Reduction of Plasma Renin Activity by Inhibition of the Fatty Acid Cyclooxygenase in Human Subjects

Independence of Sodium Retention

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SUMMARY We carried out the present studies to determine whether the suppression of plasma renin activity (PRA) that follows inhibition of prostaglandin (PG) synthesis can be dissociated from the sodium-retaining effects of these drugs. In an initial investigation we studied the effect of indomethacin on PRA in normal subjects in balance on a 10 mM Na+ diet to prevent Na+ retention. Under these experimental conditions indomethacin did not lower PRA even though the fatty acid cyclooxygenase was inhibited, as indicated by a >70% reduction in the major urinary metabolite of prostaglandin E (PGE-M). Sodium depletion leads to enhanced sympathetic activity. We therefore studied the effect of indomethacin on a group of subjects in 10 mM Na+ balance in whom the effect of increased β-sympathetic activity was blocked by the administration of propranolol. In this group, indomethacin caused 85% suppression of PGE-M and had no effect on Na+ balance, but reversibly reduced PRA in the supine and upright positions by 84% and 70%, respectively. In normal subjects in 10 mM Na+ balance, the isoproterenol-induced increase in PRA also was unaffected by indomethacin. These data establish that inhibition of the cyclooxygenase can result in a reduction of PRA that is independent of changes in Na+ balance or β-sympathetic tone. Circ Res 44: 781-787, 1979

PREVIOUS STUDIES from this (Frölich et al., 1976) and other (Gill et al., 1976; Bartter et al., 1976; Rumpf et al., 1975) laboratories have shown that indomethacin reduces plasma renin activity (PRA) in man, and we have found that this effect of indomethacin is associated with a significant reduction in renal prostaglandin synthesis. The reduction in PRA produced by indomethacin was accompanied by sodium retention in patients with hypertension as well as in normal volunteer subjects (Frölich et al., 1976). The observation that the acute rise in PRA within minutes after intravenous infusion of furosemide was completely blocked by indomethacin, whereas, the natriuretic effect of furosemide was only slightly reduced (Frölich et al., 1976), suggested that indomethacin could affect PRA by a mechanism independent of sodium retention.

The present studies were conducted to test further the hypothesis that indomethacin reduces PRA by a direct action that does not require retention of sodium. In an initial investigation in which the possibility of sodium retention was circumvented by sodium depletion with furosemide followed by a 10 mM sodium diet, indomethacin did not lower PRA. This lack of effect of indomethacin on PRA during sodium depletion was confirmed in a second group of normal volunteer subjects. Sodium depletion is a recognized stimulus for catecholamine secretion (Gordon et al., 1967; Robertson et al., 1977), and increased circulating catecholamine levels are associated with increased levels of PRA. Thus, the low sodium diet of our subjects not only may have prevented sodium retention during indomethacin therapy but also may have overcome the inhibitory effect of indomethacin by activating the β-adrenergic stimulus for release of PRA. To eliminate any situation of renin release that might result from such an activation of the sympathetic nervous system, a study was conducted in which volunteers were brought into balance on a 10 mM sodium diet and propranolol was given in a dose that was supramaximal with respect to inhibition of renin release. Under these conditions indomethacin caused a significant, reversible reduction in PRA and prostaglandin E (PGE) synthesis but did not affect sodium balance. To characterize further the
relationship between PRA, β-sympathetic tone, and sodium balance, we studied the effect of isoproterenol infusion on PRA in normal volunteers in 10 and 150 mM Na⁺ balance. The results of these studies are compatible with a role of the prostaglandin system in the regulation of renin release in man that is independent of both sodium retention and the adrenergic nervous system.

Methods

Studies of the Effect of Indomethacin on PRA in Normal Volunteers in 10 mM Sodium Balance

Five normal male volunteer subjects were brought into balance on a 10 mM sodium diet. This was accomplished by placing them on a 10 mM Na⁺, 80 mM K⁺ diet and administering 40 mg of furosemide twice on the 1st day of the study to hasten achievement of the desired Na⁺ balance. After they had achieved sodium balance as determined by urinary Na⁺ excretion of less than 10 mM/24 hours, they received in a single blind randomized crossover fashion either placebo or indomethacin, 50 mg three times a day for 2 days, and 50 mg on the morning of the 3rd day. On the 2nd day a 24-hour urine sample was collected for analysis of Na⁺, creatinine, and 7α-hydroxy-9,11-diketotetranorprost-1,16-dioic acid (PGE-M), the major metabolite of PGE₁ and PGE₂ in man (Hamberg, 1972; Frolich, 1977). On the 3rd day PRA and plasma aldosterone levels were determined according to methods previously described (Carey et al., 1972; Ito et al., 1972). The placebo and drug periods of the study were separated by 18 days, during which periods the subjects ate their normal ad libitum diet.

