Role of Prostaglandins in the Reversal of One-Kidney Hypertension in the Rabbit

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ABSTRACT

Hypertensive rabbits with a clip on the renal artery of their solitary remaining kidney show an abrupt decrease in blood pressure after the arterial constriction is released. Although the mechanism underlying this phenomenon remains controversial, some experimental evidence suggests that it could be humorally mediated. The involvement of prostaglandins was investigated by examining the effect of the release of the arterial constriction on blood pressure, renal blood flow, glomerular filtration rate, and urinary output in five conscious single-kidney hypertensive rabbits in which prostaglandin synthesis was blocked with indomethacin (priming intravenous injection of 9 mg/kg followed by a constant infusion of 1 mg/kg hour⁻¹). The results were compared with those obtained in another group of five single-kidney hypertensive rabbits submitted to the same protocol but not treated with indomethacin. The blockade of prostaglandin synthesis with indomethacin prevented the increments in renal blood flow and glomerular filtration rate seen in the control rabbits after unclipping and significantly retarded the appearance of diuresis and the fall in blood pressure. Despite these observations, the results do not indicate a major participation of prostaglandins in the reversal of single-kidney hypertension, because the decrease in blood pressure 9 hours after the removal of the arterial constriction was similar in both groups.

Fifty years ago, Fasciolo (1) and Goldblatt et al. (2) showed that the kidney was physiologically capable of counteracting high blood pressure. The concept was initially derived from the protective effect exerted by a normal kidney contralateral to a kidney that had an arterial constriction created proximal to it (1). In certain experimental circumstances, this renal antihypertensive effect seems to be the major factor in lowering the blood pressure. Hypertensive animals with a solitary remaining clipped kidney show an abrupt decrease in blood pressure following the release of the arterial constriction (3). This renal blood pressure-lowering effect does not involve the renin-angiotensin system, since neither the administration of antirenin antibodies (4), converting enzyme inhibitors (5), or angiotensin analogue competitive inhibitors (6) nor the removal of the solitary clipped kidney (7) acts to lower blood pressure in one-kidney hypertensive animals.

Such findings strongly suggest that one-kidney hypertension is not dependent on the renin-angiotensin system but rather on the lack of some renal function that is rapidly restored by removal of the renal arterial constriction. Although some evidence indicates that the increased excretion of salt and water which follows the release of the arterial constriction plays an important role in lowering blood pressure (8), other studies suggest that the antihypertensive action of the kidney is humorally mediated (9). For instance, autoexplantation of the renal medulla (10) or transplantation of renal medullary interstitial cells grown in tissue culture (11) is effective in alleviating renal vascular hypertension.

The humoral principles responsible for this antihypertensive effect have not yet been identified. However, the existence in the kidney of naturally occurring prostaglandins of the E series, which have marked vasodilator and natriuretic pharmacologic actions (12), has prompted the design of experiments aimed at defining the influence of these compounds on cardiac output, peripheral resistance, and renal function (13).

The present study was undertaken to evaluate the possible involvement of prostaglandins in the antihypertensive function of the kidney. The effect of the release of a renal arterial constriction on the blood pressure of one-kidney hypertensive rabbits was studied after prostaglandin synthesis had been blocked by indomethacin (14). The results were then compared with those obtained in a similar group of rabbits submitted to the same protocol but without blockade of prostaglandin synthesis.
Methods

Animal Protocol.—The study was conducted in New Zealand rabbits weighing 1.8-2.0 kg. After a control period of 6 days, renal hypertension was induced by constricting the left renal artery according to a procedure published elsewhere (15). However, the clip used to constrict the renal artery was slightly modified: the ends of both limbs of the U-shaped clip were bent externally to constrict the left renal artery according to a procedure described previously (17).

Thirty days after the removal of the contralateral kidney (36 days after clipping), when hypertension had definitely stabilized, 19 rabbits with systolic and diastolic readings in the range of 125-135/100-110 mm Hg were selected for unclipping and randomly divided into three groups. In the first group (N = 8), the effect of blockade of prostaglandin synthesis on the blood pressure–lowering effect of release of the arterial constriction was studied. The second group (N = 8) was used as a control. The renal artery was unclipped, but the rabbits were not treated with indomethacin. The third group (N = 3) was used as a negative control for unclipping; these rabbits were treated with indomethacin and underwent a sham-operation.

