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Influence of Captopril on Red Cell Velocity in the Vasa Recta of the Renal Medulla

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ABSTRACT

Previous studies from our laboratory have indicated an important role for angiotensin II (AII) in the regulation of renal medullary haemodynamics during normal physiological conditions. In order to investigate further the influence of endogenous AII on the juxtamedullary vascular resistance in anaesthetized rats the velocity of fluorescently-labeled red cells (v_{RBC}) was measured with a cross-correlation technique in the vasa recta before and after infusion of the angiotensin I- converting enzyme inhibitor captopril or vehicle.

Irrespective of treatment, v_{RBC} was higher in the descending vasa recta (DVR) than in the ascending vasa recta (AVR). In time control animals v_{RBC} in DVR and AVR and mean arterial blood pressure (MAP) remained stable over the 45 min study period. In animals receiving captopril (3 $mg \cdot h^{-1} \cdot kg^{-1}$ bw) v_{RBC} increased almost proportionally in DVR and AVR; by 26 % in DVR (from 1.02 ± 0.12 to 1.28 ± 0.10 $mm \cdot s^{-1}$, $p<0.05$) and by 19 % in AVR (from 0.46 ± 0.05 to 0.55 ± 0.07 $mm \cdot s^{-1}$, $p<0.05$). MAP decreased by 9 % (from 107 ± 3 to 97 ± 2 mm Hg, $p<0.05$).

These results give further support to the suggestion of an involvement of AII in the regulation of juxtamedullary vascular resistance during normal physiological conditions.

INTRODUCTION

Angiotensin II (AII) is an important mediator of volume regulation (18) and is generated intrarenally in response to changes in nephron function. All the components necessary for the intrarenal generation of AII exist in the kidneys (2, 21, 27). Previous investigations from our laboratory have demonstrated an important role for AII in the regulation of

juxtamedullary vascular resistance during normal physiological conditions. Inhibition of the angiotensin I-converting enzyme results in elevation of the juxtamedullary single nephron glomerular filtration rate (7), medullary plasma flow (30), intratubular free flow pressure in deep nephrons (8) and of the vasa recta erythrocyte flux (11). Furthermore, the effects of inhibition of the converting enzyme are much smaller on the superficial vasculature (8, 26, 30), indicating a heterogeneity in the influence of AII.

The present investigation was performed to study further the influence of endogenous AII on juxtamedullary haemodynamics. In order to measure the velocity of red cells in the vasa recta of the exposed papilla fluorescently labeled erythrocytes were utilized in combination with a cross-correlation technique.

MATERIALS AND METHODS

The study was performed on 19 young Munich-Wistar rats of both sexes weighing 132 ± 4 g (mean ± 1 SEM). Up to the time of the experiment the animals had free access to tap water and a standardized pellet chow (R3, Ewos, Södertälje, Sweden) containing 0.1 % sodium, 0.7 % potassium and 21 % protein. The animals were anaesthetized with an intraperitoneal injection of Inactin (Byk-Gulden, Constance, Germany), 100 $mg \cdot kg^{-1}$ body weight, and were then placed on a servo-controlled heating pad to maintain the rectal temperature at 37.5 °C.

Surgical procedure.

The animals were tracheostomized and the left femoral artery was cannulated for continuous recording of the mean arterial blood pressure (MAP). The left and right femoral veins were cannulated for injection of labeled erythrocytes and for continuous infusion of a Ringer solution (5.0 $ml \cdot h^{-1} \cdot kg^{-1}$ body weight), respectively.

The left kidney was exposed by a flank incision, dissected free from surrounding tissue and placed in a plastic cup. The kidney with its dorsal surface facing upwards was embedded in small pieces of cotton wool and was covered with a piece of cotton cloth soaked in mineral oil to reduce evaporation. The papilla of the kidney was exposed as described previously (5).

Cell labeling.

The red cell labeling procedure is similar to the approach of Matuhasi et al. (19) and has been described in detail previously (11). Briefly, blood obtained from young donor rats

(litter mates) was washed and labeled with fluorescein-iso-thio cyanate (FITC, 3 $mg \cdot ml^{-1}$, 2 hours incubation in room temperature). After subsequent washing the final haematocrit was adjusted to approximately 60 % with isotonic saline. If the cells were not to be used immediately, they were suspended in excess of a glucose-saline buffer solution and stored in darkness at +4 °C. The stored cells can be used for at least 2-3 days without any significant reduction of fluorescence. In a previous investigation (11) we found that the number of fluorescent cells in systemic blood did not decline up to 150 min after their injection, indicating good viability. We have also demonstrated that when using the same rationale as in the present study, injection of 50 µl of labeled cells (haematocrit about 60 %) results in a systemic labeled to native cell quotient of about 1 % (11,12).

