Cortical and Medullary Hemodynamics in Deoxycorticosterone Acetate-Salt Hypertensive Mice

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Abstract. The effect of acutely increasing renal perfusion pressure or extracellular fluid volume on renal medullary and cortical blood flow was examined in the low-renin deoxycorticosterone acetate (DOCA)-salt hypertension model in mice. A 50-mg DOCA tablet was implanted, and 1% saline was given as drinking water for 3 wk. Medullary and cortical blood flow were determined with laser-Doppler flowmetry, and whole-kidney blood flow was measured with a transit-time ultrasound flowprobe around the renal artery. In control mice, total renal blood flow ranged from 6.3 and 7.6 ml/min per g kidney weight and in DOCA-salt mice from 4.3 and 4.7 ml/min per g kidney weight, respectively, and was minimally affected as renal perfusion pressure was increased. Renal vascular resistance increased correspondingly. During stepwise increases in renal artery pressure from 90 to 140 mmHg, medullary blood flow progressively increased in control mice to 125% of baseline values, whereas cortical blood flow did not change. In DOCA-salt mice, increasing BP from 100 to 154 mmHg had no effect on either cortical or medullary blood flow. Urine flow and sodium excretion were lower in DOCA-salt mice than in controls and increased nearly to the same extent in both groups after volume expansion with isotonic saline. Total renal blood flow increased after saline loading, more in controls than in DOCA-salt mice. Increases in medullary blood flow after saline loading were up to 122% of baseline values in controls and demonstrated a significantly steeper slope than the 110% of baseline increases in DOCA-salt mice. Cortical blood flow, however, was not different between the groups. Thus, medullary blood flow is not as tightly autoregulated as cortical blood flow in normal mice. Natriuresis with acute volume loading is facilitated by increased medullary blood flow. In DOCA-salt mice, the medullary blood flow reaction to renal perfusion pressure increases is abolished, whereas flow increases with extracellular volume expansion are diminished. These results suggest that diminished pressure-natriuresis responses in DOCA-salt mice are related to perturbed medullary blood flow. (J Am Soc Nephrol 9: 346–354, 1998)

The pressure-natriuresis-diuresis phenomenon is the central mechanism by which the kidney controls long-term BP. The relationship between arterial pressure and salt and water excretion is shifted toward higher pressures in every model of hypertension that has been studied (1–3). Recently, we showed that pressure-diuresis-natriuresis can be measured in mice and that deoxycorticosterone acetate (DOCA)-salt hypertensive mice have a pressure-diuresis-natriuresis relationship that is reduced in slope and shifted rightward (4). Altered tubular sodium reabsorption could be responsible for shifting the pressure-natriuresis relationship, as it was shown for Dahl S rats (5) and possibly for transgenic (mRen2)27 rats (6). In both the Dahl S rat and in transgenic (mRen2)27 rats, the intrarenal renin-angiotensin system has been considered as possibly responsible for enhanced tubular sodium reabsorption (5,7,8). Similarly, the nitric oxide system has been implicated in shifting pressure natriuresis in the Sabra DOCA-salt hypertensive rat (9), as well as in hypertension of Dahl S rats (10). However, changes in renal medullary hemodynamics have also been implicated in resetting pressure-natriuretic response in hypertension (11). The development of laser-Doppler flow probes allows the comparison of qualitative changes in cortical and medullary blood flow simultaneously. In the course of studying the exaggerated natriuresis associated with hypertension, we recently observed that DOCA-salt rats did not increase their medullary blood flow in response to acute volume expansion compared with controls (12). In this study, we examined renal cortical and medullary blood flow in DOCA-salt mice to elucidate possible mechanisms influencing salt excretion in this low-renin model.

