Suppression of Plasma Renin Activity by Indomethacin in Man

JÖRGEN C. FRÖLICH, M.D., JOHN W. HOLLIFIELD, M.D., JOHN C. DORMOIS, M.D.,
BRIGITTE L. FRÖLICH, HANNSJÖRG SEYBERTH, M.D., ANDREW M. MICHELAKIS, M.D.,
AND JOHN A. OATES, M.D.

SUMMARY The effect of indomethacin or placebo on aldosterone, plasma renin activity (PRA), sodium excretion, and urinary prostaglandin (PG) levels was investigated in five hypertensive subjects in 100 mEq sodium balance who had experienced malignant hypertension with a disturbance of their renin-aldosterone relationship in the past. Indomethacin significantly lowered aldosterone levels by 43%, PRA by 58%, 24-hour sodium excretion by 49%, and urinary PG excretion, an indicator of renal PG synthesis, by 67%. Angiotensin infusion increased aldosterone to the same level before and after treatment with indomethacin. Similarly, in normal subjects in 150 mEq sodium balance, indomethacin lowered PRA by 47%; sodium excretion fell by 33%, and urinary prostaglandin E (PGE) excretion, by 55%. The acute elevation in PRA 10 minutes after intravenous furosemide was completely abolished by indomethacin. Five subjects with essential hypertension were classified as normal renin hypertensives according to their response to orally administered furosemide. Indomethacin pretreatment resulted in 60% reduction of PRA following furosemide, and three of these subjects now fell into the low renin category. Studies in vitro demonstrated that indomethacin has no effect on the renin-renin substrate interaction. Thus, indomethacin lowers PRA concomitantly with a reduction in renal PG synthetase activity. Whether indomethacin inhibits renin release by an intrarenal, PG-related mechanism or secondarily via sodium retention is discussed.

THE RELEASE of renin, like other secretory events, probably is mediated by chemical or electrochemical signals. Although β-adrenergic agonists are known stimuli for renin release, there is evidence that other important but undetermined mediators influence the secretion of renin. Our interest in the possibility that prostaglandin (PG) might participate in the release of renin arose from investigations on the possible role of PG's in the control of aldosterone secretion. Because some subjects who have previously experienced malignant hypertension have aldosterone hypersecretion that is not accompanied by renin excess, the PG synthetase inhibitor, indomethacin, was given to a group of postmalignant hypertensive subjects to determine whether PG's participated in the control of their aldosterone secretion. Not only did aldosterone fall after administration of indomethacin, but plasma renin activity (PRA) was suppressed in parallel. To determine whether the observed suppression of PRA by indomethacin was a general phenomenon and to examine the extent to which it related to sodium retention, the effect of indomethacin on PRA was evaluated in normal volunteer subjects. In addition, we studied the effect of indomethacin on the abrupt rise in PRA that occurs within minutes after intravenous administration of furosemide.

Because the increase in PRA after orally administered furosemide is used as a diagnostic stimulus in the characterization of hypertension with suppressed renin, indomethacin's possible interference with this test was investigated in subjects with essential hypertension. The inhibition of renal PG synthesis by indomethacin was assessed in these studies by measuring the urinary excretion of prostaglandin E (PGE).

Methods

STUDIES IN MAN

Patients with Postmalignant Hypertension. Five subjects who previously had had malignant hypertension characterized by severe hypertension (diastolic blood pressure greater than 130 mm Hg), papilledema, and signs of microangiopathic hemolytic anemia were hospitalized. At this time one subject still exhibited increased urinary aldosterone secretion without increased PRA, whereas the other subjects had normal aldosterone secretion rates. Further data on these subjects are given in Table 1. Informed consent for the study that had been approved by the Vanderbilt Clinical Investigation Committee was obtained. The subjects were brought into 100 mEq sodium balance and a placebo capsule resembling 25 mg of indomethacin was administered four times daily for 5 days. On the 4th day, a 24-hour urine was collected for determination of sodium, creatinine, and PGE.

On the 5th day, after a 4-hour control period during which the vehicle (5% glucose in water) was given intravenously, angiotensin II amide (Hypertensin, Ciba) was infused at an average rate of 1.52 ng/kg per min for 4 hours. In the middle of the control angiotensin infusion period, blood was obtained for the determination of PRA, aldosterone, and creatinine. Urine was collected during each period. Blood pressure was monitored every 15 minutes throughout the study. Subsequently, each subject received the active drug (25 mg four times a day) for 4 days and an infusion of the same dose of angiotensin was repeated on the 5th day.