Animal Preparation.—For the acute study done 36 days after renal artery constriction, the preparative procedures in the three groups of rabbits were identical. Both ureters were cannulated (PE50) at the level of the bladder through a 2-cm medial suprapubic abdominal incision performed with local anesthesia (lidocaine). A 21-gauge butterfly needle was inserted in the central artery of the right ear to collect blood samples for the determination of plasma renin activity and plasma levels of inulin and para-aminomethyluric acid (PAH). Blood pressure was recorded constantly on a Grass polygraph from a 21-gauge butterfly needle placed in the central artery of the left ear and connected to a Statham 23Db transducer.

The rabbits were placed in comfortable restrainers at room temperature (24°C). A priming injection of inulin and PAH was given in 1 ml of physiologic saline through the marginal vein of the left ear, followed by a constant maintenance infusion of 0.05 ml/min. This infusion was continued during the whole experiment.

After a 1-hour control period, the first group of rabbits was given a priming dose of 0.9 ml/kg of a phosphate buffer solution (pH 8.4) containing 10 mg/ml of indomethacin through the marginal vein of the right ear followed by an intravenous infusion of 0.2 ml/kg hour^-1 of solution containing 5 mg/ml of indomethacin in phosphate buffer. This infusion was maintained throughout the experiment by use of a Harvard infusion pump and a syringe protected from light. Since the balance of fluids is critical to these experiments, it is important to mention that the rabbits received, after 9 hours, a total of 32.5 ml.

Rabbits in the second group were submitted to the same protocol as were the rabbits in the first group except that no indomethacin was included in the phosphate buffer. The protocol for the rabbits in the third group was identical to that for those in the first group except that the clip constricting the renal artery was not removed during the surgical procedure—done 36 days after the clip was placed.

Two hours after the indomethacin or the buffer infusion was begun, the clip constricting the renal artery of the sole remaining kidney was removed in the first two groups of rabbits. This maneuver was performed under light ether anesthesia. The surgical intervention consisted mainly of removing the silk sutures of the incision that had been made 36 days previously to clip the renal artery. The rabbits usually recovered from the anesthetic in less than 10 minutes. Thereafter, changes in blood pressure and urinary output were constantly monitored for up to 9 hours.

Hourly urine samples were collected. Blood samples for the determination of inulin, PAH, and plasma renin activity were taken during the control period, 2 hours after the administration of indomethacin was started, and 1, 4.5, and 8.5 hours after the renal artery was unclipped. Red blood cells were immediately separated from the plasma and reinjected into the rabbits with an equal volume of saline.

Nine hours after the unclipping, the rabbits were anesthetized with ether, and the solitary unclipped kidney was removed and cut into equal halves. One half was dropped immediately into liquid nitrogen, but the second half was allowed to stand at room temperature for 10 minutes before it was frozen in liquid nitrogen. The rationale underlying this procedure—used to estimate the capacity of the renal tissue to synthesize prostaglandins—will be outlined later. The efficacy of the priming dose and the intravenous infusion of indomethacin in blocking the synthesis of prostaglandin 2 hours after the onset of the treatment (immediately before unclipping) was checked in kidneys removed from three rabbits from the first group and three from the second group. Thus, only five rabbits in each of these groups completed the 9-hour experiments.

Collection of Blood and Preparation of Plasma for Determination of Plasma Renin Activity.—Changes in plasma renin activity in all of the groups were studied in 2.5-ml blood samples obtained from the central artery of the right ear and collected in cooled, commercially available tubes (Vacutainer) containing ethylenediaminetetraacetic acid (EDTA). After centrifugation, the plasma was separated and kept frozen. The specified amounts allowed duplicate determinations. Plasma renin activity was estimated by radioimmunoassay (18) according to the techniques reported in a previous study (4), and values are expressed as nanograms of angiotensin I released in plasma during 1 hour of incubation. Reproducibility was studied in 20 replicate determinations on three individual 15-ml samples. The mean values were 20.6, 15.4, and 26.1 ng/ml hour^-1 with coefficients of variations of 10.3, 9.8, and 11.1%, respectively.

