Effects of Prostaglandin Inhibition on Intrarenal Hemodynamics in Acutely Saline-Loaded Rats

Rainer Düsing, Bernward Melder, and Herbert J. Kramer

SUMMARY We studied the effect of inhibition of the prostaglandin (PG)-synthesizing enzyme system in female Sprague-Dawley rats following acute expansion of the extracellular fluid volume (ECV). In 57 conscious rats expansion of the ECV with isotonic saline corresponding to an increase in body weight of 10% was induced. Prior to ECV expansion 31 rats received indomethacin (10 mg/kg of body wt) by stomach tube. In six non-ECV-expanded rats indomethacin had no effect on glomerular filtration rate (GFR) and renal plasma flow (RPF). In ECV-expanded rats pretreated with indomethacin, GFR was unaltered but 125I-hippuran clearance decreased, and filtration fraction significantly increased. Intrarenal \( ^{86} \)Rb distribution was similar in control and ECV-expanded rats. Indomethacin caused a slight increase in relative cortical \( ^{80} \)RB activity in non-ECV-expanded rats, but had no effect on intrarenal \( ^{86} \)Rb distribution in ECV-expanded rats. No difference in intracortical glomerular perfusion was noted between control and ECV-expanded rats. Indomethacin-treated ECV-expanded rats an increase in relative inner cortical perfusion was observed. Absolute perfusion remained unaltered. Thus the decrease in total RPF was entirely due to decreased perfusion of outer cortical nephrons. Renal prostaglandins therefore may play a permissive role for physical factors to promote renal sodium excretion in acute ECV expansion via changes in intrarenal hemodynamics.

THE ADAPTATION of renal tubular function with its resulting natriuresis following intravenous infusion of isotonic saline may be independent of changes in glomerular filtration rate (GFR) or aldosterone activity. \(^1\) Therefore other determinants of tubular reabsorption of sodium, such as a natriuretic hormone, have been postulated.\(^2-4\) In addition, the important role of peritubular physical factors, i.e., onotic and hydrostatic pressure, has been recognized in acute expansion of the extracellular fluid volume (ECV) with isotonic saline.\(^9,10\) Previous studies concerning the role of changes in intrarenal and intracortical distribution of blood flow in the renal response to acute saline loading have so far led to conflicting results.\(^11-14\) If such alterations in renal hemodynamics do play a role they may be mediated by intrarenal hormonal action.

Thus, infusions of either prostaglandin (PG) A or E have been shown to result in intrarenal hemodynamic changes accompanied by a marked increase in urinary sodium and water excretion in animals and in man (for review see Anderson et al.\(^15\) and Lee et al.\(^16\)). It therefore

References
has been proposed that these substances might act as intrarenal or systemic natriuretic agents. Infusions of PGs, however, might not mimic their physiological action, because renal PGs are mainly synthetized within the inner medullary tissue and high PG metabolizing enzyme activity is present in the renal cortex (for review see Lee et al.24). Furthermore, it has been shown that intrarenal PGs in the rat25 and peripheral venous PGA concentrations in normal humans18,19 decrease during high salt intake. This has been interpreted as evidence against the hypothesis that PGs are involved in the natriuresis of chronic salt load.

In contrast to the infusion of PGs, the experimental model of inhibiting PG synthetase with nonsteroidal anti-inflammatory drugs such as indomethacin or the use of substances which compete with arachidonic acid for PG synthetase activity, such as RO-20 5720,26-31 appear more appropriate to elucidate the biological function of renal PG. Previous results from this laboratory have demonstrated that inhibition of PG synthesis with indomethacin has no effect on renal function in rats on a normal salt intake but significantly reduces urinary flow rate and sodium and potassium excretion in rats which are acutely expanded with isotonic saline.25 The present results demonstrate that this retention of sodium and water occurs in the absence of changes in GFR but is associated with a significant decrease in total renal plasma flow (RPF). To further elucidate the mechanism determining renal sodium excretion after inhibition of PG synthesis in the present study the effects of indomethacin on total RPF and its intrarenal distribution as well as on GFR and glomerular perfusion of superficial and deep cortical nephrons were studied in control rats and in rats undergoing acute expansion of the ECV with isotonic saline.

Methods
Female Sprague-Dawley rats weighing 250-320 g were fed a normal rat chow diet (0.25% Na; 0.6% K) (Altromin) with free access to water. The rats were anesthetized with methohexitol-Na (12 mg/kg), and polyethylene catheters were inserted into the jugular vein, the aorta via the carotid artery, and transabdominally into the bladder. Two hours after recovery from anesthesia and surgery the rats were placed in restraining cages mounted on a triple-beam balance. In 57 conscious rats infusion of isotonic saline was started at a rate of 0.5 ml/min until an increase of body weight of 10% was achieved.23 At this time a sustaining infusion to maintain body weight constant. After an equilibration period of 45 minutes urine was collected during three 15-minute periods. The rats were then killed by iv injection of saturated KCl. Prior to ECV expansion 31 rats received indomethacin (10 mg/kg) by stomach tube. This dosage also was administered to non-ECV-expanded rats. Total RPF was estimated as 131I-hippuran clearance in six control rats before and after administration of indomethacin and in 21 expanded rats of which 11 were given indomethacin prior to ECV expansion. Intrarenal blood flow distribution to cortex, medulla and papilla was estimated using the method of Saphirstein in 10 ECV-expanded and in 10 non-ECV-expanded rats. In each group five rats were pretreated with indomethacin. With this method tissue 86Rb activity as percent of total injected activity represents a rough measure of the percentage of cardiac output perfusing this tissue. Changes in the ratio of relative activities between different sections of the kidney then may be considered as a crude estimate of changes in relative distribution of blood flow within the kidney.