The Effect of Indomethacin on PRA in Hypertensive Patients in 10 mM Na⁺ Balance during Propranolol Administration.

Initially, a single normotensive subject was studied in 10 mM Na⁺ balance while taking propranolol, 240 mg daily. Because he developed symptoms due to orthostatic hypotension, subsequent studies were carried out in hypertensive individuals. The nature of their hypertension had been evaluated previously by a rapid sequence intravenous pyelogram, aortography with selective renal arteriograms, urinary vanillylmandelic acid (VMA), and 17-keto- and 17-hydroxy steroids. In all patients, K⁺, VMA, and steroid determinations were in the normal range. They were classified into low and high renin hypertensive groups following their PRA response to oral furosemide (Carey et al., 1972; Hollifield et al., 1976). Criteria for renovascular hypertension were those of Wilson et al. (1977). The patients' diagnoses are listed in Table 1.

These seven patients and one normal subject were placed on a 10 mM Na⁺, 80 mM K⁺ diet and received two doses of 40 mg furosemide per os on the 1st day to hasten achievement of sodium balance. When sodium balance was achieved, propran-

### Table 1: Clinical Data on Hypertensive Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Highest recorded BP</th>
<th>Cl_cr (mL/min)</th>
<th>Renal arteriogram</th>
<th>PRA (ng angiotensin I/ml per hour)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>M</td>
<td>220/160</td>
<td>93</td>
<td>NL</td>
<td>R 48</td>
<td>High renin HT</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>F</td>
<td>250/150</td>
<td>70</td>
<td>Bilat. bypass, partially ste-</td>
<td>R 7.5, L 16.4, IVC 12.4</td>
<td>Renovascular HT, bilat. bypass</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>M</td>
<td>240/160</td>
<td>83</td>
<td>Bilat bypass</td>
<td>R 6.9</td>
<td>High renin HT</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>F</td>
<td>180/120</td>
<td>70</td>
<td>25% stenosis of RRA due to P</td>
<td>L 5.2, R 4.2</td>
<td>High renin HT</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>F</td>
<td>220/120</td>
<td>93</td>
<td>Severe stenosis, left</td>
<td>L R 33, IVC 19, 15</td>
<td>Renovascular HT</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>F</td>
<td>270/120</td>
<td>38</td>
<td>Three LRA's with plaque in middle artery</td>
<td>L ND</td>
<td>Low renin HT</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>M</td>
<td>220/140</td>
<td>65</td>
<td>Bilat. renal artery stenosis, arteriosclerotic</td>
<td>R 5.6, L 3.6, IVC 7.0</td>
<td>Renovascular HT</td>
</tr>
</tbody>
</table>

Abbreviations: BP = blood pressure; FMD = fibromuscular disease; Cl_cr = creatinine clearance; HT = hypertension; IVC = blood from inferior vena cava; L = left renal venous blood; LRA = left renal artery; NL = normal; ND = not done; Periph = peripheral; PRA = plasma renin activity (ng angiotensin I/ml per hour); RAS = renal artery stenosis; RRA = right renal artery; R = blood from right renal artery, bilat. = bilateral(ly). The PRA value of subject 3 was obtained following stimulation by furosemide (Carey et al., 1972).
olol was initiated with a dose of 40–160 mg four times a day (daily dose: 354 ± 164 mg/24 hours, mean ± se), and PRA in the upright and supine positions was determined 2 days later. The dose of propranolol subsequently was increased in each patient until a dose that was supramaximal with respect to renin suppression was achieved; this dose ranged from 160 to 240 mg four times daily (mean dose 566 ± 191 mg/24 hours) and was given throughout the remainder of the study. Subsequently, the subjects received, in a single blind fashion, placebo (6–8 days), indomethacin (50 mg three times daily for 6–8 days), and again placebo (4–6 days). Twenty-four-hour urine samples were collected daily and analyzed for Na⁺, K⁺, and creatinine, and, on the last day of each of the three periods, for PGE-M. On the last 2 days of each period, plasma creatinine, Na⁺, K⁺, and supine and upright PRA were determined. Blood pressure was measured four times daily throughout the study, and each patient was weighed daily.

