possible that enhanced activity of prostaglandin synthetase during a very low-sodium diet (Payakkapan et al. 1975) counteracts the diclofenac induced block. On the other hand, it cannot be excluded that sodium depletion might directly stimulate renin and aldosterone secretion without prostaglandin mediation.

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REFERENCES


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