Chronic Effects of Lovastatin and Bezafibrate on Cortical and Medullary Hemodynamics in Deoxycorticosterone Acetate-Salt Hypertensive Mice

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Abstract. Cholesterol synthesis inhibitors and fibrates both exercise effects that could influence BP and renal function in hypertension. To test this issue, transit-time ultrasound flow probes, implanted optical fibers, and laser-Doppler flowmetry were used for measurements of total and regional renal blood flows in lovastatin (40 mg/kg body wt) and bezafibrate (50 mg/kg body wt) chronically treated deoxycorticosterone acetate (DOCA)-salt hypertensive mice. Total renal blood flow was well autoregulated between 70 and 150 mmHg (approximately 3.5 ml/min per g kidney weight in DOCA-salt mice). Both lovastatin and bezafibrate increased renal blood flow to a range between 4.7 and 5.5 ml/min per g kidney weight. In the renal perfusion pressure ranges investigated, renal vascular resistance increased in lovastin- and bezafibrate-treated DOCA-salt mice, but not as steeply as in vehicle-treated DOCA-salt mice. During a stepwise increase in renal perfusion pressure in lovastatin-treated DOCA-salt mice, medullary blood flow increased up to 130% of baseline values, which was not seen in vehicle- or bezafibrate-treated mice. After extracellular volume expansion with 1% saline, 1 ml over 1 min, total renal blood flow was also higher in lovastatin- or bezafibrate-treated DOCA-salt mice, whereas medullary blood flow increased more steeply in lovastatin-, compared with bezafibrate- or vehicle-treated mice. Systemic BP was significantly decreased in lovastatin-treated DOCA-salt mice compared with vehicle-treated mice. Lovastatin prevented histologic evidence for hemostasis in the medullary circulation of DOCA-salt mice. The results suggest that both lovastatin and bezafibrate diminished DOCA-salt-induced reductions in total renal blood flow. Lovastatin also abolished the perturbed medullary blood flow reactions to increased perfusion pressure or to volume expansion. Finally, lovastatin decreased systemic BP in DOCA-salt mice. These data suggest that cholesterol synthesis inhibition or fibrate treatment improve disturbed renal function in a mouse model of salt-dependent hypertension.

Hypertension and lipid disturbances are both important risk factors for cardiovascular disease and often occur concomitantly (1–3). BP lowering and lipid reduction with medication have both proved highly successful in reducing the risk of stroke and cardiac and vascular disease. 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors and fibrates have both been successful in lowering the risk of myocardial infarction (4,5). A putative negative effect of HMG-CoA reductase inhibitors on BP regulation has been raised by Roullet *et al.* (6,7), who found that inhibition of mevalonate synthesis increased the responses of both rat and human resistance vessels to vasopressors and who also reported that lovastatin increased BP in the spontaneously hypertensive rat (SHR). Subsequently, Roullet *et al.* (8) were able to show that farnesol, which is produced in lesser amounts in the presence of HMG-

CoA reductase inhibition, blocks the L-type voltage-dependent calcium channel. Such an effect would provide a putative mechanism by which HMG-CoA reductase inhibition might increase BP.

In contrast to the findings of Roullet *et al.* (6), other investigators observed that lovastatin decreased systemic BP in SHR (9). The decrease in BP in SHR was associated with a leftward shift in the relationship between renal medullary blood flow, renal interstitial pressure, sodium excretion, and renal perfusion pressure to lower levels of arterial pressure. A decrease in BP was also described in Dahl salt-sensitive rats after clofibrate treatment (10). We showed earlier that medullary blood flow is perturbed in mice with deoxycorticosterone acetate (DOCA)-salt hypertension (11). In the present study, we sought to reexamine the effect of HMG-CoA reductase inhibition in this model of hypertension, particularly in terms of altered renal medullary blood flow. We included a fibrate, to concomitantly examine a second lipid-lowering agent in the DOCA-salt mouse model.

