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## SPECIFIC FEATURES AND ROLES OF RENAL CIRCULATION: ANGIOTENSIN II REVISITED

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The status of intrarenal circulation determines in part renal excretion, affects body fluid homeostasis and has a role in long term control of arterial blood pressure. The vascular resistance in the renal cortex and medulla is determined by interaction of a vast array of vasoactive hormones and paracrine factors; among these the role of constrictor angiotensin II and dilator prostaglandins and nitric oxide may appear to be dominating. The focus of this review and underlying studies is on the mechanisms whereby the microcirculation of the renal medulla is protected against the vasoconstrictor action of angiotensin II. In anaesthetized normal rats the three mentioned active agents or their inhibitors were applied and total renal blood flow and cortical, outer- and inner medullary laser-Doppler fluxes were determined; in some studies renal tissue nitric oxide was measured using selective electrodes. We conclude that angiotensin II, acting *via* AT1 receptors, constricts the renal cortical vasculature; in the medulla its action is effectively buffered by prostaglandin E2 but most probably not by nitric oxide.

*Key words: intrarenal circulation, angiotensin II, prostaglandins, nitric oxide*

Considering the role of the kidney in the regulation of body fluid homeostasis and direct linking of renal excretion to renal haemodynamics, for many decades the special features of intrarenal circulation and mechanisms of its control have been the subject of maintained interest and research. It may appear less obvious why a particular effort has been made to explore the function and microcirculation of the renal medulla which constitutes a relatively small fraction of the whole kidney mass and receives less than 10% of the blood delivered to the kidney *via* the renal artery. At least two circumstances justify the special interest for the medulla and its perfusion.

First, any decrease in blood and oxygen supply to this zone could present a serious danger because, even at normal perfusion, oxygen tension in the medullary tissue is low; indeed, the medulla appears to function at the edge of anoxia (1). Thus, an impairment of medullary circulation, *e.g.* following a release of renin and increased generation of angiotensin II, could seriously endanger the medullary integrity and function.

Second, over the years evidence has accumulated indicating that the renal medulla and the status of its circulation have a pivotal role in long term control of arterial pressure; more specifically, a decrease in renal medullary perfusion may lead to an increase in arterial pressure and an increase in medullary perfusion has an opposite effect (2, 3).

Under normal and pathophysiological conditions the renal microcirculation is maintained at an adequate level owing to an interplay of numerous hormonal and autocrine/paracrine agents, both vasodilators and vasoconstrictors (*Table 1*). Among these, the role of angiotensin II (Ang II) exerting a powerful vasoconstrictor action *via* AT1 receptors, seems at the first sight well established, similar as is the role of the renin-angiotensin-aldosterone system in cardiovascular homeostasis and control of arterial pressure. However, a close inspection of the literature reveals many controversies regarding the actual role of Ang II in the control of perfusion of the renal cortex and medulla. In the present paper the authors' recent

*Table 1.* A review of hormonal and paracrine agents affecting intrarenal vascular resistance

| Renal Vasoconstrictors  | Renal Vasodilators   |   |
|---|--|---|
| <ul style="list-style-type: none"> <li>• Norepinephrine - <math>\alpha</math> 1</li> <li>• <b>Angiotensin II</b> – AT1</li> <li>• Vasopressin (AVP) – V1</li> <li>• Endothelin 1 – ET<sub>A</sub></li> <li>• Adenosine – A1</li> <li>• 20-HETE (hydroxyeicosatetraenoic a.)</li> <li>• ATP – P2X</li> <li>• Thromboxan A (TXA2) - TP</li> </ul> | <ul style="list-style-type: none"> <li>• <b>NO</b> → cGMP, <b>20-HETE</b></li> <li>• <b>Prostaglandins (PGE2, PGI2)</b> – EP, IP</li> <li>• Acetylcholine (Ach) → NO</li> <li>• Kinins (bradykinin, Bk) – B2</li> <li>• Adenosine – A2</li> <li>• EET (epoxyeicosatrienoic a.)</li> <li>• ATP – P2Y</li> <li>• ANP → cGMP</li> </ul> |   |
| <p><b>Angiotensin II</b></p> <pre> graph TD     A[Angiotensin II] --&gt; B[AT1]     A --&gt; C[AT2]     </pre>  | <p><b>NO</b></p> <pre> graph TD     A[NO] --&gt; B["↑ cGMP"]     A --&gt; C["↓ 20-HETE"]     </pre>  | <p><b>PGE<sub>2</sub></b></p> <pre> graph TD     A[PGE2] --&gt; B["EP2, EP4"]     A --&gt; C["EP1, EP3"]     </pre> |
| <p><b>AT1</b>      <b>AT2</b></p> <p>vasoconstrictor      vasodilator</p>   | <p>↑ cGMP      ↓ 20-HETE</p>   | <p><b>EP2, EP4</b>      EP1, EP3</p> <p>vasodilator      vasoconstrictor</p>  |
| <p>Regional constrictor effect:</p> <p>Cortex +++</p> <p>Medulla ?</p>  | <p>Regional dilator effect:</p> <p>Cortex +++</p> <p>Medulla +++</p>   | <p>Regional dilator effect:</p> <p>Cortex ++ ?</p> <p>Medulla +++</p>   |