Normal Volunteers. Five male volunteers were screened by history, physical examination, sequential multiple anal-
Hypertensive Subjects with Normal PRA. Five subjects with essential hypertension and normal PRA (who also had normal renal arteriograms, normal urinary excretion of 17-keto steroids and 17-hydroxy steroids, creatinine clearance, vanillylmandelic acid, and catecholamines) were selected for evaluation of the effect of indomethacin on the PRA response to furosemide administered orally according to the procedure used in the categorization of hypertensive subjects with low renin hypertension. According to this test these subjects were found to have normal PRA. They also had normal PRA according to the criteria established by Brunner et al.,4 which are based on the sodium-renin index. In the former test, the subject receives 40 mg of furosemide orally 15, 9, and 3 hours prior to PRA sampling and is kept upright after administration of the third dose. A PRA value >1.67 ng of angiotensin I/ml per hour is considered indicative of normal renin hypertension. The responsiveness of each subject's PRA to this test had been established on at least two occasions prior to our present study and on each occasion the PRA was >1.67 ng angiotensin I/ml per hour. The PRA response to the same dose of furosemide in these subjects was tested when they were on no drugs, after they had received 50 mg of indomethacin every 6 hours starting 45 hours prior to PRA sampling, and 1 week later while receiving no drugs.

STUDIES IN VITRO
Studies in vitro were performed to determine whether indomethacin or its metabolites would interfere with PRA determination. To duplicate samples of pooled human plasma, indomethacin was added in increasing concentrations (0, 10, 100, and 10,000 ng/ml) and the PRA was assayed. To investigate whether any metabolites of indomethacin found in plasma might interfere with PRA measurement, a patient with primary aldosteronism was treated with 50 mg of indomethacin four times daily for 2 days. Two hours after the last dose, blood was collected and to the plasma of this blood were added 2 x 10⁻⁴ Goldblatt units¹ of human kidney renin. The generation of angiotensin I from this plasma was compared to angiotensin I generation in the plasma of this subject obtained prior to indomethacin treatment to which an identical amount of human renal renin had been added.

PRA was determined as described previously.³ Aldosterone was determined by radioimmunoassay⁴ and urinary PGE by competitive protein-binding assay.⁵ Furosemide was determined by high performance liquid chromatography (unpublished observations). Statistical analysis was accomplished by Student's t-test.

Results

EFFECT OF INDOMETHACIN ON PLASMA ALDOSTERONE AND PRA IN POSTMALIGNANT HYPERTENSION
Indomethacin reduced plasma aldosterone levels measured during the control period by 43% from 11.4 ± 6.8 (mean ± SD) to 6.5 ± 2.9 ng/100 ml (P < 0.05) (Fig. 1). The simultaneously determined PRA fell in each of four subjects from 5.7 ± 2. to 2.4 ± 0.89 ng of angiotensin I/ml per hour (P < 0.05, Wilcoxon test) (Fig. 1). In the fifth subject the effect of indomethacin on PRA could not be evaluated because he had suppressed PRA (PRA < 0.17 ng of angiotensin I/ml per hour) throughout the study. Indomethacin reduced urinary sodium excretion from 110.9 ± 20.2 (average of the 2 days preceding indomethain) to 56.8 ± 18.9 (average of the first 2 days on indomethacin) mEq/24 hours (P < 0.05) (Fig. 1); this was accompanied by an average weight gain of 0.8 ± 0.1 kg (P < 0.02). Urinary
PGE excretion fell from 25.7 ± 16.5 to 8.4 ± 5.6 ng/hour (P < 0.05). The results of angiotensin II infusion are summarized in Table 2. Angiotensin infusion resulted in an increase in plasma aldosterone to the same levels before and after indomethacin pretreatment. Indomethacin reduced urinary PGE excretion during angiotensin infusion (P < 0.05) (Table 2).

**EFFECT OF INDOMETHACIN ON PRA BEFORE AND AFTER INTRAVENOUSLY ADMINISTERED FUROSEMIDE IN NORMAL VOLUNTEER SUBJECTS**

In the normal volunteer subjects in balance on a 150 mEq sodium diet indomethacin caused a fall in control PRA from 5.3 ± 1.4 to 2.8 ± 1.6 ng of angiotensin I/ml per hour (P < 0.05) (Fig. 2), and sodium excretion fell from 174 ± 15.6 to 115.9 ± 51.5 mEq/24 hours (0.1 > P > 0.05) in parallel with urinary PGE excretion from 80.1 to 36.4 ng/hour (P < 0.05, Wilcoxon test).