Materials and Methods

Experiments were performed on NMRI mice purchased from Tierzucht Schoenwalde (Schoenwalde, Germany). The mice were allowed

free access to standard chow (0.25% sodium; SNIFF 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 (12) for removing the right kidney. A 50-mg DOCA pellet (Innovative Research of America, Sarasota, FL) was implanted subcutaneously in the abdominal area after which the incision was sutured. The mice were allowed to recover in a warm cage. Thereafter, they received 1% NaCl solution as drinking water for 3 wk and randomly allocated to the three groups. One group served as controls and received as vehicle Na-carboxylmethylcellulose (1% solution, 2 μl/g body wt) daily over 3 wk by gavage. A second group received lovastatin (40 mg/kg) dissolved in the vehicle daily by gavage. The third group received bezafibrate (50 mg/kg body wt) by gavage over a period of 3 wk.

Protocol 1: Blood Pressure, Serum Chemistries, and Histology

BP was measured intra-arterially in DOCA-salt mice treated for 3 wk with vehicle ($n = 18$; body wt 33.3 $\pm$ 1.1 g), lovastatin ($n = 12$; body wt 34.0 $\pm$ 1.7 g), or bezafibrate ($n = 12$; body wt 32.4 $\pm$ 0.8 g). Mice were prepared and BP measurements were conducted as described by Krege *et al.* (12). Mice were anesthetized with a mixture of ketamine/xylazine. The right carotid artery was exposed through a cervical incision and isolated. A catheter (PE-10) mounted on the tip of a silicon tubing (inner diameter 0.508 mm, outer diameter 0.9398 mm; Ulrich, St. Gallen, Switzerland) was inserted into the vessel. The catheter was filled with 0.9% saline solution and heparin (50 IU/ml). The catheters were tunneled subcutaneously to exit at the back of the neck. Thereafter, the incisions were carefully closed with sutures. Between 4 and 5 p.m., 6 to 7 h after surgery when mice had recovered from anesthesia, BP was measured and recorded on a TSE computer system (Technical & Scientific Equipment, Bad Homburg, Germany). The comparison of BP measured by this approached with BP measured in mice by the tail-cuff method showed good agreement (12). All mice were free to move and not restrained during the measurements. Mice were usually resting quietly during the measurements. Representative mean arterial pressure (MAP) values were calculated for each mouse by averaging all of the values recorded during that period (usually a 5- to 10-min period). Requirements for inclusion of data were a pulsatile wave form, and survival of the mouse until the following day.

Serum Chemistry

On the following day, mice (vehicle $n = 14$; lovastatin $n = 14$; bezafibrate $n = 8$) were anesthetized with inactin (100 mg/kg) and blood was drawn by puncture of the heart for measurements of plasma electrolytes, lipids, and creatinine. Plasma sodium and potassium concentrations, plasma cholesterol, triglyceride, and plasma creatinine (Jaffe method) were determined using a Hitachi 912 machine (Boehringer-Mannheim, Mannheim, Germany).

Histology

For conventional morphology, the kidney was removed, cut sagittally, and fixed in 4% buffered formaldehyde at room temperature. Subsequently, the tissue was embedded in paraffin, and after deparaffinizing and rehydrating it was stained with hematoxylin and eosin and Masson's trichrome.

Protocol 2: Cortical and Medullary Blood Flow in DOCA-Salt Mice

The effect of acutely increased renal perfusion pressure (RPP) or extracellular fluid volume on total renal blood flow (RBF) and renal medullary and cortical blood flow were examined in eight vehicle-treated mice weighing 32.7 $\pm$ 0.8 g (kidney weight 0.503 $\pm$ 0.03 g), in nine lovastatin-treated mice weighing 31.5 $\pm$ 0.7 g (kidney weight 0.446 $\pm$ 0.02 g), and in eight bezafibrate-treated mice weighing 32.9 $\pm$ 1.1 g (kidney weight 0.482 $\pm$ 0.02 g).

Surgical Preparations and Hemodynamic Measurements

We relied on techniques described earlier (11). The mice were anesthetized with a mixture of ketamine (50 μg/g intraperitoneally; Parke-Davis, Berlin, Germany) and inactin (100 μg/g intraperitoneally; Res Biochemical Inc., Natick, MA) and were placed on a heated table for maintenance of body temperature at 37°C. During surgery, the mice were given occasional small supplemental doses of inactin to maintain stable anesthesia levels. Cannulas (PE 90) were placed into the trachea for facilitating breathing and into the carotid artery (PE 10) for measurement of systemic MAP and RPP, in the jugular vein (PE 10) for infusion of 0.9% NaCl solution (1.5 μl/min per g body wt), and in the urinary bladder (PE 50) for urine collection. After midline and flank incisions, a 0.5-mm V-series flowprobe (Transonic Systems, Inc., Ithaca, NY) was placed around the left renal artery to measure RBF. The flowprobe was kept in place on the position of the highest sensitivity by a micromanipulator and connected to a flowmeter (T206; Transonic Systems, Inc). 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 loosely placed around the celiac and mesenteric arteries, as well around the abdominal aorta below the kidney for later occlusion, so that RPP could be varied.