Materials and Methods
Experiments were performed on nuclear magnetic resonance imaging mice purchased from Tierzucht Schoenwalde (Schoenwalde, Germany). The mice were allowed free access to standard chow (0.25% sodium, SNIF Spezialitäten, Soest, Germany) and drinking water *ad libitum*. The experimental protocol was approved by the local council on animal care, whose standards correspond to those of the American Physiological Society. Four-week-old mice were anesthetized with a mixture of ketamine/xylazine, and the right kidney was removed. A 50-mg DOCA pellet (Innovative Research of America, Saratoga, FL) was implanted subcutaneously in the abdominal area.

Received October 12, 1997. Accepted November 20, 1997.
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1046-6673/99-03460$03.00/0
Journal of the American Society of Nephrology
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Thereafter, the incision was sutured and the mice were allowed to recover in a warm cage. Afterward, they received 1% NaCl solution as drinking water for 3 wk. Control mice were only uninephrectomized and received tap water to drink. To characterize the effect of DOCA-salt treatment in a subgroup of mice (DOCA-salt, n = 5; controls, n = 5), sodium and water excretion were measured using metabolic cages (UNO Roestvaststaal, Zevenaar, The Netherlands). BP were determined by tail plethysmography using a computerized tail-cuff system (BP-2000, Visitech Systems, Napa, NC), as described by Krege et al. (13). The mice were trained for 7 d; measurements were then recorded daily (two sets of 10 measurements) for 4 to 5 d. In our earlier study (4), uninephrectomy did not induce hypertension in nuclear magnetic resonance imaging mice (119 ± 7 mmHg), whereas DOCA-salt increased BP significantly to 159 ± 4 mmHg. In the present study, we relied on the tail-cuff measurements to make certain that the DOCA pellets and salt loading were indeed inducing hypertension.

Cortical and Medullary Blood Flow

In seven DOCA-salt mice weighing 30 ± 2 g (kidney weight, 0.45 ± 0.05 g) and 12 control mice weighing 36 ± 0.8 g (kidney weight, 0.33 ± 0.01 g), total, cortical, and medullary blood flow were measured when renal perfusion pressure was increased step-by-step, and thereafter a volume load was given. The mice were anesthetized with a mixture of ketamine (50 μg/g intraperitoneally, Parke-Davis, Berlin, Germany) and inactin (100 μg/g intraperitoneally, Research Biochemicals, Natick, MA) and were placed on a heated table for maintenance of body temperature at 37°C. During surgery, the mice were infrequently administered small supplemental doses of inactin, given to maintain stable anesthesia levels. Cannulas (PE 90) were placed into (1) the trachea to facilitate breathing; (2) the carotid artery (PE 10) for measurement of systemic mean arterial pressure (MAP) and renal perfusion pressure (RPP); (3) the jugular vein (PE 50) for infusion of 0.9% NaCl solution (1.5 μl/min per g body wt); and (4) the urinary bladder (PE 50) for urine collection. After midline and flank incisions, a 0.5-mm V-series flowprobe (Transonic System, Ithaca, NY) was placed around the left renal artery to measure renal blood flow (RBF). The flowprobe was kept in place by a micromanipulator and connected to a flowmeter (T206, Transonic System). The Transonic flowmeter used transit-time principles of ultrasound to measure volume flow directly with the perivascular flowprobe. The renal artery was nestled within the deepest angle of the V-reflector and kept by the micromanipulator in the position of the highest sensitivity. Thereafter, all manipulations were done without moving the position of the flowprobe. A lubricating jelly (TC-90952-02, Carter Products, New York, NY) acted as an acoustical coupler and replaced all air space around the probe’s acoustic window. Ligatures were placed loosely around the celiac and mesenteric arteries, as well as around the abdominal aorta below the kidney for later occlusion, so that RPP could be varied. A laser-Doppler flowmeter (model ALF 21D, Transonic System) with implanted fibers was used in each mouse to measure blood flow in the cortex and the medulla. The implanted fibers consisted of 500-μm-diameter fiber-optic strands (Mitsubishi Cable America) and were connected to an external probe specifically designed for such applications. The loss of light at the connection between the implanted optic fibers and the external probe was minimized by introduction of fused silica-matching liquid (no. 50350, Cargille Laboratories, Cedar Grove, NJ) into the connection. The fibers were secured on the surface of the kidney with cyanoacrylate glue. The location of the implanted fibers was confirmed in each experiment by dissecting the kidney and viewing the regions surrounding the fiber tip. If the implanted fibers were incorrectly positioned or if excessive bleeding or tissue damage occurred caused by movement of the fibers during the experiment, the data were discarded.