Intravenous furosemide caused a steep rise in PRA that reached maximum 10 minutes after administration of the drug and was completely abolished by indomethacin (Fig. 2). Sodium excretion during the 2 hours after administration of furosemide was 122 ± 8 mEq for the placebo period and 88 ± 12.6 mEq for the indomethacin period (P < 0.01).

Furosemide plasma levels during the placebo period, 10 and 30 minutes after its administration, were 4.56 ± 0.59 µg/ml and 1.38 ± 0.32 µg/ml, respectively. During the indomethacin period the levels were 5.3 ± 1.3 and 1.64 ± 0.3 µg/ml, respectively (P not significant). Furosemide concentration in the urine excreted during the 2 hours following administration of the drug was 11.44 ± 2.46 µg/ml during the placebo period and 12.2 ± 1.98 µg/ml during the indomethacin period. The amount of furosemide excreted in the urine during this period was 11.9 ± 1.7 mg during the placebo period and 9.8 ± 1.3 mg during the indomethacin period (P < 0.01).

**EFFECT OF INDOMETHACIN ON THE TEST USED TO CATEGORIZE LOW RENIN HYPERTENSION (PRA RESPONSE TO ORALLY ADMINISTERED FUROSEMIDE)**

The effects of indomethacin on the furosemide-induced PRA increase and weight loss are shown in Table 3. The PRA increase after furosemide was decreased by indomethacin by 60% (P < 0.01). During the indomethacin treatment period, three out of five subjects failed to increase their PRA to a level greater than 1.67 ng of angiotensin I/ml per hour and thus appeared to fall into the category of low renin hypertensives. Indomethacin reduced furosemide-induced weight loss by 63% (P < 0.01). Both responses returned to control levels 1 week after indomethacin was discontinued.

**STUDIES TO EXCLUDE AN EFFECT OF INDOMETHACIN ON THE IN VITRO GENERATION OF ANGIOTENSIN I BY RENIN**

The effect of three concentrations of indomethacin on the synthesis of angiotensin I in plasma is shown in Table 4. Indomethacin did not inhibit PRA in concentrations ranging from 10 to 10,000 ng/ml of plasma.

The synthesis of angiotensin I in the plasma of a subject with primary aldosteronism to which exogenous human renal renin had been added was 3.46 ± 0.55 ng of angiotensin I/ml per hour before and 3.88 ± 0.41 ng during treatment with indomethacin (n = 6, P not significant).
TABLE 2  Effect of Indomethacin on Aldosterone (ng/100 ml), Plasma Renin Activity (PRA) (ng Angiotensin I/ml per hour), and Prostaglandin E (PGE) Excretion Rate (ng/hour) Before (Control) and During Angiotensin Infusion in Five Subjects with Postmalignant Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Angiotensin</th>
<th>Placebo</th>
<th>Angiotensin</th>
<th>Placebo</th>
<th>Angiotensin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aldosterone (n = 5)</td>
<td>PRA (control/angiotensin)</td>
<td>PGE (ng/hour)</td>
<td>Aldosterone (n = 5)</td>
<td>PRA (control/angiotensin)</td>
<td>PGE (ng/hour)</td>
</tr>
<tr>
<td>Placebo</td>
<td>11.4 ±6.8</td>
<td>171 ±30.2</td>
<td>25.7 ±16.5</td>
<td>19.8 ±7.9</td>
<td>173.3 ±127</td>
<td>32.1 ±0.5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6.5 ±6.6</td>
<td>69.8 ±24.6</td>
<td>8.4 ±5.6</td>
<td>21.2 ±7.4</td>
<td>69 ±29</td>
<td>3.9 ±2.6</td>
</tr>
<tr>
<td>P (placebo/indomethacin)</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. NS = not significant.

Discussion

Indomethacin was found to lower PRA in subjects with hypertension, both essential and postmalignant, and in normal volunteer subjects. This effect was associated with a substantial reduction in the urinary excretion of PGE, a finding that probably mirrors a reduction in the synthesis of PGE in the kidney. In all instances the changes in PRA were associated with some degree of retention of sodium; this finding made it difficult to ascertain whether indomethacin lowered PRA by a mechanism confined to the kidney, by an increase in plasma volume, or both.

