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# Renal Vasodilation after Unilateral Renal Ablation

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## ABSTRACT

Renal blood flow was studied in rats 120 minutes after unilateral renal ablation. The influence of endogenous prostaglandin formation was evaluated by indomethacin treatment prior to the ablation. Radioactive microspheres were used for estimation of the total renal and cortical blood flow, and the renal medullary blood flow was determined with the 86-Rb chloride extraction method. The total blood flow in the remaining kidney was increased by 80 % following contralateral ablation, with augmentation in all areas, particularly in the deep medullary region. Indomethacin treatment in intact rats evoked increased blood flow in the renal cortex and in the various medullary regions. Unilateral renal ablation in indomethacin-treated rats caused no further change in renal blood flow as compared with the indomethacin control group. The results indicated a) that the renal blood vessels respond to ablation of the contralateral kidney with dilation in all kidney regions, and b) that this vascular dilation may be prostaglandin-mediated.

## INTRODUCTION

Shortly after unilateral renal ablation (URA) the remaining kidney shows increased urine flow and electrolyte excretion rates (11,26). The mechanism underlying this swift adaptation to nephron loss has not yet been fully elucidated, though it has frequently been investigated (1,9,10,12,17).

At this laboratory we have found that the interstitial hydrostatic pressure rises and the interstitial oncotic pressure falls within 30 minutes after URA in the rat. These changes were prevented by prostaglandin synthesis inhibitors (18). Moreover, we noted that the sensitivity of the tubuloglomerular feedback control is decreased 30 minutes after URA (25). This phenomenon, which is a prerequisite for acute adaptation, was not observed after nephron loss in animals treated with indomethacin (19).

Our previous findings, and those of other authors (11) suggested that prostaglandin or prostaglandin-like substances might be involved in the acute adaptive process that takes place after URA. Prostaglandins are synthesized in all areas of the kidney, prostaglandin $E_2$ ($PGE_2$) primarily in the medulla and prostacyclin ($PGI_2$) both in the renal cortex and in the medulla (2). The prostaglandins are highly vasoactive substances, and altered prostaglandin activity in the kidney may influence the renal circulation. The aims of the present study were to observe the renal blood flow (RBF) and its cortical and medullary distribution in the rat 2 hours after URA and to evaluate the influence of the cyclooxygenase inhibitor indomethacin on these blood flow rates.

## MATERIAL AND METHODS

The experiments were conducted on male Sprague-Dawley rats (Möllegaard, Denmark) weighing 200-300 g. Anaesthesia was induced with Inactin$^R$ in a dose of 120 mg/kg body weight (BW) given intraperitoneally. All rats were tracheotomized and placed on a servo-controlled heating pad. The renal pedicles were carefully dissected and loops of silk were loosely placed around them. The right carotid artery was catheterized for injection of tracers at the level of the aortic valves and the right femoral artery for drawing of reference blood samples. Two additional catheters were inserted, one into the left femoral artery for continuous monitoring of arterial blood pressure (MAP) and the other into the left femoral vein for injection of drugs and for saline infusion, initially at a rate of 1.2 ml/h, which was reduced to 0.6 ml/h after URA.

Four groups of rats were used. Group I served as controls and consisted of seven rats in which blood flow was determined in the left kidney after 120 minutes of saline infusion. In group II (8 rats) ablation of the right kidney was performed by tightening the silk loop around the pedicle, and the blood flow in the left kidney was determined 120 minutes later. In group III (4 rats) the haemodynamic effects of indomethacin (Confortid$^R$, Dumex, Copenhagen, Denmark) were studied. The indomethacin was dissolved in sterile water (2 mg/ml, pH 7.4). A dose of 2 mg/kg BW was given initially, and 1 mg/kg BW was given after 60 minutes. The blood flow in the left kidney was estimated 120 minutes after the first indomethacin injection. Group IV (7 rats) received the same doses of indomethacin as group III, but 30 minutes after the first injection the silk loop was tightened to occlude the right kidney, and the blood flow in the left kidney was estimated 120 minutes after this manoeuvre. The saline infusion in the indomethacin-treated rats was adjusted to ensure that all groups received the same amount of fluid. All measurements of blood flow were made at comparable times after induction of anaesthesia.