After surgery and a 45-min equilibration period, MAP, RBF, and cortical and medullary blood flow signals were recorded continuously. RPP was then increased approximately 25 mmHg in both groups to 116 mmHg in controls and 127 mmHg in DOCA-salt mice by ligating the mesenteric and celiac arteries. After this maneuver, RBF and cortical and medullary flow signals were recorded as soon as a steady state was reached. RPP was then increased by an additional 20 to 25 mmHg to approximately 140 mmHg in controls and 154 mmHg in DOCA-salt mice by occluding the aorta below the kidney. After an equilibration period, RBF were again recorded. At the end of these procedures (at the highest pressure levels) and a period of approximately 10 min, urine was collected for two 5-min periods, and all mice were given a bolus of 0.9% NaCl solution (1 ml per mouse over 1 min). Thereafter, urine flow was collected for 2-min periods over 20 min. Total and regional RBF, heart rate, and systemic BP were recorded during this procedure continuously. At the beginning and at the end of the experiment, blood was drawn to measure hematocrits levels. MAP and RBF measurements were recorded on a computer system (Technical & Scientific Equipment, Bad Homburg, Germany). Representative MAP and RBF values were calculated for each period by averaging all recorded values during that time period. Urine flow was determined gravimetrically. Urinary sodium and potassium concentrations were determined by flame photometry (FLM3, Radiometer, Copenhagen, Denmark). Urine flow, sodium excretion, potassium excretion, and RBF were normalized per gram kidney weight (kwt).

RNase Protection Assay and Morphology

To assure ourselves that the DOCA-salt mouse model was indeed a low-renin model, we relied on RNase protection assay to determine the components of the renin-angiotensin system in the kidney. For RNase protection assay, the kidneys were deep-frozen. Total RNA was isolated from the kidneys, and the RNase protection assay was performed with an Ambion RPA III kit (ITC Biotechnology, Austin, TX) according to the manufacturer’s instructions as described previously (9). For the detection of the renin and angiotensin type 1 (AT1) receptor mRNA, the same mouse renin-2d probe and a rat AT1 receptor probe were used as described earlier (8). A 345-bp cDNA fragment containing exons I, III, IV, and V of the mouse angiotensinogen gene was subcloned into the transcription vector pGEM5Zf(+) (Promega, Madison, WI) and linearized with BamHI for antisense RNA transcription, using the SP6 promoter. The angiotensin-convert- ing enzyme (ACE) probe was generated by PCR and inserted into the vector pCR<sup>TM</sup>II (9). The plasmid was linearized with BamHI to obtain a 263-bp-long antisense RNA by in vitro transcription using the T7 promoter.

For conventional morphology, kidneys were removed after the experiment, cut sagittally, and fixed in 4% buffered paraformaldehyde at room temperature. Tissue fixation and staining techniques are detailed elsewhere (6).