A laser-Doppler flowmeter (model ALF 21D; Transonic Systems, Inc.) with implanted fibers was used to measure blood flow in the cortex and medulla. The implanted fibers consisted of 500-μm diameter fiber optic strands (Mitsubishi Cable America, New York, NY) and were connected to an external probe specifically designed for such applications. The loss of light at the connection between the implanted optical 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.

RPP and Renal Hemodynamics

After surgery and a 45-min equilibration period, MAP, RBF, and cortical and medullary blood flow signals were recorded continuously. Under these baseline conditions, RPP in vehicle-treated mice measured 74 $\pm$ 5 mmHg, in lovastatin-treated mice 78 $\pm$ 6 mmHg, and in bezafibrate-treated mice 79 $\pm$ 6 mmHg. RPP was then increased by ligating the mesenteric and celiac arteries by approximately 36 to 40 mmHg to values of 113 $\pm$ 7 mmHg in vehicle-mice, 116 $\pm$ 6 mmHg in lovastatin-treated mice, and 116 $\pm$ 7 mmHg in bezafibrate-treated mice. After this maneuver, RBF and cortical and medullary flow signals were recorded as soon as a steady state was reached. RPP was then approximately increased by an additional 25 to 35 mmHg to

148 $\pm$ 4 mmHg in vehicle-treated mice, to 146 $\pm$ 3 mmHg in lovastatin-treated mice, and to 142 $\pm$ 8 mmHg in bezafibrate-treated mice by occluding the aorta below the kidney. After another equilibration period, renal blood flows were again recorded. The BP levels reached by tying off celiac and mesenteric arteries and occluding aorta below the renal artery were not significantly different between the groups.

Volume Expansion and Renal Hemodynamics

At the highest pressure levels and a period of about 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, equivalent to 3% body weight). Thereafter, urine flow was collected for 2-min periods over 20 min. In this part of the experiment, urine was collected to quantify the effect of volume expansion in the different experimental groups. Total and regional renal blood flows, heart rate, and systemic BP were continuously recorded during this procedure. At the end of the experiment, blood was drawn to measure hematocrit. MAP and RBF measurements were recorded on a computer system (Technical & Scientific Equipment). Representative MAP, total RBF, and cortical and medullary flow values were calculated for each period by averaging all recorded values during that time period. The laser-Doppler flowmeters produce a voltage signal proportional to the flux of red blood cells in the tissue beneath the tip of the optical fibers. This situation implies that the voltage signal, which is a qualitative index of blood flow, depends on the position of the fiber to the vessel geometry of the kidney region investigated. This limitation of the method is caused by scattered interindividual values. To standardize the readings from different animals, percent changes from baseline values are given, as well as absolute laser-Doppler flow readings. For percentage from control calculations, the cortical and medullary flow readings before increasing RPP or volume expansion were set at 100%. Urine flow (UV) was determined gravimetrically. Urinary sodium and potassium concentrations were determined by flame photometry (FLM3; Radiometer, Copenhagen, Denmark). Urine flow, sodium excretion (UNaV), potassium excretion (UKV), and RBF were normalized per gram kidney weight (kwt).

Statistical Analyses

Values are expressed as means $\pm$ SEM. Statistically significant differences in mean values were evaluated by ANOVA and the Duncan multiple range test. A value of $P < 0.05$ was considered statistically significant.

Results

Mean arterial pressures measured in conscious, unrestrained mice with in-dwelling catheters in the carotid artery averaged 130 $\pm$ 2 mmHg in vehicle-treated, 114 $\pm$ 7 mmHg in lovas-

tatin-treated, and 122 $\pm$ 5 mmHg in bezafibrate-treated mice. The BP difference between vehicle- and lovastatin-treated DOCA-salt mice was significant, whereas the heart rates of 600 $\pm$ 19 and 574 $\pm$ 29 beats/min were not different between vehicle- and lovastatin-treated mice. The heart rate of bezafibrate-treated mice was with 649 $\pm$ 12 beats/min slightly increased compared with the other groups. In bezafibrate-treated mice, both plasma values of triglycerides and cholesterol were decreased, but only the decrease in plasma cholesterol was significant compared with the other groups. Plasma levels of electrolytes and creatinine were not different between the groups (Table 1).