The blood flow estimations were performed as follows. Blood sampling was started from the right femoral artery at a rate of 0.75 ml/min, using a constant speed reference pump Then an injection of about $7 \cdot 10^5$ Bq 86-RbCl and 200 000 microspheres (10 µm 113-Sn) suspended in saline solution was given into the right carotid artery. Precisely 30 seconds after half of the tracer had been injected, the reference blood sampling was stopped and the renal pedicle was ligated. The kidney was removed and dissected under a microscope into specimens of cortex and medulla. The renal medulla was further microdissected into the outer and inner stripe of the outer zone and the inner zone (22). The specimens and the rest of the kidney were weighed and the radioactivity was measured in a gamma spectrophotometer (Nucab, Gothenburg, Sweden). The samples were kept in a brass cylinder in the well crystal in order to avoid the beta radiation from the rubidium.

The blood flow (F) was determined from the equation $\frac{M}{M_{ref}} = \frac{F}{F_{ref}}$ for both isotopes, where $M_{ref}$ is the amount of indicator in the reference blood sample, M the amount of indicator in the specimens and $F_{ref}$ the sampling rate. The microsphere method was used for estimations of cortical and total RBF and of cardiac output (CO). CO was estimated with the same equation, the factor M denoting the total amount of activity injected and F denoting CO. The rubidium extraction method was used for determinations of renal medullary blood flow in the different areas. (For further information and detailed discussion of the methods, readers are referred to previous reports - 21, 22).

Statistics

Differences in CO and in blood flow between the treatment groups were tested with a one-way analysis of variance, and for differences in regional blood flow changes a two-way analysis of variance was used. MAP changes during the different treatment periods within the various groups were analyzed with the aid of Student`s t-test for paired means. A p-value of less than 0.05 was accepted as significant.

## RESULTS

The general condition of all the rats remained good. MAP and CO values are presented in Table 1. Transient MAP increase occurred after the indomethacin infusion in groups III and IV. The MAP values at the termination of the experiments did not differ from the control period values in either of these groups. No significant differences in CO were found between the groups.

The blood flow values are given in Table 2 and Fig. 1. The flow rates in control conditions (group I) were in accordance with earlier observed values

Table 1. Mean arterial blood pressure (MAP) during different periods and cardiac output (CO) at termination of the experiments (means ± SEM)

| Group | Period | n | MAP (mm Hg) | p | CO (ml/min/100 g BW) |
|---|---|---|---|---|---|
| I | | 7 | 135 ± 5 | | 21.0 ± 0.6 |
| II | control | 8 | 128 ± 5 | | |
| | post-URA | 8 | 130 ± 5 | | 25.8 ± 2.8 |
| III | control | 4 | 129 ± 2 | | |
| | post-indomethacin | 4 | 136 ± 2 | < 0.02 | |
| | post-experiment | 4 | 129 ± 4 | < 0.05 | 21.8 ± 2.5 |
| IV | control | 7 | 114 ± 1 | | |
| | post-indomethacin | 7 | 123 ± 2 | < 0.001 | |
| | Post-indomethacin + URA | 7 | 111 ± 3 | < 0.001 | 23.6 ± 1.8 |

URA = unilateral (right) renal ablation, n = no of rats, p = significance level v. preceding period

in rats of the same strain (22).

After URA (group II) there was a highly significant increase of RBF in all areas. The total renal and cortical blood flow rates rose by 80 %. The relative increase in RBF was significantly greater in the inner medulla than in the outer medulla or the cortex (Fig. 2). This relationship was tested by two-way analysis of variance. An interaction between the flow increases in the different regions was found ($p = 0.0208$).

The rats of group III, indomethacin-treated controls, showed increased RBF in all regions after this treatment, though the blood-flow increment was less than in the rats with renal ablation. The flow rates in the indomethacin-treated rats with subsequent URA (group IV) did not differ from the corresponding rates in group III. As shown in Table 2 and Fig. 1, the mean values of total RBF in groups III and IV were higher than the corresponding value in group II, though none of these differences reached the 5 % level of significance.