Statistical Analyses

Values are expressed as mean ± SEM. Statistically significant differences in mean values were evaluated by ANOVA for repeated-measures ANOVA and the Duncan’s multiple-range test. A P value < 0.05 was considered statistically significant.
Results

The tail-cuff BP values for DOCA-salt mice in the third week of treatment averaged 153 ± 4 mmHg and were in the range we described in our earlier study (4). The renal regions from which blood flows were measured showed undamaged renal tissue beneath the tip of the fiber-optic flow probes, as shown in Figure 1. Thus, we do not believe that implanting the fibers affected whole-kidney or regional blood flow. Figure 2 shows the expression levels of the renin-angiotensin system genes in mouse kidneys before and after DOCA-salt treatment. The figure demonstrates mRNA values for renin, angiotensinogen, ACE, and AT₁ receptor in the kidneys of mice with and without DOCA-salt treatment. Renin mRNA was significantly decreased by DOCA-salt treatment; the values were reduced by half (P < 0.05). Angiotensinogen gene expression was not different in untreated and DOCA-salt-treated mice. ACE mRNA was significantly decreased after DOCA-salt treatment compared with controls (P < 0.05). AT₁ receptor mRNA showed a similar pattern; i.e., DOCA-salt treatment reduced the mRNA expression by half.

Figure 3 shows sodium excretion, measured with metabolic cages, in DOCA-salt and control mice. Sodium excretion was fourfold to 10-fold higher in DOCA-salt mice than in controls. This difference remained stable until the final experiment. In contrast, potassium excretion was not different between both groups (data not shown).

MAP at baseline averaged 91 ± 4 and 101 ± 8 mmHg in anesthetized control and DOCA-salt surgical preparations (P < 0.05). Corresponding to these values, total RBF levels were 7.6 ± 0.5 ml/min per g kwt in controls and 4.7 ± 0.5 ml/min per g kwt in DOCA-salt mice (P < 0.05). The renal vascular

Figure 2. Semiquantitative analysis of renin mRNA (top left), angiotensinogen mRNA (top right), angiotensin-converting enzyme (ACE) mRNA (bottom left), and AT₁ receptor mRNA (bottom right) expression in kidneys of control and deoxycorticosterone acetate (DOCA)-salt hypertensive mice. DOCA-salt decreased renin, ACE, and AT₁ receptor mRNA expression (P < 0.05), whereas angiotensinogen mRNA expression was not affected.

Figure 1. Hemalaun and eosin stains of kidneys in which optic fibers were implanted. The renal tissue beneath the tip of the fiber was not damaged, suggesting that even in these very small kidneys the method did not interfere with the results. Magnification, ×200. * fiber canal.
Figure 3. Sodium excretion during the development of hypertension in DOCA-salt compared with control mice. The effect of the 1% NaCl diet is reflected by the increase in sodium excretion of DOCA-salt mice.

The resistance (RVR) calculated from these values was $12.5 \pm 0.8$ mmHg/ml per min per g kwt in control mice and $22.8 \pm 3.0$ mmHg/ml per min per g kwt in DOCA-salt mice ($P < 0.05$). Both in controls and DOCA-salt mice, cortical laser-Doppler blood flow signals were approximately 3.5 V, whereas medullary laser-Doppler flow signals were much smaller, i.e., $1.22 \pm 0.16$ and $1.45 \pm 0.19$ V in control and DOCA-salt mice, respectively. These differences in cortical and medullary blood flow readings reflect the relative differences in blood supply to the cortex and the medulla. The cortical and medullary flow readings were taken as 100% values to calculate cortical and medullary flow changes as RPP was increased.

Figure 4 shows the effects of changing RPP on RBF (top panel) and RVR (bottom panel). RBF ranged from 6.3 to 7.6 ml/min per g kwt in control mice and declined slightly when RPP was increased. In DOCA-salt mice, RBF was lower ($P < 0.05$) and ranged from 4.4 to 4.7 ml/min per g kwt. The RBF was autoregulated throughout the entire range of RPP. The autoregulation of RBF was caused by an increase in RVR as RPP was increased. RVR increased in DOCA-salt mice from $22.8 \pm 3$ to $36.7 \pm 4.4$ mmHg/ml per min per g kwt as RPP was increased from 100 to 154 mmHg ($P < 0.05$). In control mice, RVR was $12.5 \pm 0.84$ mmHg/ml per min per g kwt, much lower than in DOCA-salt mice ($P < 0.05$), and increased to $23.4 \pm 1.7$ mmHg/ml per min per g kwt as RPP was increased from 90 to 140 mmHg ($P < 0.05$).