Evidence favoring a direct effect of PG's on renin release stems from our earlier observation that PGE stimulates the release of renin from cell suspensions of rabbit renal cortex. Also, an acute inhibitory effect of indomethacin on renin release has been observed in the rabbit; the rapid reduction in PRA following the parenteral administration of indomethacin is not consistent with an exclusive role of sodium retention in the reduction of PRA. The time course of the PRA response to furosemide in the present study is of interest in that the highest (2.6-fold) rise in PRA was observed 10 minutes after drug administration. Thirty minutes after furosemide, at a time when much more sodium had been excreted, PRA was only 1.3-fold higher than during the control period. This suggests that part of the rise in PRA following the intravenous administration of furosemide is caused by factors other than reduction in the blood volume. This acute response is most significantly affected by blockade of PG synthesis and, thus, PG synthetase may be of importance in bringing about the rise in PRA following furosemide. Recently it has been shown that indomethacin blocks the acute increase in renal blood flow in the dog that follows bumetanide and furosemide administration.

This observation suggests that furosemide can also alter intrarenal hemodynamics by a PG-mediated mechanism which is independent of blood volume. Current evidence is insufficient to determine whether indomethacin affected the acute release of renin by furosemide via a direct influence on the renin-releasing cell or indirectly via a change in renal hemodynamics or tubular-macula densa transport of electrolytes. Whatever the mechanism, indomethacin clearly produces significant changes in the body's volume control mechanism, and this is correlated with suppression of PG synthetase activity.

The possibility that indomethacin blocked the tubular secretion of furosemide and prevented its intraluminal action was considered. Probenecid, by inhibiting the secretion of furosemide, will antagonize its diuretic effect. If such an inhibition of furosemide's transport into the tubule were responsible for the total inhibition of early PRA release, then the renal elimination of furosemide should have been similarly prevented. However, in the present study the slight decrease in the rate of elimination of furosemide by indomethacin cannot account for the complete blockade of the PRA response.

The effect of indomethacin on PRA prompted us to investigate whether the reaction between renin and its
substantially was inhibited by indomethacin. Neither indomethacin nor its metabolites were found to interfere with the PRA assay. Others have shown that prostaglandin A₃ (PGA₃) may affect the renin reaction, and it might be that indomethacin affected circulating levels of PGA₃, thereby acting on the renin reaction in a way undetectable in our experiments in vitro. Recent evidence, however, indicates that PGA₃ is not a biosynthetic product of the mammalian kidney, or a circulating hormone in man. The effect of indomethacin on plasma aldosterone levels observed in the subjects with postmalignant hypertension is most likely secondary to a reduction in PRA, because the adrenal remained fully responsive to exogenously administered angiotensin II. Thus, inhibition of PG synthetase by indomethacin does not block the adrenal steroidogenesis stimulated by angiotensin. Others have found a stimulating effect of PG on adrenal steroid production in vitro, but responses to exogenously administered PG's in vivo were variable. The reduction of PRA by indomethacin has practical implications in the diagnosis of low renin hypertension. In the subjects with normal renin essential hypertension, indomethacin caused a significant reduction in the PRA response to orally administered furosemide, a test used to categorize hypertensives into low and normal renin classes. Since this classification carries with it therapeutic implications, the subject's drug history is of importance in the assignment into low or normal renin categories.

In summary, indomethacin has been shown to lower PRA in subjects with postmalignant hypertension, and in normal volunteer subjects. It blunted the response to orally administered furosemide in subjects with essential hypertension and abolished the response to intravenous furosemide in normal volunteer subjects. This effect of indomethacin was accompanied by a reduction of renal PGE excretion, which we have shown to reflect predominantly renal PG synthesis.

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**Table 3: Effect of Orally Administered Furosemide on Weight and Plasma Renin Activity (PRA) (3 Hours Upright) Before, During, and After Indomethacin Treatment in Five Subjects with Normal Renin Essential Hypertension**

<table>
<thead>
<tr>
<th>Weight loss (kg)</th>
<th>Before indomethacin</th>
<th>P</th>
<th>Plus indomethacin</th>
<th>P</th>
<th>After indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.18 ± 0.49</td>
<td>&lt;0.01</td>
<td>0.8 ± 0.41</td>
<td>&lt;0.02</td>
<td>2.04 ± 0.34</td>
</tr>
<tr>
<td>PRA (ng A I/ml per hr)</td>
<td>6.24 ± 0.91</td>
<td>&lt;0.01</td>
<td>2.5 ± 0.74</td>
<td>&lt;0.01</td>
<td>6.8 ± 3.75</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. A I = angiotensin I

Indomethacin also caused sodium retention and reduced the natriuretic response to oral or intravenous furosemide by a mechanism other than pharmacokinetic drug interaction. It is not clear to what degree sodium retention contributed to the reduction in PRA; however, the complete suppression of the PRA response to furosemide by indomethacin suggests that a mechanism other than sodium retention is involved.