Table 2. Blood flow (ml/min/g) in different kidney regions (means ± SEM)

| Group * | | I | II | III | IV |
|---|---|---|---|---|---|
| Cortex | | 7.0 ± 0.4 | 12.4 ± 1.4 | 10.7 ± 0.2 | 10.2 ± 1.1 |
| p v. group | I | | < 0.01 | < 0.05 | < 0.05 |
| | II | | | NS | NS |
| | III | | | | NS |
| Outer stripe | | 1.8 ± 0.3 | 2.9 ± 0.5 | 2.8 ± 0.1 | 2.7 ± 0.3 |
| p v. group | I | | < 0.01 | < 0.05 | < 0.05 |
| | II | | | NS | NS |
| | III | | | | NS |
| Inner stripe | | 1.2 ± 0.1 | 2.8 ± 0.2 | 2.6 ± 0.1 | 2.5 ± 0.3 |
| p v. group | I | | < 0.001 | < 0.001 | < 0.001 |
| | II | | | NS | NS |
| | III | | | | NS |
| Inner zone | | 0.6 ± 0.1 | 1.7 ± 0.2 | 1.2 ± 0.1 | 1.1 ± 0.1 |
| p v. group | I | | < 0.001 | < 0.02 | < 0.02 |
| | II | | | | < 0.01 |
| | III | | | | NS |
| Total kidney | | 4.1 ± 0.4 | 7.4 ± 0.8 | 6.1 ± 0.3 | 5.7 ± 0.6 |
| p v. group | I | | < 0.01 | NS | NS |
| | II | | | NS | NS |
| | III | | | | NS |
| n | | 7 | 8 | 4 | 7 |

* I = untreated controls, II = unilateral renal ablation, III = indomethacin-treated controls, IV = indomethacin + unilateral renal ablation.
n = no of rats, NS = $p > 0.05$.

## DISCUSSION

The dissection of the left renal pedicle could have involved risk of altering the flow pattern by traumatic nerve stimulation. Special care was taken not to touch the renal vessels, and the kidneys were not dissected free from the fat capsule. However, more vigorous dissection has been shown not to affect RBF (21).

In this study we combined the microsphere and the rubidium uptake method. In this way we were able to measure whole kidney blood flow and cortical blood flow with microspheres and medullary blood flow with rubidium. The use of microspheres for regional flow studies involves a risk of uneven distribution because of axial streaming (6). Consequently this method was used only to determine cortical and total renal blood flow. One critical point when using the rubidium method is the sampling time, which must be long enough for the rubidium to reach the tissue under study but short enough to prevent washing out of