The active agents and their receptors or second messengers are shown. Note that one agent may have opposed effects, depending on the actual receptor mediating the action. The focus of this review is on constrictor action of angiotensin II and vasodilator action of nitric oxide and prostaglandin E2 (bold type). For these, effects on the cortex and medulla are estimated separately.

studies attempting to resolve some of these controversies are reviewed and discussed in the context of the information available earlier. Attention is focused on the interplay of Ang II, nitric oxide and vasodilator prostaglandins.

The previous data on the response of intrarenal circulation to angiotensin II were not uniform. All studies reported a decrease in total or cortical blood flow; in one study Ang II was found to decrease the flow in dog renal medulla (4). A number of workers reported no change in medullary or papillary blood flow (5 - 8), but a medullary vasodilatation in response to Ang II was also seen (7, 9). The results of attendant studies using inhibitors of angiotensin converting enzyme or of AT1 receptors were also discrepant.

In order to find out if changing activity of Ang II would affect the renal cortical and medullary circulation in a parallel fashion, and high hormone activity would, indeed, compromise perfusion and, potentially, the function of the medulla, we re-examined recently the effects of moderately pressor doses of Ang II in anaesthetised Wistar rats (10). The total renal blood flow (renal artery flow, RBF) was used as a measure of cortical perfusion or this perfusion was measured by laser-Doppler probe placed on kidney surface. The medullary blood flow was measured by another I-D probe placed in the inner (white) medulla, close to the border with the outer medulla. The laser-Doppler system used was Periflux 4001, PERIMED, Jarfalla, Sweden.

The data of *Fig. 1* show that Ang II infusion at a dose that induced an increase of arterial pressure of 10 mm Hg distinctly decreased perfusion of the cortex but increased perfusion of the medulla. The same was observed when renal perfusion pressure was maintained constant by controlled suprarenal aortic constriction. In other experimental series (see below) MBF did not change after Ang II but a decrease was never seen. Mirror responses (an increase in RBF or CBF and a decrease in MBF) were seen when AT1 type receptors of Ang II were inhibited by losartan, even more distinctly so after the baseline Ang II activity was raised by infusion of exogenous hormone. These results clearly show that circulating Ang II is a powerful renal cortical vasoconstrictor but the hormone does not reduce perfusion of the renal medulla *in vivo*.

The absence of a depression of medullary circulation after Ang II does not accord with the evidence from the work using a variety of experimental preparations, such as split hydronephrotic kidney, isolated perfused juxtaglomerular nephron and from measurements of blood cell velocity in visualized *vasa recta* of an exposed papilla. All of these studies showed constriction of medullary vessels after Ang II (11 - 13). Moreover, the resistance vessels determining perfusion of the medulla (afferent and efferent arterioles of the juxtamedullary glomeruli as well as outer medullary descending *vasa recta*) are known to express angiotensin II receptors of AT1 type mediating vasoconstriction. This suggests that the medullary vasculature has the potential to respond to the hormone in the same way as is seen in the cortex, however, vasoconstriction does not manifest itself in whole animal