**Acknowledgments**

The skillful technical assistance of Kathy Davis and Dr. Marshall Frazer is gratefully acknowledged.

**References**


**Table 4: Effect of Indomethacin on Plasma Renin Activity (PRA) in Pooled Plasma in Vitro**

<table>
<thead>
<tr>
<th>Indomethacin (ng/ml plasma)</th>
<th>PRA (ng A I/ml per hr)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.42 ± 0.63</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>6.22 ± 0.49</td>
<td>NS</td>
</tr>
<tr>
<td>100</td>
<td>5.92 ± 1.04</td>
<td>NS</td>
</tr>
<tr>
<td>10,000</td>
<td>6.07 ± 0.62</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD; n = 6; A I = angiotensin I; NS = not significant.
Red Cell Velocity and Plasma Transit Time in the Cerebral Microcirculation of Spherocytic Deer Mice

WILLIAM I. ROSENBLUM, M.D.

SUMMARY Spherocytic deer mice provide a model of human spherocytosis. Their erythrocytes are abnormal in shape and are more rigid than normal red blood cells (RBC). Like their human counterparts, spherocytic mice are anemic. Measurements of RBC velocity in microvessels on the cerebral surface failed to reveal a difference between the velocity of cells in spherocytic as compared to normal deer mice. However, plasma transit, as measured by fluorescein microangiography, was faster than normal. Both decreased plasma transit time and increased RBC velocity are expected in nonspherocytic, anemic mice. Since the former, but not the latter, was found in the spherocytic, anemic mice, it appears that increased RBC rigidity has a greater effect on RBC movement than on plasma movement within the cerebral microcirculation. Thus it would seem that this increased RBC rigidity prevents the increased RBC velocity that otherwise would be observed in anemia but does not prevent the decrease in plasma transit time.

APPROXIMATELY 20 years ago hereditary spherocytosis was recognized in the deer mouse. The hematological, pathological, and physiological features of this syndrome were shown to be similar to those of spherocytosis in man. In spite of the interest one might expect in this model of a disease with abnormal red blood cells (RBC), little use appears to have been made of the affected animals for studies of blood rheology or of the microcirculation.

Our interest in the animal model of spherocytosis was prompted by our previous studies of the cerebral microcirculation in other models of rheological disorders. In these earlier investigations on mice we demonstrated that two hyperviscous conditions, macroglobulinemia and polycythemia, exerted a greater effect on plasma flow than on RBC velocity. Thus plasma flow was retarded more than RBC velocity, a phenomenon that appeared to accentuate the normal difference between the velocity of plasma and RBC. We ascribed this accentuation to an increase of plasma skimming in the cerebral microcirculation. On the other hand, we also investigated acutely anemic mice and found that both plasma velocity and RBC velocity were accelerated, without a demonstrable difference in the degree to which the plasma was accelerated in relation to the RBC. The studies just referred to were investigations of conditions in which blood viscosity was altered either by increased plasma viscosity, increased hematocrit (Hct), or decreased Hct. The availability of deer mice with spherocytosis provided us with an opportunity to study the effects of abnormal RBC shape and rigidity in a small rodent resembling the mouse of our earlier investigations. The new study employed the same techniques and measured the same parameters as the old. Unfortunately, the availability of deer mice was severely limited by the loss of one of two colonies maintained in this country, and by the small size of the other. Nevertheless, the data presented below appear to provide a basis for valid conclusions which, in retrospect, fit a hypothesis that could have been constructed from our data concerning the other models of rheological abnormality.

Methods

Deer mice (Peromyscus maniculatus) of both sexes were used. Spherocytosis was established on the basis of splenomegaly, increased RBC fragility, and examination of a blood smear. Studies of RBC velocity and plasma transit time were made by observing vessels on the cerebral surface. These vessels were exposed by craniotomy in anesthetized mice, prepared as previously described. Measurements of RBC velocity were made with ultra high speed microcinematography which permits RBC tracking from frame to frame. Plasma transit time from arteriole to venule was determined by analyzing movie film exposed at lower framing rates (40/sec) during passage of fluorescein microangiography. Blood pressures were measured by employing a tail cuff and pulse transducer.
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