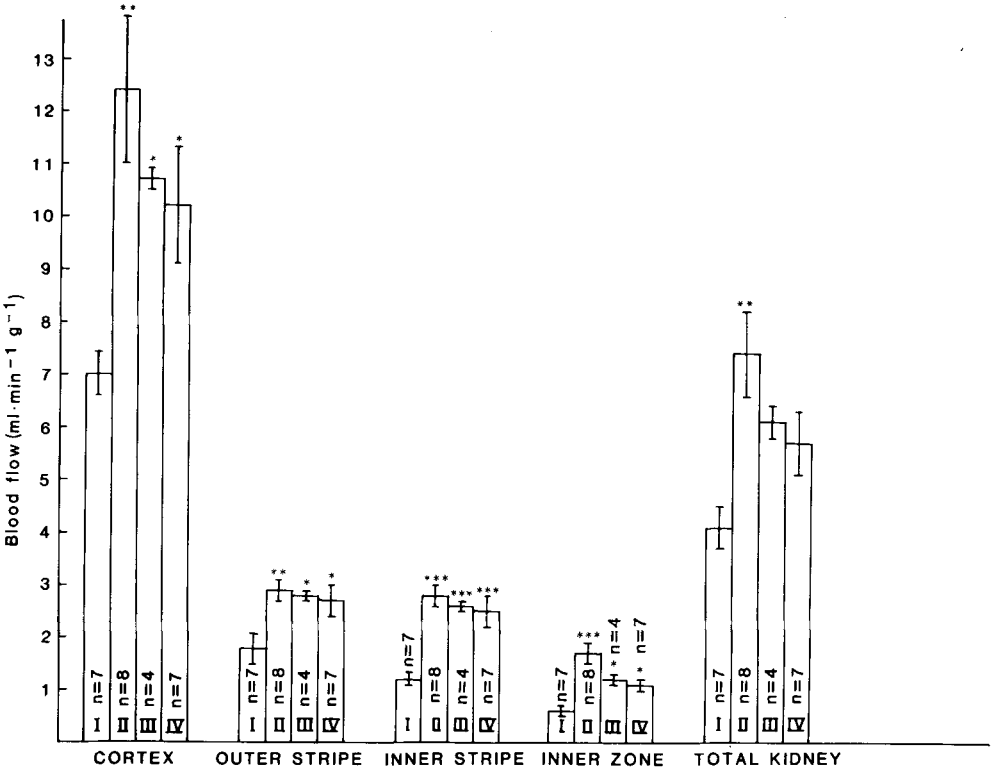


Fig. 1. Mean regional and total renal blood flow in control group (I), unilateral renal ablation (II), indomethacin-treated controls (III) and indomethacin + unilateral renal ablation (IV). Bars = ± SEM, n = no of rats in each group. *** $p < 0.001$ v. group I, ** = $p < 0.01$ v. group I, * $p < 0.05$ v. group I.

rubidium. Because of the differing transit times of cortical and medullary blood flow, the rubidium extraction method is not suitable for combined cortical and medullary flow studies. In evaluation of the rubidium method (22), a sampling time of 30 seconds in medullary blood flow measurements resulted in almost complete medullary extraction.

Earlier studies showed that immediately after unilateral kidney loss the remaining kidney responds by increasing its excretion of water and electrolytes. In the rat this response has been attributed to decreased fractional reabsorption of sodium and water in the proximal tubules and increased potassium secretion in the distal tubules (7,8,9,11,12). The transient rise in blood pressure regularly seen after unilateral renal ablation has been excluded as

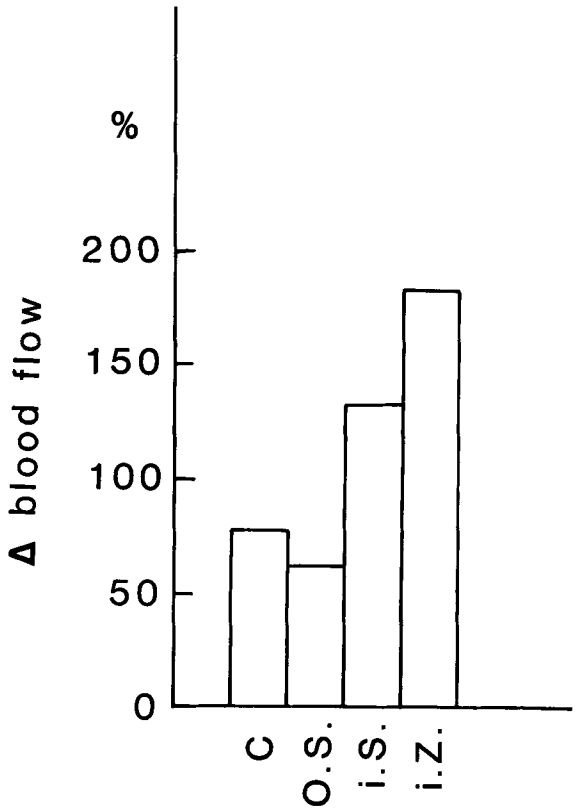


Fig. 2. Relative blood flow change in the renal cortex (c), the outer stripe (o.s.) and inner stripe (i.s.) of the outer zone and the inner zone (i.z.) of the renal medulla following contralateral renal ablation.

the sole cause of this response (10,11,31). In the present investigation no significant increase in MAP was seen after URA. MAP showed transient increase in association with indomethacin treatment, but regained the control level in less than 30 minutes. This transient blood pressure rise therefore seemed unlikely as the cause of the RBF increase in group III.