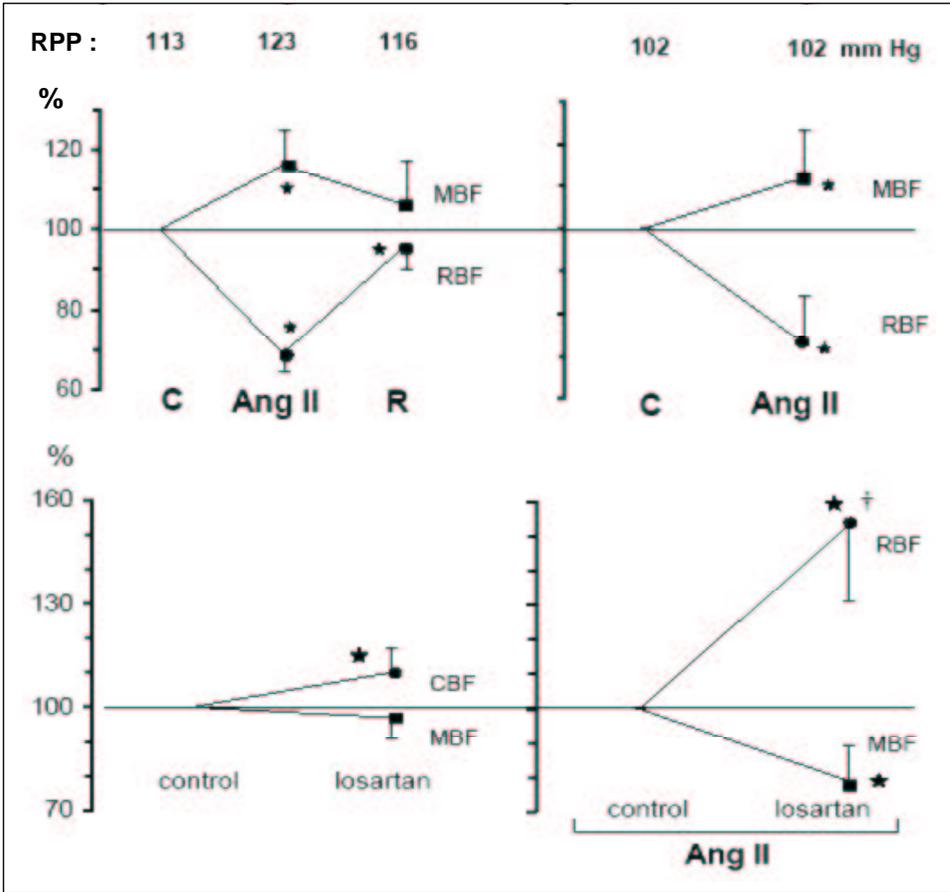


Fig. 1. Upper panel: Effect of Ang II infusion on total renal blood flow (RBF) and medullary blood flow (MBF, laser-Doppler flux). The renal perfusion pressure (RPP) was allowed to increase (A) or was maintained constant (B).

Lower panel: Effects of losartan (10 mg kg<sup>-1</sup> i.v.) on cortical blood flow (CBF), RBF and MBF. Losartan was given alone (A) or in rats infused Ang II (30 ng kg<sup>-1</sup> min<sup>-1</sup> i.v.) (B). Data represent percentage of the initial control value, means ± S.D.

\* P<0.05 vs preceding control value; † p<0.001 vs CBF value measured after losartan alone. Reproduced from ref. (10).

studies with the kidney functioning in a natural milieu of the organism. This observation has led us to the hypothesis that the renal medullary vasculature does not constrict in response to Ang II because its effect is offset by the action of a vasodilator.

In a subsequent study we examined a possible role of two potential vasodilator agents known to operate in the renal medulla: nitric oxide (NO) and vasodilator prostaglandins (PG) (14). The experimental set-up used in the study was similar

as before except that superficial cortical laser-Doppler probe was not used (the cortical perfusion was estimated simply as total renal blood flow, RBF). On the other hand, two laser-Doppler probes were used to measure separately perfusion of the outer medulla (OMBF) and inner medulla (IMBF), at the depth in the rat kidney of 3 mm or 5 mm, respectively.

Fig. 2 shows that in untreated rats Ang II infusion caused an almost 40% decrease in cortical perfusion (RBF) and a modest (less than 10%) decrease in outer medullary perfusion (OMBF) whereas inner medullary perfusion (IMBF) did not change. This response pattern was also seen when the synthesis of nitric oxide (NO) was inhibited by pre-treatment with L-NAME, which suggests that NO has no role in buffering renal medullary hypoperfusion in response to Ang II. On the other hand, after elimination PG synthesis with indomethacin the usual decrease in RBF and OMBF was seen but was also associated with a definite 10% decrease in the perfusion of the inner medulla. These results indicate that, first, by analogy with the well established separate control of renal cortical and medullary circulation, the perfusion of the inner medulla can be controlled independent of blood flow through the outer medulla. Second, since with intact synthesis of vasodilator PG the perfusion of the inner medulla is maintained despite a reduction of cortical and outer medullary blood flow, and after