In anesthetized vehicle-, lovastatin-, and bezafibrate-treated DOCA-salt mice, baseline RPP averaged between 74 and 79 mmHg. Corresponding to these values, total RBF in vehicle-treated mice was 3.6 $\pm$ 0.7 ml/min per g kwt. In lovastatin-treated and bezafibrate-treated DOCA-salt mice, RBF values were 30 to 40% higher and averaged 4.7 $\pm$ 0.4 and 5.0 $\pm$ 0.3 ml/min per g kwt, respectively ($P < 0.05$). The renal vascular resistance (RVR) calculated from these values was 20.8 $\pm$ 2.1 mmHg/ml per min per g kwt in vehicle-DOCA-salt mice. RVR was slightly but not significantly decreased under baseline conditions in lovastatin- and bezafibrate-treated DOCA-salt mice and averaged 18 and 15 mmHg/ml per min per g kwt, respectively. Cortical laser-Doppler blood flow signals measured between 1.7 and 2.6 V, whereas medullary laser-Doppler flow signals were much smaller and measured between 0.7 and 0.9 V. The differences in cortical and medullary blood flow readings reflect the relative differences in blood supply to the cortex and the medulla.

Figure 1 shows the effects of changing RPP on RBF (top panel) and RVR (bottom panel). RBF was well autoregulated in all groups, and the higher RBF values of lovastatin- and bezafibrate-treated DOCA-salt mice observed at baseline RPP were preserved throughout the entire range of RPP values investigated. The autoregulation of RBF was caused by an increase in RVR as RPP was increased. RVR increased in vehicle-treated DOCA-salt mice from 20.8 $\pm$ 2.1 to 50.9 $\pm$ 5.5 mmHg/ml per min per g kwt as RPP was increased from 74 to 148 mmHg ($P < 0.05$). In lovastatin- and bezafibrate-treated DOCA-salt mice, the increase in RVR was much lower than in vehicle-treated DOCA-salt mice, so that at RPP levels of 115 and 140 mmHg, RVR was significantly lower in these groups than in the vehicle-treated DOCA-salt mice ($P < 0.05$). RVR

Table 1. Plasma concentrations of triglycerides, cholesterol, creatinine, sodium, and potassium in vehicle-, lovastatin-, and bezafibrate-treated deoxycorticosterone acetate-salt mice[a]

Treatment	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	Na (mmol/L)	K (mmol/L)
Vehicle	74.64 $\pm$ 6.41	188.14 $\pm$ 11.56	0.45 $\pm$ 0.05	153.29 $\pm$ 1.05	4.21 $\pm$ 0.20
Lovastatin	78.29 $\pm$ 8.45	182.86 $\pm$ 12.27	0.47 $\pm$ 0.06	153.69 $\pm$ 1.83	4.23 $\pm$ 0.29
Bezafibrate	60.63 $\pm$ 3.95	128.50 $\pm$ 18.13[b]	0.39 $\pm$ 0.03	155.38 $\pm$ 2.17	4.78 $\pm$ 0.52

[a] Bezafibrate decreased plasma cholesterol significantly compared with vehicle or lovastatin mice.