Microscopy.

For intravital microscopy of the vasa recta in the papilla, a Ploemopak system (Leitz Orthoplan, Wetzlar, Germany) with a 32x objective (NA 0.4) was used. The microscope image was transferred via a highly sensitive video camera (TYC 9A, Bosch, Germany) to a monitor (Panasonic WV-5360/G, Matsushita Industry, Japan) and recorded on tape (Panasonic AG 6200). The magnification from tissue to monitor was about 900x. A time reference was stored on each frame (VTG-33, FOR.A, Japan).

The excitation of the FITC-labeled red cells was achieved by epi-illumination of the tissue with a 50-W mercury lamp and a filter system (Leitz filter block I2: exciting filter BP 450-490, suppression filter LP 515, beam splitting mirror RKP 510).

Choice of vessels.

Vasa recta capillaries offering maximal contrast to the papillary background and displaying steady flow were selected for velocity determination. All the examined vessels were located on the surface of the lower half of the extrarenal papilla. Measurements were made on two to five vessels per animal. This subjective choice of vessels implies that emphasis should be directed towards the relative change in velocity rather than on the absolute velocity or absolute change in velocity.

Experimental protocol.

Thirty minutes after the surgical procedure, 50 µl of FITC-labeled red cells (haematocrit about 60 %) were slowly injected into the left femoral vein. After allowing 30 min for stabilization the control velocities in two to six vessels were recorded on videotape. When the control measurements were complete the continuous Ringer infusion was either

allowed to go on (time control) or changed for a Ringer solution containing captopril (3 $mg \cdot kg^{-1} \cdot h^{-1}$) after a priming dose of 3 $mg \cdot kg^{-1}$. After 45 min of infusion the red cell velocities in the same vessels as under control conditions were recorded. The recording time was 60-75 seconds each for the control and experimental periods.

Group 1 (10 rats) served as a time control group and received continuous Ringer infusion during the whole experiment. The animals of group 2 (9 rats) were given captopril (SQ 14,225, Squibb, Princeton, NJ) dissolved in the Ringer solution after the control period; first a priming dose of 3 $mg \cdot kg^{-1}$ in 0.1 ml and then a continuous infusion at a rate of 3 $mg \cdot kg^{-1} \cdot h^{-1}$. The dose of captopril utilized in the present study has previously been shown to block completely the hypertensive effect of up to 150 ng of angiotensin I (8).

A total of 32 and 29 vessels were examined in the control and captopril-treated group, respectively.

Velocity measurement.

The velocity of the FITC-labeled red cells in the vasa recta was measured off-line from the video recording using a dual-window cross-correlation technique based on the method described by Wayland & Johnson (31). A video photometric analyzer was used to align two electronic windows over the vessel to be analysed. The size of the windows were identical and the width was selected to exceed slightly the diameter of the red cell column of the vessel. The separation of the windows was varied between 10 and 20 µm. The signals from the windows were automatically cross-correlated in the frequency domain, and the velocity was determined from the time delay between the signals (CapiFlowR, CapiFlow AB, Kista, Sweden). The fluorescent cells produced discrete signal pairs providing high correlation coefficients which were only accepted if they were higher than 0.7.

The length of each sampling period was 45-55 seconds. In each vessel the sampling was repeated twice and the average was used to obtain one value per vessel for each experimental condition.

Statistics.

Only those vessels which allowed both control and experimental measurements were included in the study. Differences in velocities between control and experimental conditions were tested for significance by paired Student's *t* test. A p value of less than 0.05 (two-tailed test) was accepted as significant. All values are expressed as means ±

1 SEM.

RESULTS

In Table 1 v_{RBC} and MAP are given in both time control rats and in rats receiving captopril. In Fig. 1 the percentage changes in v_{RBC} (Δv_{RBC}) in DVR and AVR are plotted before and after 45 min infusion of captopril or vehicle.

During the control period v_{RBC} in DVR and AVR and MAP was not different between the groups. Irrespective of the treatment, v_{RBC} was higher in DVR than in AVR.

In the animals of the time control group body weight (BW) and kidney weight (KW) was 129 ± 5 g and 0.69 ± 0.06 g, respectively, and MAP and v_{RBC} remained stable over the 45 min study period.