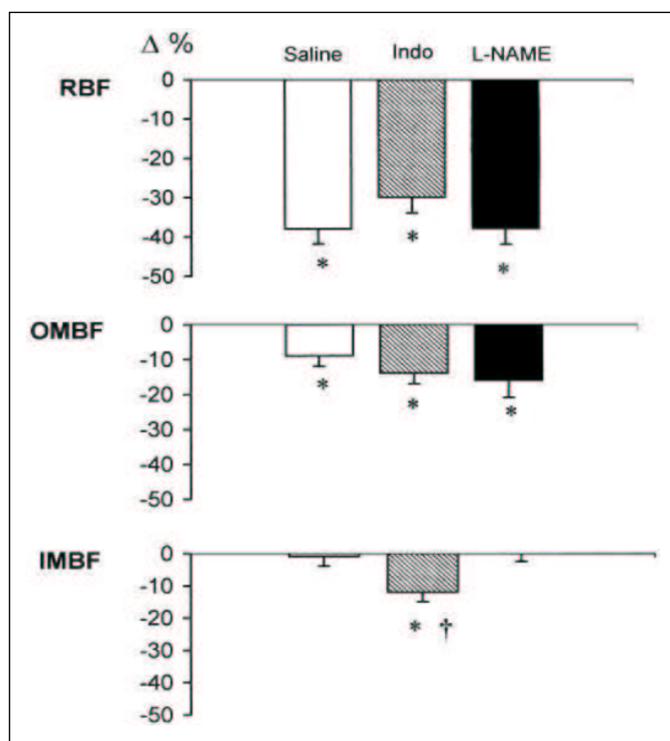


Fig. 2. A comparison of Ang II ( $30 \text{ ng kg}^{-1} \text{ min}^{-1}$ ) effects on total RBF, OMBF and IMBF in control rats (Saline) and animals pretreated with indomethacin (Indo) or L-NAME. Data represent percentage of the initial control value, means  $\pm$  S.E.M. \* Significantly different from pre-Ang II control at  $P < 0.05$ ; † significantly different from the change measured after Ang II alone (empty bars) at  $P < 0.05$ . Reproduced from ref. (14).

elimination of PG the IMBF decreases in parallel with cortical and outer medullary perfusion, we conclude that PG offset the vasoconstrictor action of Angiotensin II. It can be speculated that the abrogation of Ang II effects by PG is confined to the inner medulla because the antagonistic action of the two agents occurs at the descending medullary *vasa recta* and not upstream, at the afferent or efferent arterioles of the juxtamedullary glomeruli. A dilatation of the outer medullary segments of the *vasa recta* (OMDVR) could help offset the effect of reduced inflow of blood to the medullary vasculature. Alternatively, a constriction of OMDVR located at the periphery of the medullary vascular bundle, those which supply blood to the capillary plexus of the outer medulla (reflected by decrease in OMBF), could divert blood to the conduit type *vasa recta* located centrally in the bundle and supplying the inner medulla and the papilla (11).

Our results indicating no role of NO in buffering the potential Ang II dependent vasoconstriction cannot be easily reconciled with earlier indirect evidence that Ang II can stimulate NO production in the kidney *via* AT<sub>2</sub> receptors (15). More important, Zou *et al.* (16) reported an increase in both cortical and medullary tissue NO after subpressor doses of Ang II. In order to examine if NO could still have a role in buffering the Ang II dependent vasoconstriction in the renal medulla, possibly together with prostaglandins, we examined the effect of PG, simultaneously on renal tissue NO and intrarenal circulation (17).

NO current was measured by constant voltage amperometry (ISO-NO MARK II system, World Precision Instruments, Sarasota, FL, USA). Large-surface selective electrodes were placed on kidney surface, to record the signal from the cortex, or inserted into the kidney to the depth of about 5 mm, to record from the inner medulla. *Fig. 3* shows the results obtained in two groups of rats: in one NO was measured in the cortex and in the other one in the medulla (for technical reasons we avoided at first simultaneous application of two medullary probes: for NO and IMBF measurement). The studies confirmed an Ang II dependent reduction in perfusion of the cortex and no change in perfusion of the inner medulla. However, the most important finding was a significant decrease in NO signal recorded both in the cortex and in the medulla. It is unlikely that this decrease was a nonspecific consequence of the post-angiotensin decrease in RBF *per se*: the changes in NO and in RBF were not correlated and in still another group of rats a significant decrease in cortical perfusion was not associated with a decrease in tissue NO (no change was seen) (17). In conclusion, the demonstration of a decrease in medullary (and cortical) tissue NO speaks against the proposal that NO is the agent buffering the Ang II dependent vasoconstriction in the renal medulla. The reason for the discrepancy between our results and the demonstration by Zou *et al.* (16) of an Ang II induced increase in tissue NO is unclear. It will be noticed that these workers did not measure NO directly: instead, in microdialysates of renal interstitium, NO induced methaemoglobin formation from oxyhaemoglobin (infused *via* microdialysis tube) was assayed. A