Figure 5 shows the relationship between RPP and cortical (top panel) and medullary (bottom panel) blood flow. In control and in DOCA-salt mice, cortical blood flow was well autoregulated across the entire RPP ranges investigated. However, increasing RPP from 91 to 140 mmHg in control mice led to an increase in medullary blood flow to 120% of baseline ($P < 0.05$). Thus, a 25-mmHg increase in perfusion pressure increased medullary blood flow by 10%. On the other hand, in DOCA-salt mice, medullary blood flow did not change when RPP increased.

To assess the effects of volume expansion on these preparations, we next abruptly infused 1 ml of normal saline. Sys-
Figure 5. Relationship between RPP and cortical (top) and medullary (bottom) blood flow in DOCA-salt hypertensive and control mice. *P < 0.05, values compared at equivalent RPP levels. Step-by-step increase in RPP increased medullary flow signals in control mice and had no effect on medullary flow in DOCA-salt mice. Cortical blood flow was not affected by changes in RPP levels.

Figure 6. Diuresis (top) and natriuresis (bottom) in DOCA-salt hypertensive and control mice after volume loading (0.9% saline, intravenously, 1 ml in 1 min). Volume loading increased urine flow and sodium excretion in both groups.

temnic BP and heart rate dropped initially after intravenous sodium loading, but returned within 1 min to the baseline values and stabilized at those levels. Diuresis and natriuresis after volume expansion are shown in Figure 6. The urine flow values, the peaks in volume and sodium excretion of DOCA-salt mice were 234 ± 23 and 205 ± 22%, respectively. These values were significantly higher than in control mice, which achieved values of 187 ± 12 and 167 ± 11%, respectively. Volume expansion also resulted in an abrupt kaliuresis in both groups (data not shown).

RBF (top panel) and RVR (bottom panel) values in response to acute volume expansion are shown in Figure 7. Bolus volume expansion increased RBF in control mice from 6.57 ± 0.49 to 8.86 ± 0.74 ml/min per g kwt and in DOCA-salt mice from 4.48 ± 0.51 to 6.17 ± 0.56 ml/min per g kwt, respectively. The RBF of control mice was higher than DOCA-salt mice at every time point (P < 0.05). The increase in RBF was caused by a sharp decrease in RVR, as shown in the bottom panel of Figure 7. Although the RVR of control mice was invariably lower than DOCA-salt mice, the percent changes in RVR were similar.
Figure 7. RBF (top) and RVR (bottom) in DOCA-salt hypertensive and control mice after volume loading (0.9% saline, intravenously, 1 ml in 1 min). RBF increased in control mice more than in DOCA-salt mice after volume loading. RVR decreased to a similar extent in both groups.

Cortical and medullary blood flows after volume loading are shown in Figure 8. Cortical blood flow did not change significantly in either group after acute volume expansion. However, acute volume expansion resulted in a greater increase in medullary blood flow (bottom panel) in control mice compared with DOCA-salt mice (P < 0.05). In control mice, medullary blood flow increased after volume loading to 122% of baseline values and declined over 20 min to baseline values. The corresponding increase in medullary blood flow of DOCA-salt mice was to only 110% of baseline values.

In control mice, the hematocrit level at the beginning of the experiments was 0.34 ± 0.03 and increased to 0.46 ± 0.03 at the end of the experiments. In DOCA-salt mice, these values were 0.40 ± 0.01 and 0.47 ± 0.03, respectively.