The total RBF was significantly increased 120 minutes after URA, with augmentation found in the cortex and in all areas of the medulla. The relative increase was most striking in the inner medulla. Fig. 2 shows that the blood flow increase was 180 % in the inner zone, 80 % in the cortex and 60 % in the outermost part of the medulla. This indicates a redistribution to the inner medulla following URA. The present study was not designed to reveal if redistribution occurs also within the renal cortex.

In calculations of RBF from inulin extraction measurements, the mean

glomerular blood flow was found to have doubled in the remaining kidney 4 weeks after URA in rats (24). In canine experiments using 85 Kr disappearance curves and silicone rubber casts, no corresponding RBF increase was found after unilateral nephrectomy (4). The discrepancy may have been due to species difference and/or differing experimental conditions. In remnant kidneys retaining 20-30 % of the renal arterial supply, however, 85 Kr radioactivity rapidly appeared and disappeared in the medulla, and silicone rubber injection specimens revealed marked vasodilation (4).

Other authors found only moderate changes in RBF in the contralateral kidney two days after unilateral nephrectomy and no response to indomethacin treatment (29). On the other hand, they observed total RBF increase in the contralateral kidney 24 hours after release of unilateral ureteral occlusion, and this RBF increase was blocked by indomethacin treatment. These authors used microspheres. In our laboratory, studies of RBF using the joint method of microspheres and rubidium revealed increased flow rates in all areas of the contralateral kidney 24 hours after a 45-minute period of unilateral renal artery clamping (23). Seven days after clamping the flow rates in the contralateral kidney were normal again. These earlier reports (23,29) are not necessarily in conflict with the results of the present investigation. The significant RBF increase we found after 2 hours may be almost abolished after 48 hours. The RBF changes appearing at different times in the period of acute adaptation after URA and during the subsequent compensatory hypertrophy merit further investigation.

Species differences may explain differing effects of indomethacin administration on RBF in dogs and rats. No change was observed in rat RBF during indomethacin treatment in earlier experiments (14). In conscious or anaesthetized dogs indomethacin likewise did not influence RBF, though anaesthetized, laparotomized dogs responded with a 40 % reduction in RBF (30). Interestingly, we found significant increase in rat RBF on administration of indomethacin (group III), most pronounced in the inner medulla. In indomethacin-treated rats with URA (group IV) the RBF increase was of the same magnitude as in the indomethacin-treated controls. No additional vasodilation was seen after URA. Our findings can be interpreted in two ways: Either the vasodilation during indomethacin treatment is maximal, so that URA cannot further increase blood flow, or the prostaglandins are involved in the mediation of this vasodilatory response after URA. Differentiation between these two alternatives is not yet possible.

Prostaglandin $E_2$ synthesis in the rat kidney takes place primarily in the medulla (13). In several species, including dog, $PGE_2$ exerts renal vasodilatory effects. In rat, however, $PGE_2$ and arachidonic acid were found to be vasoconstrictors (16). Accordingly, $PGE_2$ was noted in rat micropuncture studies to evoke angiotensin II release and vasoconstriction (28). On the other hand, low-dose intrarenal infusion of $PGE_2$ was associated with reduced renal vascular

resistance in rats (20). Whether $PGE_2$ is predominantly a vasodilator or a vasoconstrictor in the rat thus remains an open question. Thromboxan $A_2$ ($T_xA_2$) is a potent vasoconstrictor which was found to decrease renal plasma flow in anaesthetized Sprague-Dawley rats (27). Prostacyclin ($PGI_2$) has been reported to be a renal vasodilator in rats (16). In dogs, intraarterial $PGI_2$ infusions resulted in renal vasodilation, natriuresis and kaliuresis (15). In recent studies in our laboratory we found enhanced urine flow, reduced renal interstitial oncotic pressure and raised interstitial hydrostatic pressure in rats during and after intraarterial infusion of a nonhypotensive dose of $PGI_2$ (3). $PGI_2$ or some other vasodilating prostaglandin may be involved in the acute vascular changes following unilateral renal ablation.

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