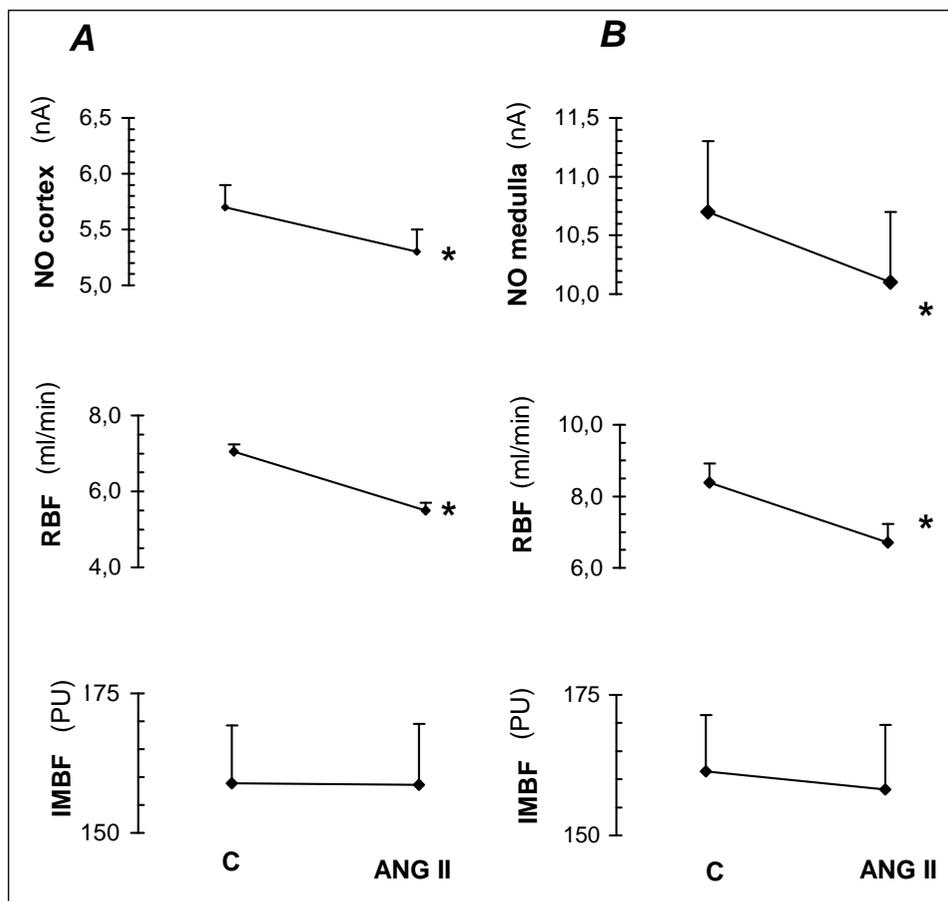


Fig. 3. Responses of the nitric oxide (NO) signal, RBF and inner medullary blood flow (IMBF) to Ang II infusion ( $30 \text{ ng kg}^{-1} \text{ min}^{-1}$ ) in two groups of rats. In A the signal was recorded from kidney surface (NO cortex) and in B from the inner medulla (NO medulla). PU - perfusion units. Means  $\pm$  S.E.M., \* significantly different from control at  $P < 0.002$ . Reproduced from ref. (17).