In animals receiving captopril the BW and KW were 134 ± 3 g and 0.60 ± 0.03 g, respectively. v_{RBC} increased in both DVR and AVR by 26 % and 19 %, respectively ($p<0.05$). MAP decreased by 9 % from 107 ± 3 to 97 ± 2 mm Hg ($p<0.05$).

Table 1. Vasa recta red cell velocity and mean arterial blood pressure in time control rats and in rats receiving captopril during 45 min.

	v_{RBC} (mm·s^{-1}) DVR contr.	AVR contr.	v_{RBC} (mm·s^{-1}) DVR exp.	AVR exp.	MAP (mm Hg) contr.	exp.
TC	0.98±0.10†	0.49±0.07	1.04±0.11†	0.51±0.06	105 ± 2	103 ± 2
CA	1.02±0.12†	0.46±0.05	1.28±0.10*†	0.55±0.07*	107 ± 3	97 ± 2*

Abbreviations: TC, time control rats; CA, captopril treated rats; v_{RBC}, red cell velocity; MAP, mean arterial blood pressure; AVR and DVR, ascending and descending vasa recta, respectively; contr., values before vehicle or captopril; exp., values 45 min after vehicle or captopril;* $p<0.05$ vs control conditions;† $p<0.05$ vs AVR;

DISCUSSION

The present study has demonstrated that attenuation of angiotensin II-generation, by use of the angiotensin I-converting enzyme inhibitor (CEI) captopril, increases the red cell velocity in the vasa recta of the inner medulla in conjunction with a decrease in the arterial blood pressure. These results are consistent with the suggestion of an involvement of angiotensin II in the regulation of juxtamedullary vascular resistance during normal physiological conditions.

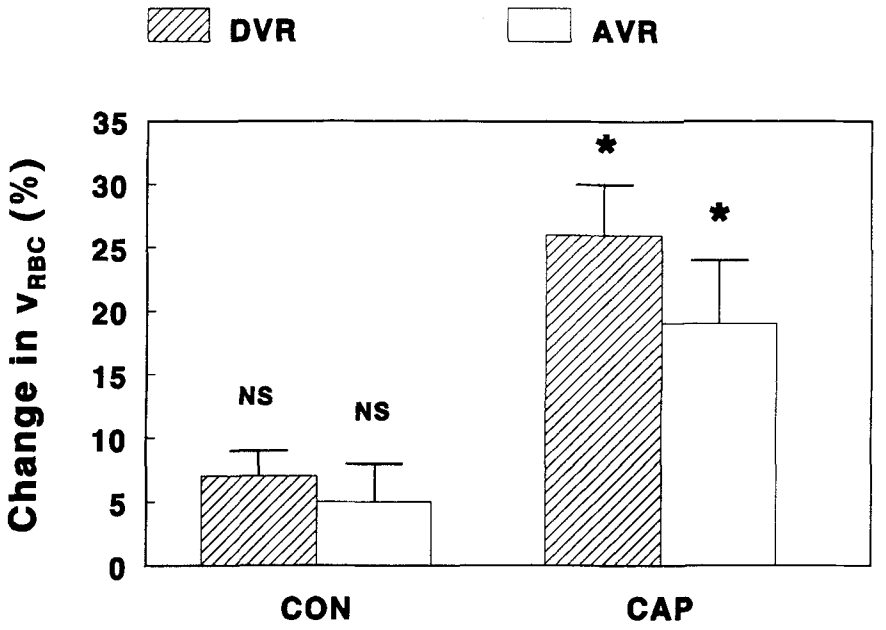

Fig. 1. Percentage change in red cell velocity (Δv_{RBC}) before and after captopril (CAP) or vehicle (CON) in descending (DVR, hatched bars) and ascending (AVR, open bars) vasa recta. * denotes statistically significant change ($p<0.05$), NS = non statistically significant change.

In previous investigations from our laboratory we have demonstrated that AII exerts a tonic vasoconstrictor influence on medullary haemodynamics during normal physiological conditions. Interference with AII-generation increases the juxtamedullary single nephron glomerular filtration rate (7), medullary plasma flow (30), intratubular free flow pressure in deep nephrons (8) and vasa recta erythrocyte flux (11). The results of the present investigation would thus further strengthen the suggestion of an important involvement of AII in the regulation of renal medullary haemodynamics.