Effect of Isoproterenol on PRA in Normal Subjects in 10 and 150 mM Sodium Balance

One group of four normal subjects was brought into 10 mM Na⁺ balance as described above, and another group of four normal subjects into 150 mM sodium balance. They then received, in randomized crossover sequence separated by 1 week, either placebo or indomethacin, 50 mg three times a day for 2 days and one dose of 50 mg on the morning of the 3rd day. A 24-hour urine sample was collected on day 2 and analyzed for Na⁺, creatinine, and PGE-M. On the 3rd day after supine PRA and plasma Na⁺ were determined, an infusion of isoproterenol was started at an initial rate of 1 μg/min. The rate was increased after 10 minutes to 1.5 μg/min if the heart rate had not increased by 20 beats/min. In none of the subjects was it necessary to increase the infusion rate above 1.5 μg/min to achieve the desired increase in heart rate. When the subject was restudied during administration of the alternate drug 1 week later, the same dosing pattern of isoproterenol infusion was repeated as in the first study, regardless of the heart rate response. After isoproterenol infusion was begun, PRA, blood pressure, and heart rate were measured every 10 minutes for 60 minutes. Statistical analysis was accomplished by paired t-test (Snedecor and Cochran, 1967). All of the above studies were carried out at the Clinical Research Center of Vanderbilt Hospital and were approved by the Committee for the Protection of Human Subjects.

Results

Effect of Indomethacin on PRA, Aldosterone, and PGE-M in Normal Volunteers in 10 mM Sodium Balance

Indomethacin had no effect on PRA or plasma aldosterone in sodium-depleted normal subjects in the supine and upright positions (Fig. 1). Sodium (in mM/24 hours, mean ± se) and creatinine excretion (in mg/24 hours) were 7.75 ± 2.6 and 1020.5 ± 3.5, respectively, in the placebo period, and 10.87 ± 3 and 1055 ± 373, respectively, in the indomethacin period. Body weight was 73.1 ± 1.6 kg in the placebo period and 74.5 ± 1.6 kg in the indomethacin period. PGE-M was significantly suppressed by indomethacin (Fig. 1).

Effect of Indomethacin on PRA and PGE-M in Sodium-Depleted Hypertensive Subjects Taking Propranolol

The initial average dose of propranolol of 354 mg/24 hours was associated with a plasma concentration of propranolol of 388 ± 125 ng/ml and PRA in the supine and upright positions of 2.35 ± 0.9 and 4.08 ± 1.62 ng angiotensin I (A I)/ml per hour, respectively. Increasing the dose of propranolol to an average of 568 mg/24 hours significantly increased the plasma propranolol levels to 728 ± 198
ng/ml (P < 0.01) but did not further lower PRA in the supine or upright positions (2.3 ± 0.6 and 6.2 ± 1.9 ng A I/ml per hour, respectively).

When indomethacin was added to treatment with the larger dose of propranolol, PRA in both the supine and upright positions was reduced significantly, an effect that reverted when indomethacin was discontinued (Fig. 2). The reductions in PRA for the supine and upright positions were 84% and 70%, respectively, and the return of PRA to pre-indomethacin levels was virtually complete when indomethacin was replaced by placebo. Indomethacin lowered PGE-M from a control value of 4.3 ± 0.8 to 1.5 ± 0.3 μg/g creatinine (P < 0.01). After discontinuation of indomethacin, PGE-M returned to 4.9 ± 1.3 μg/g creatinine (P < 0.05; Fig. 2). The reduction in PRA by indomethacin was not accompanied by retention of sodium (Fig. 2).

Consistent with the known inhibition of water excretion by indomethacin (Frolich et al., 1978), there was a modest average weight gain of 0.9 kg when indomethacin replaced placebo (P < 0.05) and an average loss of 1.1 kg when the second placebo was substituted for indomethacin (P < 0.05); there were corresponding changes in urine volume (Table 2). Serum sodium fell slightly but not significantly during indomethacin treatment (Table 2). However, the reduction in PRA in the upright position that resulted from substitution of indomethacin for placebo, and its subsequent rise on reinstitution of placebo, were not significantly correlated with the changes in body weight (P > 0.1). The change in PRA measured in the supine position did not correlate with the change in body weight when indomethacin was substituted for placebo (P > 0.1); when the second placebo replaced indomethacin, the probability that PRA correlated with body weight was 4.2%. Thus, consistent reduction in PRA during indomethacin administration was not usually correlated with the changes in body weight. There was no significant change in creatinine excretion with indomethacin.