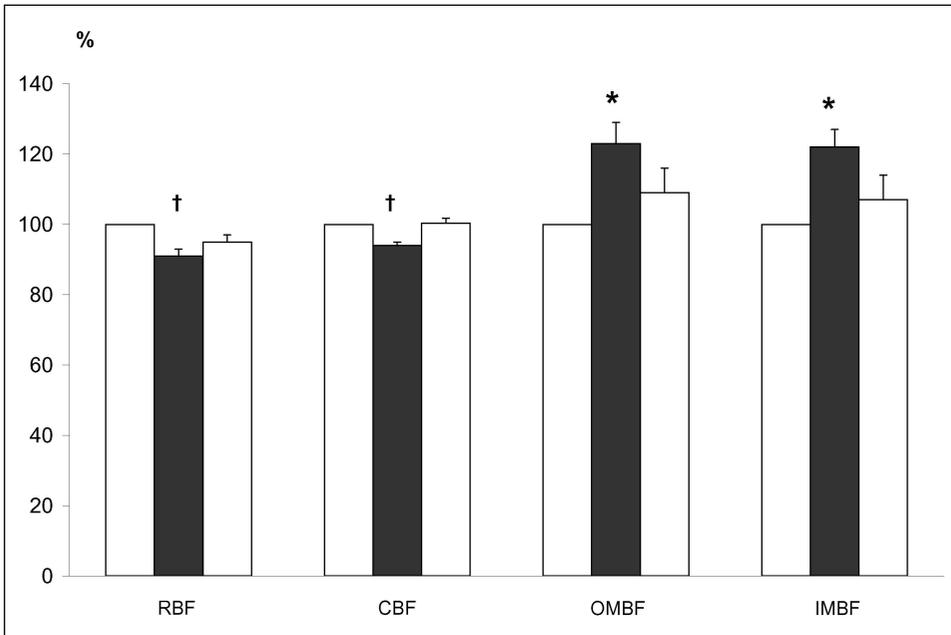
comparison of the results of oxyhaemoglobin-trapping methodology and of direct determination of tissue NO by the electrochemical method (selective electrodes) has never been made *in vivo*. Moreover, unlike in the present study, the dose of Ang II used was subpressor.

Despite strong evidence against the role of NO in buffering the potential vasoconstrictor action of Ang II in the renal medulla, this issue should not perhaps be regarded as finally resolved. In a recent study with mice, Ang II was found to increase medullary blood flow, in agreement with our study in the rat (10). However, no increase was seen in the strain deficient in the gene for neuronal NO synthase (nNOS  $-/-$ ) or in animals pre-treated with L-NAME (18).

This indicates that in the mouse Ang II stimulates medullary nNOS and the generated NO does protect the medulla against hypoperfusion. However, the discrepancy between these results and ours may simply reflect a species difference.

Our results indicate that, at least in the anaesthetised rat, the protection of the renal medulla against Ang II induced vasoconstriction is provided by vasodilator prostaglandins (14). However, this conclusion was so far based on the data derived exclusively from experiments with blockade of the synthesis of prostaglandins whereas their vasodilator *in vivo* potency in the medulla has never been examined directly. It will be noticed that PG induced renal hyperperfusion, as established in early studies and occasionally reported to include also the medulla (4), may have reflected dilatation of arterioles of the juxtamedullary glomeruli i.e. the effect on the cortex. In a recent study we examined the effect of PGE<sub>2</sub>, the chief vasodilator prostaglandin in the rat kidney, when infused directly into the renal medullary interstitium (19). Using this approach a selective vasomotor influence within the medulla is obtained, with little or no effect on cortical circulation.

The medullary interstitial infusion of PGE<sub>2</sub> in animals pretreated with indomethacin induced significant increases in outer- and inner medullary blood



*Fig. 4.* Effect of prostaglandin E<sub>2</sub> infusion into the medullary interstitium ( $15 \mu\text{g kg}^{-1} \text{h}^{-1}$ ) on the parameters of renal circulation. Denotations as in *Figs 1-2*. Data represent percent of the baseline control (100%), means  $\pm$  S.E.M. Empty columns – control and recovery, black columns – PGE<sub>2</sub> infusion. \* significantly higher than control; † significantly lower than control ( $P < 0.05$ )

flow but did not increase perfusion of the cortex (*Fig. 4*). This finding indicates that PGE<sub>2</sub> is a potent vasodilator of the renal medulla and strengthens its postulated role of an agent which could offset Ang II dependent vasoconstriction in this zone. Associated with the PGE<sub>2</sub> dependent medullary hyperperfusion was a modest yet significant decrease in total renal and cortical blood flow (*Fig. 4*), suggesting vasoconstriction in the cortex. It is unclear how increased PG activity in the medulla and/or the consequent local vasodilatation could trigger cortical vasoconstriction. Considering the role of intrarenal circulation in body fluid and cardiovascular homeostasis, this puzzling finding deserves an appropriately focused study.

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