Removal and Preparation of Renal Tissue for Prostaglandin Determinations.—Prostaglandin concentrations in excised tissue allowed to remain at room temperature are many times higher than those in tissue samples frozen immediately on removal (19). This phenomenon has been confirmed in our laboratory, although increases noticed after incubation have never exceeded five-
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sixfold. Since the measurements of prostaglandin concentrations in renal tissue in this study were intended to evaluate the degree of inhibition produced by indomethacin rather than to estimate true changes in tissue concentrations, the lack of postremoval enhancement of prostaglandin synthesis can be used to ensure that the selected dose of indomethacin was effective in blocking prostaglandin synthesis (20). Thus, the kidneys from all of the rabbits were rapidly removed and cut into equal halves (transverse section through the hilus). One half was immediately immersed in liquid nitrogen and the second half was kept at room temperature for 10 minutes before it was frozen in the same manner.

After the completion of the experiments, frozen kidneys were thawed at 3°C (cold room), and the prostaglandins were extracted and measured by radioimmunoassay according to procedures reported previously (21).

Renal Clearance Determinations of Inulin and PAH.—Inulin and PAH concentrations were determined in plasma and urine by AutoAnalyzer techniques, and clearances were calculated according to the standard formula. Data for each group in each time period were averaged. Statistical analysis was performed using Student's t-test for paired and unpaired groups as appropriate. Statistical significance was considered as \( P < 0.05 \).

Results

Figure 1 shows that no significant changes occurred in systolic and diastolic blood pressures during the first 2 hours after the removal of the arterial constriction in either the indomethacin-treated or the untreated group. The average values recorded during this period remained within the range of those seen during the control period. However, at 2 hours diastolic pressure in the group of rabbits that was not treated with indomethacin began to show a progressive decrement; the pressure reached its lowest level at 4.5 hours and remained at this level for the rest of the observation period (up to 9 hours). The average decrease in diastolic blood pressure was approximately 23 ± 3 mm Hg \( (P < 0.05) \).

The direction of the blood pressure changes recorded in the group of rabbits that was unclipped after receiving indomethacin was similar to that seen in the control group, but the time course of the recorded changes was different. At 4.5 hours after unclipping, that is, when blood pressure in the untreated group had reached its lowest level, the systolic and diastolic pressures of the rabbits treated with indomethacin were not significantly different from their values during the period before unclipping. A significant decrease in blood pressure was not observed in the treated group until 6 hours after unclipping, and the lowest values were not reached until the eighth hour after the unclipping. At this time, the recorded values of systolic and diastolic blood pressure in both groups were not statistically different.

The effect of removing the arterial constriction on renal blood flow and glomerular filtration rate in the two groups of rabbits was also different (Fig. 2). In the untreated group, removing the arterial constriction resulted in increases in the clearances of inulin and PAH measured 1 hour after unclipping of 28% and 37%, respectively. However, the average values recorded 4.5 and 8.5 hours after unclipping had returned to the level seen before unclipping. These transient increases in renal blood flow and glomerular filtration rate observed in the untreated group were absent in the group of rabbits treated with indomethacin. In this group, values for both measurements recorded 1, 4.5, and 8.5 hours after clipping remained unchanged at levels below the average values recorded before treatment with indomethacin was commenced.

The hourly measured urinary output in the group of rabbits that was not treated with indomethacin (Fig. 3) was significantly higher than that in the indomethacin-treated group during the first 4 hours after the unclipping. When the average urinary output seen during this period is related to blood pressure, it becomes apparent that for a similar systemic blood pressure the rabbits of the untreated group excreted a significantly higher urine volume than did the rabbits treated with indomethacin. The capability of the untreated rabbits to excrete more urine in proportion to renal perfusion pressure was still present 5 hours after unclipping; at this time, the urinary outputs of the two groups were identical despite the fact that the systolic and diastolic pressures of the untreated rabbits were, respectively, 20 and 17 mm Hg below those of the group treated with indomethacin.
Renal blood flow (top) and glomerular filtration rate (bottom) in indomethacin-treated one-kidney hypertensive rabbits (open circles) and control non-indomethacin-treated one-kidney hypertensive rabbits (solid circles) after the renal artery was undipped at time 0. Indomethacin treatment prevented the increase in renal blood flow and glomerular filtration rate observed in the untreated rabbits 1 hour after unclipping.

At 4.5 hours after unclipping, the average difference in total cumulative urinary output between the group of indomethacin-treated rabbits and the untreated group never exceeded 9 ml (Fig. 4). However, at this time the average decrease in systemic pressure in the untreated group was 20–23 mm Hg greater than that in the experimental group.