Discussion

This study found that in normotensive mice, medullary blood flow was not strictly autoregulated as RPP was increased. Furthermore, in these mice, medullary blood flow increased more steeply than cortical blood flow after a bolus volume expansion with 0.9% NaCl solution. Induction of DOCA-salt hypertension resulted in significantly lower RBF and increased RVR compared with controls. Furthermore, DOCA-salt hypertension prevented pressure-dependent medullary blood flow increases and diminished medullary blood flow reactions after volume loading. Adjustments in medullary blood flow may be necessary to excrete acute volume loads and to conduct pressure-natriuresis in mice. Furthermore, impaired medullary blood flow regulation may be involved in this form of salt-sensitive hypertension.

Renin-angiotensin system gene expression has not been studied in DOCA-salt-treated mice to our knowledge. Because the system is important for RBF regulation and for the development of nephrosclerosis, we relied on RNase protection.
assays to assess components of the system within the mouse kidney. We found that DOCA-salt treatment reduced renin, AT$_1$ receptor, and ACE gene expression, whereas angiotensinogen expression remained unchanged. The renin mRNA decrease after DOCA-salt corresponds to observations in DOCA-salt-treated rats (9,14). The ACE gene downregulation we observed in the kidneys of our DOCA-salt mice may differ from the situation in rats, where an inverse relationship between plasma renin activity and ACE activity in kidney was described in a study involving 2-kidney/1 clip, 1-kidney/1-clip, and DOCA-salt-treated rats (15). On the other hand, in another study, manipulation of dietary salt intake did not appear to influence tissue ACE levels in rats (16). AT$_1$ receptor gene expression was examined in 2-kidney/1-clip hypertensive rats and was found to be inversely correlated with renin renin mRNA expression (17). This relationship was not apparent in our DOCA-salt-treated mice, because expression of both genes was decreased. We had expected an upregulation of the AT$_1$ receptor gene.

The pathogenesis of DOCA-salt hypertension remains unclear, although several mechanisms have been implicated, including alterations in electrolyte metabolism (18), vasopressin responses (19), attenuation of vasodilatory responses such as bradykinin (20), the nitric oxide system (9), and increased sympathetic nervous system activity (21). In any event, the final common pathway of hypertension in the DOCA-salt model is a shift in the pressure-natriuresis-diuresis relationship rightward. In our earlier study in DOCA-salt mice (4), we demonstrated a flattened, rightwardly shifted pressure-natriuresis relationship characteristic of salt-sensitive hypertension. In that study, we did not address mechanisms responsible for the decreased sodium and water excretory capacity.

Our findings are not the first to show that medullary blood flow is not strictly autoregulated across the pressure ranges. In the volume-expanded rat, cortical blood flow is highly autoregulated across a wide range of pressures, whereas the medullary pattern adjusts to increasing pressures in a manner very similar to that reported here (22). Impairment of this phenomenon may be responsible for resetting the relationship between sodium excretion and BP toward higher arterial pressure values. Data obtained in the spontaneously hypertensive rat (23,24) and in hypertension induced by long-term infusion of the nitric oxide synthase inhibitor NO$_2$-nitro-L-arginine methyl ester (25,26) would support that point of view. The blood flow in the inner renal medulla may comprise only 1% of the total RBF. Nevertheless, small changes induced via changes in renal interstitial pressure, for example, may have profound effects on sodium and water homeostasis and therefore also on BP. This mechanism may apply primarily to rats; in other species, the mechanism remains less clearly defined. In dogs, blood flow to the inner medulla appears to be as efficiently autoregulated in the outer medulla as it is in the cortex of the kidney (27). Thus, the mechanism for arterial pressure-induced changes in sodium excretion may not depend on a coincident alteration in medullary blood flow in this species (28). On the other hand, work performed in dogs using the noninvasive technique of fast-computed tomography (29) showed that a decrease in RPP within the range of total RBF autoregulation was associated with a decrease in inner medullary blood flow. In contrast, flow to other renal regions was well autoregulated (30). These results are at variance with studies finding preserved medullary regulation (27,28), in which the authors suggested that adjustments in inner medullary blood flow could be involved in pressure natriuresis and subsequently in BP regulation.