Because the study was designed to provide a generalization regarding any effect of indomethacin on PRA, subjects were selected with hypertensions of various etiologies, including unilateral and bilateral renovascular hypertension and primary hypertension with low and high renin. Accordingly, it was not the objective of the study to evaluate the effect of indomethacin on arterial pressure. In light of the consistent reduction of PRA by indomethacin in this heterogeneous group, however, it is pertinent to note that the administration of indomethacin was associated with varied effects on arterial pressure, which fell significantly in subject 2 (Table 1) and rose slightly in others (Table 3). Clearly, the uniform reduction in PRA cannot be attributed to any change in arterial pressure.

In the normal subject taking propranolol (240 mg/day), indomethacin also caused a reduction of PRA in the supine and upright positions from 9.0 and 4.1 to 2.1 and 2.7 ng A I/ml per hour, respectively. Excretion of PGE-M was reduced by indomethacin from 3.5 to 1.1 μg/g creatinine, and indo-

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**Table 2. Effect of Indomethacin in Hypertensive Subjects in 10 mM Na⁺ Balance while Taking Propranolol**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Indomethacin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>U Na⁺ (mM/24 hr)</td>
<td>7.75 ± 2.62</td>
<td>10.78 ± 2.96</td>
<td>13.41 ± 2.9</td>
</tr>
<tr>
<td>U Na⁺ (ml/24 hr)</td>
<td>2538 ± 506.98</td>
<td>2347 ± 464</td>
<td>2567 ± 444</td>
</tr>
<tr>
<td>U Na⁺ (mg/24 hr)</td>
<td>1019 ± 119</td>
<td>1005 ± 141</td>
<td>1073 ± 161</td>
</tr>
<tr>
<td>Serum Na⁺ (mM/liter)</td>
<td>139 ± 1.2</td>
<td>137 ± 1.8</td>
<td>141 ± 2.7</td>
</tr>
</tbody>
</table>

Values given (mean ± se, n = 7) are means of the last 2 days of each period.

* P < 0.05 as compared to first placebo period.
indomethacin had no effect on plasma Na+, urinary Na+ excretion (always less than 1 mM/24 hours), urinary creatinine and volume, or body weight.

**Effect of Indomethacin on Isoproterenol-Stimulated PRA in Normal Subjects in 10 mM Na+ Balance**

As was the case in the initial study of the effect of indomethacin on PRA during sodium deprivation (Fig. 1), indomethacin did not lower the level of PRA measured in supine subjects receiving a 10 mM sodium diet (Table 4A). Furthermore, the approximately 3-fold increase in PRA produced by isoproterenol infusion was unaltered by indomethacin pretreatment, even though this cyclooxygenase inhibitor reduced the excretion of PGE-M from 5.21 ± 0.57 to 2.56 ± 1.2 µg/g creatinine (P < 0.05). The chronotropic response to isoproterenol as well as its effect on blood pressure were unaltered by indomethacin (Fig. 3). Urinary sodium (in mM/24 hours) and creatinine (in mg/24 hours) excretions were 6.3 ± 0.3 and 1704 ± 55, respectively, during placebo administration and 5.6 ± 4.5 and 1825 ± 120 during treatment with indomethacin.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Body weight (kg)</th>
<th>PRA supine</th>
<th>PRA upright</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43.0</td>
<td>43.1</td>
<td>41.9</td>
</tr>
<tr>
<td>2</td>
<td>56.9</td>
<td>58.8</td>
<td>55.5</td>
</tr>
<tr>
<td>3</td>
<td>56.5</td>
<td>57.4</td>
<td>56.6</td>
</tr>
<tr>
<td>4</td>
<td>84.4</td>
<td>85.5</td>
<td>83.7</td>
</tr>
<tr>
<td>5</td>
<td>46.6</td>
<td>47.8</td>
<td>48.0</td>
</tr>
<tr>
<td>6</td>
<td>55.1</td>
<td>56.7</td>
<td>56.1</td>
</tr>
<tr>
<td>7</td>
<td>74.5</td>
<td>74.0</td>
<td>73.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PRA supine</th>
<th>PRA upright</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.57</td>
<td>5.12</td>
</tr>
<tr>
<td>3.65</td>
<td>4.99</td>
</tr>
<tr>
<td>0.17</td>
<td>0.60</td>
</tr>
<tr>
<td>0.43</td>
<td>1.43</td>
</tr>
<tr>
<td>2.43</td>
<td>3.84</td>
</tr>
<tr>
<td>4.44</td>
<td>14.55</td>
</tr>
<tr>
<td>2.55</td>
<td>7.63</td>
</tr>
</tbody>
</table>