GFR was determined as 51Cr-EDTA clearance in six non-ECV-expanded rats before and after administration of indomethacin and in 16 ECV-expanded rats of which 10 were pretreated with indomethacin.

In another 10 ECV-expanded and 10 nonexpanded rats we pretreated five rats in each group with indomethacin. Glomerular perfusion to the outer and inner renal cortex was studied using 131I-macroalbumin aggregates (131I-MAA). This method was previously described by Flohr and Hoppe and is based on the same experimental principle as the recently described use of microspheres to determine single glomerular perfusion.26 For determination of intrarenal 86Rb distribution and of intracortical glomerular perfusion, kidneys were removed 2 minutes after intravenous injection of 200 µCi of 86Rb and after intraaortal injection of 131I-MAA (500 µCi/kg of body wt), respectively. They were immediately frozen and tissue slices were prepared with a heavy sledge microtome. For autoradiography of intracortical 131I-MAA distribution tissue slices 0.5 mm thick were placed on Agfa Gevaert Graphic film 053 with an exposure time of approximately 48 hours. A representative example of intracortical 131I-MAA distribution in kidneys from expanded rats is shown in Figure 1. For quantitative evaluation 1.0-mm slices of renal cortex were dissected into an inner and outer half and 131I-MAA activities were determined and expressed per milligram of tissue wet weight. Relative inner and outer cortical tissue activity then was calculated as percent of activity in the total cortex.

Mean absolute inner and outer cortical blood flow was estimated from the mean intracortical 131I-MAA distribution and total 131I-hippuran clearance per gram of cortical tissue in individual rats. 86Rb activity in cortex, medulla, and papilla also was measured by direct tissue counting and expressed per milligram of tissue wet weight. Cortical, medullary, and papillary tissue activities then were calculated as percent of total activity in the three tissues. Activities of 51Cr-EDTA and 131I-hippuran in plasma and urine as well as 131I-MAA and 86Rb activities in renal tissue were measured in an automatic gamma counting system (Nuclear-Chicago).

Statistical analysis of results was performed using a double-tail Student's t-test. Data are presented as mean ± SE.

Results

RFF AND GFR IN CONTROL AND ECV-EXPANDED RATS

Total RPF in non-ECV-expanded rats was not altered by inhibition of PG synthesis. Following acute ECV expansion RPF, estimated as 131I-hippuran clearance, de-
Intracortical distribution of $^{131}$I-labeled macroalbumin aggregates ($^{131}$I-MAA).

creased significantly when rats were pretreated with indomethacin (Table 1). GFR in nonexpanded rats was unaffected by pretreatment with indomethacin and rose significantly during ECV-expansion to the same degree in animals that had and had not been pretreated with indomethacin (Table 1).

RELATIVE INTRARENAL DISTRIBUTION OF $^{86}$Rb IN CONTROL AND ECV-EXPANDED RATS

In nonexpanded rats indomethacin caused a slight but significant increase in relative $^{86}$Rb activity in cortical tissue with a concomitant decrease in medullary activity, while relative papillary activity remained unchanged (Table 1).

INTRACORTICAL GLOMERULAR PERFUSION IN CONTROL AND ECV-EXPANDED RATS

In nonexpanded control rats indomethacin had no effect on intracortical glomerular perfusion. The distribution of $^{131}$I-MAA was unaltered during acute expansion of the ECV with isotonic saline (Table 1). However, when ECV-expanded rats were pretreated with indomethacin a significant decrease in relative outer cortical perfusion and an increase in relative deep cortical perfusion was noted (Table 1). When intracortical perfusion was calculated from $^{131}$I-hippuran clearances obtained in ECV-expanded rats with and without pretreatment with indomethacin, absolute inner cortical perfusion was unaffected by PG inhibition. Thus the decrease in total RPF was entirely due to the decrease in absolute outer cortical perfusion (Fig. 2).