The type of vessel in which changes in flow resistance occur cannot be determined from the results of the present study. However, since our laboratory in previous publications (7, 8, 11, 30) has demonstrated simultaneous increments in glomerular filtration rate, intratubular pressure and medullary plasma flow after CEI-infusion it seems likely that both afferent and efferent arteriolar resistance decreases. It is, furthermore, possible that the vasa recta themselves are targets for AII. Mendelsohn et al. (22) demonstrated AII receptors in the vasa recta bundles and Fried & Simpson (6) observed prominent expression of angiotensinogen mRNA in the outer medulla of the rat. It is thus apparent that within the medulla a whole array of components are present

locally (9, 29) for the renin-angiotensin system to execute on-site regulation of blood flow.

The v_{RBC}-values obtained in the present study for DVR and AVR are both quantitatively and qualitatively in good agreement with those previously published (4, 13, 15, 32, 33). Even if the v_{RBC} is higher in DVR than that in AVR the AVR outnumber the DVR by a factor of about four (13) resulting in a higher total outflow through the AVR. The difference in total blood flow between AVR and DVR is an estimate of the net uptake of water into the ascending vessels. In the present study after captopril treatment v_{RBC} in DVR and AVR increased by 26 and 19 %, respectively, which is in good agreement with the results of a study by Cupples et al. (4). In the latter study v_{RBC} in DVR increased by 23 % whereas no measurements were reported on AVR. Previous studies using captopril have, furthermore, demonstrated a 30 % increase in papillary plasma flow (3) and a 40 % increase in vasa recta red cell flux (11) which are well in line with the results of the present study.

CEI results in smaller effects on the superficial circulation in contrast to that on the juxtamedullary circulation (8, 26, 30). This heterogeneity in response can partly be explained by the prevailing pressure drop along the interlobular artery (14) which creates different haemodynamic conditions for the superficial and juxtamedullary vasculature. The afferent arterioles of superficial glomeruli are almost completely dilated while those of the juxtamedullary glomeruli are significantly constricted in normohydrated, normotensive rats. The higher tonic influence of AII on the juxtamedullary vessels may be necessary if the deep nephron population is to maintain glomerular capillary pressure at a level similar to that in the superficial nephrons (1). Since the vasa recta originate from the efferent arterioles of juxtamedullary nephrons changes in vasa recta flow parameters will reflect changes in juxtamedullary nephron haemodynamics.

Red cell velocity constitutes one variable in the measurement of blood flow and the other, vessel diameter, was not estimated in the present study due to technical difficulties. Lemley et al. (17) demonstrated that the vasa recta are elliptical in shape and that AVR and DVR differ in their cross-sectional shape. Thus, the measurements of diameters to yield the cross-sectional area in the calculation of blood flow from linear velocity and vessel diameter will introduce an error which can result in an overestimate of blood flow by about 25 % (17). Provided that diameters are unchanged before and after captopril treatment in the present study changes in v_{RBC} will directly reflect changes in vasa recta blood flow. In a study by Cupples et al. (4) it was demonstrated that DVR diameters were not altered by captopril treatment inferring a comparable change between v_{RBC} and blood flow in the present study.

A prerequisite for measurements made with fluorescently labeled red cells is that the cells must have normal rheological properties. From the findings in the present and other studies (11, 19, 32, 28) considered together, it seems likely that the labeled red cells resemble native cells in their flow properties: they have the same linear velocity, microscopically they have normal biconcave shape, and they have long survival time in the circulation.

CEI blocks the formation of intrarenal and circulating angiotensin II. However, CEI also decreases the degradation of circulating and renal tissue kinins through its attenuating action on kinase II. Some investigators have suggested that kinin accumulation may contribute to the vasodilator effect of CEI (20), whereas others have reported that administration of an angiotensin II antagonist results in vasodepressor effects of the same degree as those produced by CEI (10, 16). Furthermore, Ploth et al. (25), Ploth & Roy (24) and Mimran et al. (23) have demonstrated that the angiotensin II antagonist saralasin has effects on single nephron function and whole kidney function similar to that of CEI. Finally, Cupples & Sonnenberg (3) have demonstrated reversal of the CEI effects on medullary plasma flow by infusing AII and Cupples et al. (4) showed that saralasin and captopril resulted in a similar increase in vasa recta flow. The above discussion supports the view that the observed responses in the present study are predominantly due to the reduced levels of AII.

In conclusion, the angiotensin I-converting enzyme inhibitor captopril increases the red cell velocity in the vasa recta in conjunction with a depression of arterial blood pressure. The results confirm the suggestion of an involvement of angiotensin II in the regulation of juxtamedullary vascular resistance during normal physiological conditions.

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