**Effect of Indomethacin on Renin Release by Isoproterenol in Normal Subjects**

Isoproterenol infusion during the control period resulted in a rapid rise of PRA that was maintained at levels 3- to 5-fold above control throughout the infusion (Table 4B). Indomethacin treatment reduced 24-hour Na+ excretion from 135.8 ± 13 to 97.6 ± 19 mM/24 hours but had no measurable effect on creatinine excretion (1708 ± 89 mg/24 hours during placebo treatment and 1818 ± 272 mg/24 hours during indomethacin treatment). Indomethacin reduced PRA in the control period in each subject, so that the values prior to isoproterenol infusion were less than the lower limits of quantification by radioimmunoassay (<0.17 ng/ml per hour). Isoproterenol infusion caused an increase in PRA into the measurable range (Table 4B), but because of the unquantifiable levels in the preinfusion period, the fractional increase in PRA caused by isoproterenol could not be determined. The chronotropic response of isoproterenol and its effect on blood pressure were unaltered by indomethacin (Fig. 4).
Discussion

The effect of inhibition of the fatty acid cyclooxygenase on renin release was investigated during balance on a 10 mM sodium diet to eliminate sodium retention as a contribution to any reduction in renin. When supramaximal levels of $\beta$-blockade were combined with the 10 mM Na$^+$ diet to block the high level of adrenergic activity that accompanies sodium deprivation (Gordon et al., 1967; Robertson et al., 1977), there remained a substantial level of renin release. This nonadrenergic release of renin was inhibited markedly by indomethacin. Sodium retention did not occur during indomethacin administration. Although water retention was seen in some of the volunteers, it usually was slight and did not correlate consistently with the uniform and substantial reduction in PRA. The dose of indomethacin given reduced total body PGE synthesis but did not block it entirely, suggesting that more complete blockade of the fatty acid cyclooxygenase would have almost completely inhibited the release of renin evoked by the powerful stimulus of sodium deprivation.

Because indomethacin did not lower PRA during sodium deprivation when $\beta$-receptors were not blocked, it is inferred that an augmentation of the stimulation of $\beta$-adrenergic receptors under those conditions must have compensated for removal of the nonadrenergic mediator of renin release.

The cyclooxygenase-derived metabolite of arachidonic acid that stimulates release of renin probably exerts its effects within the kidney itself. In these studies, the release of renin was blocked by inhibitors of the fatty acid cyclooxygenase regardless of whether the subject's arterial pressure fell significantly, rose, or did not change. The demonstration in this study that the reduction in the release of renin by inhibitors of the cyclooxygenase cannot be explained by sodium retention is consistent with our previous observation that the rapid release of renin 10 minutes after intravenously administered furosemide can be blocked by indomethacin (Frolích et al., 1976). These findings implicating an intrarenal site of renin release by the cyclooxygenase metabolite(s) of arachidonic acid in man are in accord with data for experimental animals that demonstrate a direct intrarenal stimulation of renin release by arachidonic acid (Larsson et al., 1974) and an inhibition of the release of renin evoked by clamping of the renal artery by the acute administration of indomethacin (Anggard et al., 1976). The likely intrarenal locus of the formation and action
of the cyclooxygenase metabolite(s) is in the cortex, as we have demonstrated in studies in the nonfiltering kidney (Data et al., 1978). Recently, we have found that PGI$_2$ is one of the major metabolites of arachidonic acid in the renal cortex (Whorton et al., 1978a), and that PGI$_2$ is the most potent stimulator of renin release both in vivo and in vitro of any metabolite of arachidonic acid tested to date (Whorton et al., 1978b).

The findings in the present study suggest that stimulation of the release of renin by the cyclooxygenase metabolite operates in parallel with the release mechanism evoked by the $\beta$-receptor, in contrast to a release mechanism in which these two mediators might function in sequence. Since indomethacin almost completely inhibits the component of renin release that persists after doses of propranolol that are supramaximal for suppression of renin release, it is concluded that its action is independent of the adrenergic stimulus. Furthermore, the failure of indomethacin to block the release evoked by isoproterenol in the studies conducted on subjects in 10 mM sodium balance is inconsistent with a participation of the cyclooxygenase metabolite in the adrenergic stimulation of the release of renin.

It is concluded that a cyclooxygenase-derived metabolite of arachidonic acid is a mediator of the nonadrenergic component of the release of renin in man.

Acknowledgments

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References


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