Discussion

Previous results from this laboratory have demonstrated that acute expansion of the ECV in the rat by intravenous infusion of isotonic saline equal to a 10% increase in body weight causes a significant increase in fractional excretion of sodium up to 20%. 22>23 In addition, we have shown that inhibition of the PG synthesizing enzyme system with indomethacin reduces this fractional excretion by more than 40%. - Although previous studies in vitro suggested that PGA₂ but not PGE₂ may reduce active tubular reabsorption of sodium via inhibition of Na-K-ATPase,27 our data did not demonstrate any change in cortical, medullary, and papillary Na-K-ATPase activity in ECV-expanded rats following inhibition of PG synthesis.28 In the present study we therefore further investigated the possi-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Non-ECV-expanded</th>
<th>ECV-expanded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without INDO</td>
<td>With INDO</td>
</tr>
<tr>
<td>RPF (ml/min/kg body wt)</td>
<td>27.8 ± 2.4 (n = 6)</td>
<td>26.0 ± 2.2 (n = 6)</td>
</tr>
<tr>
<td>GFR (ml/min/kg body wt)</td>
<td>6.1 ± 0.4 (n = 6)</td>
<td>5.2 ± 0.4 (n = 6)</td>
</tr>
<tr>
<td>$^{86}$Rb distribution (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>53.5 ± 0.7 (n = 5)</td>
<td>60.7 ± 2.4 (n = 5)</td>
</tr>
<tr>
<td>Medulla</td>
<td>28.5 ± 0.7 (n = 5)</td>
<td>23.3 ± 1.4 (n = 5)</td>
</tr>
<tr>
<td>Papilla</td>
<td>18.0 ± 0.3 (n = 5)</td>
<td>16.0 ± 1.5 (n = 5)</td>
</tr>
<tr>
<td>$^{131}$I-MAA distribution (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td>77.2 ± 1.1 (n = 5)</td>
<td>73.4 ± 1.8 (n = 5)</td>
</tr>
<tr>
<td>Inner cortex</td>
<td>22.8 ± 1.1 (n = 5)</td>
<td>26.6 ± 1.8 (n = 5)</td>
</tr>
</tbody>
</table>

* Expanded vs. nonexpanded rats: P < 0.01.
bility that changes in intrarenal hemodynamics due to endogenous PG release may at least in part be responsible for the natriuresis in acutely ECV-expanded rats. This was indirectly assessed by the use of indomethacin, a potent inhibitor of the PG synthesizing enzyme system. In the present study indomethacin had no effect on total GFR in nonexpanded rats. In addition, GFR, which significantly increased during ECV-expansion, was unaffected by inhibition of PG biosynthesis. RPF, estimated as $125^1$I-hippuran clearance, was unaffected by indomethacin in nonexpanded rats. In expanded rats, however, RPF, which only slightly increased with acute expansion of the ECV, was significantly reduced by this drug. Such reduction in total RPF following inhibition of PG synthesis was described by several groups of investigators in anesthetized animals that were not acutely salt loaded, whereas in the nonanesthetized dog no change in RPF was detected following administration of indomethacin (INDO).

Thus, the observation in the present study, that inhibition of PG synthesis has no effect on RPF in non-ECV-expanded conscious rats but decreases RPF during acute salt loading may point to a permissive role of PG in the adjustment of renal hemodynamics during acute saline administration. In nonexpanded rats the mean ratio of ECV-expanded rats decreased outer cortical blood flow accompanied by a significant relative increase in juxtamedullary glomerular perfusion was observed after pretreatment with indomethacin.

However, the calculated absolute deep cortical perfusion remained constant. Since intrarenal $86^Rb$ distribution following indomethacin was unchanged in ECV-expanded rats, medullary perfusion must have decreased in parallel with the decrease in total RPF. The discrepancy between unchanged absolute perfusion of deep cortical nephrons and the decrease in medullary plasma flow therefore may be explained if increased total kidney filtration fraction following inhibition of PG synthesis in ECV-expanded rats would essentially reflect an increase in filtration fraction of juxtamedullary glomeruli. A tendency of indomethacin to increase the filtration fraction of juxtamedullary nephrons, although to a minor degree, can also be derived from the data obtained in non-ECV-expanded rats.

Since PGs seem to be involved in the natriuresis of acute saline loading the effect of indomethacin observed in ECV-expanded rats may be interpreted as analogous to the observation that saline infusion in the dog is associated with a fall in filtration fraction in deep cortical nephrons. In conclusion, our data support the concept that a major role of renal PGs may be to act as local vasodilators of efferent juxtamedullary arterioles. In addition, the reduction in outer cortical perfusion following inhibition of PG synthesis during acute saline loading suggests a role of PG in regulating outer cortical resistance possibly via modulating pressor stimuli.

Thus, PGs may at least in part play a permissive role for physical factors to promote renal sodium excretion via changes in intrarenal hemodynamics. However, since no significant changes in intrarenal and intracortical blood flow distribution were observed with acute ECV expansion only, it may be that the hemodynamic changes following administration of indomethacin are to some degree a pharmacological rather than a physiological effect of the inhibition of PG biosynthesis.
Acknowledgments

We gratefully acknowledge the technical advice of A. Hoppe, M. D., and the technical assistance of A. Bläcker and S. Hinzen.

References

Effects of prostaglandin inhibition on intrarenal hemodynamics in acutely saline-loaded rats.
R Düsing, B Melder and H J Kramer

doi: 10.1161/01.RES.41.3.287

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/41/3/287