Changes in plasma renin activity induced by the release of the renal artery constriction in both groups of rabbits are presented in Figure 5. One hour after unclipping, the average plasma renin activity in the control group was significantly decreased compared with the average obtained immediately before unclipping. This 40% decrease was associated, in this group, with concomitant 30% and 25% increases, respectively, in renal blood flow and glomerular filtration rate but with no significant changes in systolic or diastolic pressures. The average values of plasma renin activity recorded at 4.5 and 8.5 hours in the untreated control group tended to return to levels similar to those recorded during the control period. The average plasma renin activity at 4.5 hours was still significantly lower than that during the control period, whereas the average activity recorded at 8.5 hours had returned to about the control value. These progressive increases in plasma renin activity from 4.5 hours on, compared with the values recorded 1 hour after unclipping, were accompanied by a reduction in systemic blood pressure, renal blood flow, and glomerular filtration rate.

The group of rabbits treated with indomethacin...
Plasma renin activity fell significantly and progressively during the 9 hours after the renal artery was unclipped at time 0 in the indomethacin-treated rabbits, whereas it did not change in non-indomethacin-treated rabbits after unclipping.

The changes cited for the indomethacin-treated group were associated with a significant blockade of renal prostaglandin synthesis. Figure 6 shows that the control increase in prostaglandin concentration measured in kidney tissue after 10 minutes of incubation was completely prevented 2 and 11 hours after the indomethacin treatment was begun.

Finally, Table 1 shows that the direction and the magnitude of the changes in urinary output, renal blood flow, and glomerular filtration rate elicited 2 hours after the administration of indomethacin in the sham-operated group were identical to those observed in the unclipped group treated with indomethacin. However, the surgical procedure performed in the sham-operated rabbits was not followed by any significant change in blood pressure, which remained within the range of the control values. In the treated sham-operated group, the observed changes in plasma renin activity were similar to those measured in the unclipped group treated with indomethacin in that the average values decreased by 80% below those recorded during the control period.

**Discussion**

If prostaglandins contained in the solitary remaining clipped kidney participate substantially in reversing hypertension, one can theorize that the sudden exposure of the kidney to high systemic blood pressure after unclipping results in a significant passage of PGE into the circulation (since its rate of synthesis presumably has been enhanced because of the previous ischemia). This release of PGE then induces hypotension by either modifying the venous tone (of the major systemic veins or of the arterial side of the pulmonary circulation) or causing systemic arterial vasodilation (after it is converted in the plasma to prostaglandins of the A series and passes through the lungs). Moreover, the natriuretic action of PGE may complement the vasodepressor hemodynamic effect if the clip removal produces diuresis leading to an extracellular fluid contraction.

This conception constitutes our testing hypothesis, which was suggested not only because PGE has demonstrated vasodilator and natriuretic actions (12) but also because the notions of a humorally mediated hemodynamic adjustment (9) and salt and water depletion (8) are the two mechanisms currently proposed to explain the reversal of one-kidney hypertension.

The results of this study showed that the integrity of prostaglandin synthesis is not strictly necessary to ensure the reversal of one-kidney hypertension, because 8–9 hours after unclipping the decreases in blood pressure in the treated and untreated groups were similar. However, the length of time required for the treated rabbits to reach normal levels of blood pressure was significantly longer than that for the untreated rabbits, thus indicating that, although the presence of prosta-
Changes in Various Physiological Measurements Made during Studies on Rabbits Given Indomethacin and Subjected to Sham-Operations

<table>
<thead>
<tr>
<th></th>
<th>Control (N=3)</th>
<th>2 hours after indomethacin</th>
<th>After sham-unclipping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>120 ± 2</td>
<td>123 ± 2</td>
<td>122 ± 3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>95 ± 2</td>
<td>96 ± 2</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>30.4 ± 5.9</td>
<td>18.9 ± 2.5</td>
<td>18.5 ± 1.9</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min)</td>
<td>6.0 ± 0.7</td>
<td>4.2 ± 1.2</td>
<td>4.1 ± 1.1</td>
</tr>
<tr>
<td>Urine volume (ml/hour)</td>
<td>1.8 ± 0.3</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Plasma renin activity (mg/ml min⁻¹)</td>
<td>21.4 ± 3.5</td>
<td>13.0 ± 3.8</td>
<td>9.8 ± 3.0</td>
</tr>
</tbody>
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All values are means ± SE.

glandins is not crucial in reversing one-kidney hypertension, it can be a contributory factor. In this respect, it is pertinent to analyze the significance of the changes measured in different parameters in treated and untreated rabbits.