A diminished ability to increase total RBF and medullary RBF at high perfusion pressures may be causally related to sodium excretion in DOCA-salt hypertensive mice. Total RBF, either measured absolutely or calculated as percentage changes from baseline readings, increased after sodium loading in control mice more than in DOCA-salt mice. Furthermore, increases in medullary blood flow were larger in control mice than in DOCA-salt mice, whereas changes in cortical blood flow were not different between the groups. Therefore, normal mice reacted to extracellular fluid volume expansion similar to rats, in which an increase in medullary blood flow was also observed by the laser-Doppler flowmetry technique after intravenous saline infusion (31). Because this change in medullary blood flow was diminished in DOCA-salt mice, medullary washout of the solute gradient may have occurred, and a lesser increase in renal interstitial pressure may have developed in DOCA-salt mice compared with controls. However, the morphological changes induced by the hypertension we observed earlier may also have been responsible for the derangement of sodium excretion in DOCA-salt mice (4). Because mRNA for renin and ACE were downregulated in the kidneys of DOCA-salt mice, we assume that renal levels of angiotensin II were also decreased. Thus, we do not believe that components of the renin-angiotensin system were responsible for development of nephrosclerosis on DOCA-salt hypertensive mice, as has been suggested for rats (32). The failure of DOCA-salt hypertensive mice to demonstrate an exaggerated antiurea in response to acute volume expansion may have been related to differences in the protocols (12). In the present study, volume expansion was conducted under highly contrived conditions. The perfusion pressures were already set at maximum in the mice. In fact, when calculated as peak percentage changes in urine flow and sodium excretion after sodium loading, the values were significantly higher in DOCA-salt mice compared with controls.

The mechanisms responsible for the effects on medullary blood flow as RPP or extracellular fluid volume were increased in control mice, and the reasons for their failure to function normally in DOCA-salt mice cannot be answered by this study. In an earlier study of DOCA-salt hypertension (33), we found that factors extrinsic to the kidney were more important for renal regulation than factors intrinsic to the kidneys. We assume that changes in the neurohormonal regulation of the kidneys, such as altered renal sympathetic nerve activity (34) and responsiveness to plasma vasopressin levels (35), may have had an impact on the observed blood flow changes in DOCA-salt mice. In this context, we would especially stress vasopressin. Direct infusion of vasopressin into the renal medulla reduces medullary blood flow without affecting cortical blood flow (36), and small increases in plasma vasopressin.
levels decrease renal blood medullary blood flow and attenuate pressure-medullary blood flow and pressure-natriuretic responses (37). We observed these same changes in DOCA-salt hypertensive mice. However, the powerful vasoconstrictive actions of endothelin-1 may have been involved. Endothelin-1 production in vascular tissues is increased in DOCA-salt hypertensive rats (38). Furthermore, DOCA plus salt induces overexpression of the endothelin-1 gene in blood vessels (39). On the other hand, nitric oxide release appears to be impaired in DOCA-salt hypertension (40). The atrial natriuretic factor receptor-mediated signaling is defective in DOCA-salt hypertensive rats (41). Finally, we retain an important role for structural changes. Nephrosclerosis with obsolescent glomeruli was prominent in our mice and may have contributed to altered medullary function (4).

In summary, our study is the first to indicate that DOCA-salt hypertension is associated with an impaired ability to increase medullary blood flow as RPP or extracellular fluid volume are increased. These data underscore the importance of renal medullary blood flow for sodium excretion and BP behavior in salt-sensitive hypertension. Our experiment also demonstrates that the mouse is amenable to physiological experimentation similar to the rat. Because gene-targeting experiments can be done in mice but not in rats, our model may have great relevance in the phenotypization of new hypertensive and vascular disease models.

Acknowledgments

This study was supported by a Grant-In-Aid to Volkmar Gross and Friedrich C. Luft from the Deutsche Forschungsgemeinschaft. We are grateful to Edith Richter, Anita Müller, and Gabriele N’diaye for technical assistance.

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