The earliest significant change, recorded 1 hour after the release of the arterial constriction, in the group of rabbits not treated with indomethacin was a highly significant increase in both renal blood flow and glomerular filtration rate compared with the values recorded immediately before unclipping (Fig. 2). Can these early and significant differences in renal blood flow and glomerular filtration rate account for the chronologic differences in the fall of blood pressure between the treated and untreated groups? Theoretically, a sudden increase in renal blood flow and glomerular filtration rate following the exposure of the solitary clipped kidney to high systemic blood pressure can elicit natriuresis, a decrease in renin release, and, thereby, a decrease in peripheral resistance, all of which can effectively lower systemic blood pressure. Since these changes were in fact observed after the release of the arterial constriction in untreated rabbits, it is of interest to analyze to what degree they can account for the faster relief of hypertension in this group.

At 4.5 hours after unclipping, when the differences in blood pressure between the two groups of rabbits reached the maximum (Fig. 1), the cumulative urinary volume of the group of rabbits treated with indomethacin was 19.2 ml of urine (Fig. 4), whereas the total urinary output of the group of rabbits that was not treated with indomethacin was 27.0 ml. Considering that the amount of saline solution infused with inulin, PAH, and indomethacin during these 4.5 hours was 18.1 ml, it is difficult to accept the idea that such a small difference in fluid balance can account for the difference of 21 ± 3 mm Hg in diastolic pressure seen at 4.5 hours after unclipping.

That the reduction in blood pressure in the control rabbits was induced by a decreased renin release produced by the exposure of the clipped kidney to high systemic blood pressure is suggested by the fact that 1 hour after unclipping there was a significant 33% decrease in plasma renin activity (Fig. 5). However, the time courses of the changes in blood pressure and plasma renin activity depicted in Figures 1 and 5 show that as diastolic blood pressure decreased to 75 ± 1 mm Hg the average plasma renin activity recovered to values similar to those recorded before unclipping, thus suggesting that the changes recorded for plasma renin activity follow the changes in renal perfusion pressure rather than cause definite changes in systemic blood pressure.

This notion agrees with observations in previous studies. In one-kidney Goldblatt hypertension, blockade of the renin-angiotensin system does not result in any significant decrease in blood pressure (4-6). Therefore, changes in plasma renin activity do not seem to be implicated in the fall in blood pressure that followed the release of the arterial constriction. Nor can such changes account for the time differences in the reversing of hypertension between the groups, because at 4.5 hours the average plasma renin activity of the rabbits treated with indomethacin was significantly lower than that of the untreated rabbits, yet blood pressure of the treated group was higher than that of the untreated controls. From Figure 5, it is apparent that the administration of indomethacin to un-clipped or sham-un-clipped rabbits, besides blocking the synthesis of prostaglandins, induces a
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progressive decrease in plasma renin activity. Preliminary studies designed to further elucidate the mechanism by which the administration of indomethacin lowers plasma renin activity indicate that such a response is due to an interference with the release of renin from the kidney, but at the present time it is not clear whether this effect is exerted by indomethacin or by its degradation products or is related to the blockade of prostaglandin synthesis (22).

The persistence of hypertension associated with similar decrements in plasma renin activity and prostaglandin synthesis in indomethacin-treated sham-unclipped rabbits gives further support to the concept explicit in the experimental group that none of these factors is conspicuously involved in the reduction in blood pressure that follows the unclipping. The absence of significant changes in blood pressure in the sham-operated group also rules out the possibility that the hypotensive effect seen after clip removal is due to surgical maneuvers.

In analyzing the factors that may account for the delayed decrease in blood pressure in the treated rabbits, it is important to remember that the blockade of prostaglandin synthesis by indomethacin affected the formation of prostaglandins uniformly throughout the body. Hence, the persistence of hypertension after clip removal in the treated group probably reflects suppression of the local vasodilator action of prostaglandins being synthesized at the level of the resistance vessels.

In summary, the higher and significant increments in renal blood flow, glomerular filtration rate, and urinary output as well as the earlier decrease in blood pressure that occurred in the untreated rabbits in response to the release of the arterial constriction tend to support the theory that prostaglandins may play a facilitatory role in reversing one-kidney hypertension. But a major participation of prostaglandins in this phenomenon seems improbable, because the decrease in blood pressure 9 hours after unclipping was similar in both the treated and the untreated group.

References

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