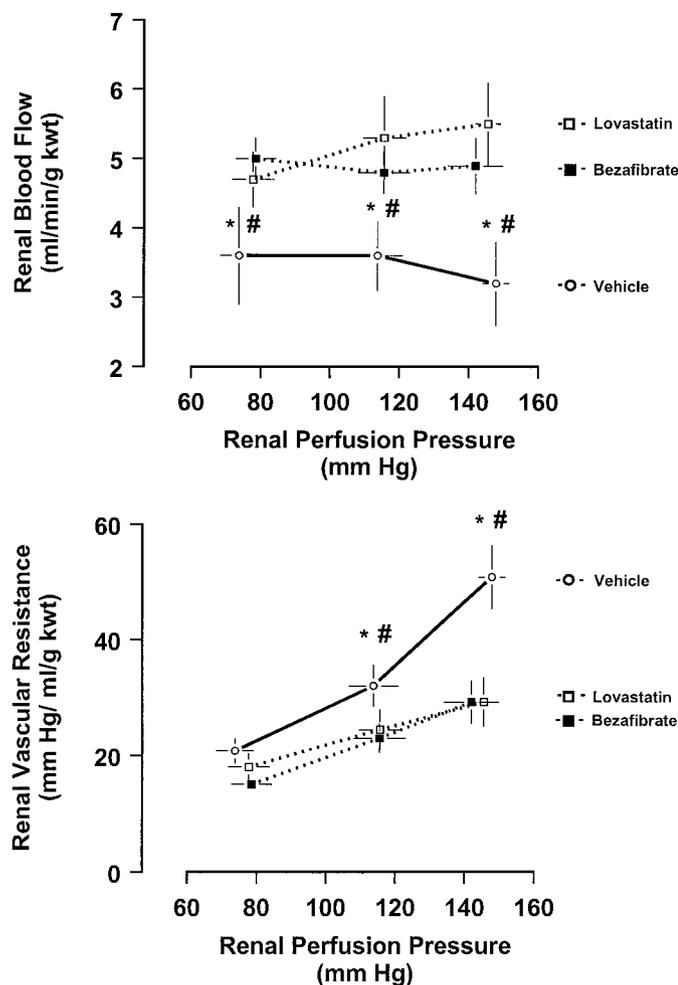[b] $P < 0.05$, bezafibrate *versus* vehicle/lovastatin.

Figure 1. Relationship between renal perfusion pressure (RPP) and renal blood flow (RBF, top panel) and renal vascular resistance (RVR, bottom panel) in lovastatin- (□), bezafibrate- (■), and vehicle- (○) treated deoxycorticosterone acetate (DOCA)-salt hypertensive mice. *$P < 0.05$, lovastatin *versus* vehicle; #$P < 0.05$, bezafibrate *versus* vehicle. Values are compared at equivalent RPP levels. The RBF values for given RPP were increased in lovastatin- and bezafibrate-treated DOCA-salt mice. The RVR for a given RPP relationship was flat compared with DOCA-salt hypertensive control mice.

of lovastatin- and bezafibrate-treated DOCA-salt mice averaged approximately 60% of the RVR calculated in vehicle-treated DOCA-salt mice at the RPP level of 140 mmHg.

Figure 2 shows the relationship between RPP and cortical (top panels) and medullary (bottom panels) blood flow. On the left are shown the fiber readings in absolute values and on the right as percentage from baseline values. Absolute cortical flow readings in vehicle-treated DOCA-salt mice ranged between 2.4 and 1.8 V. The readings of bezafibrate-treated DOCA-salt mice were in the same range, whereas the corresponding values of lovastatin-treated DOCA-salt mice were approximately 1.6 V lower than in the other groups. In vehicle-treated DOCA-salt mice, cortical blood flow decreased slightly across the RPP ranges investigated. On the other hand, in lovastatin- and bezafibrate-treated DOCA-salt mice, the corti-

cal blood flow was stable and did not change as RPP was increased from about 80 to 140 mmHg. However, the most striking and unexpected result was that increasing RPP from 78 to 146 mmHg in lovastatin-treated DOCA-salt mice led to an increase in medullary blood flow, as seen in the lower part of Figure 2. Increasing RPP was associated with an increase in medullary flow readings from 0.73 ± 0.06 to 0.90 ± 0.07 V ($P < 0.05$). In vehicle- and bezafibrate-treated DOCA-salt mice, absolute medullary flow readings were slightly higher; however, in contrast to lovastatin-treated mice, the same increase in RPP had no effect on medullary blood flow. This situation is also clearly seen when medullary flow changes are calculated as percentage of baseline values (Figure 2, bottom right panel). During stepwise increases in RPP, medullary blood flow progressively increased in lovastatin-treated mice, whereas medullary blood flow in vehicle- and bezafibrate-treated mice did not change.

To assess the effects of volume expansion on these preparations, we next abruptly infused 1 ml of normal saline intravenously. Systemic BP and heart rate initially decreased after intravenous sodium loading, but returned within 1 min to baseline values. Thereafter, systemic BP decreased slowly over time in vehicle-treated mice from 150 to 133 mmHg, in lovastatin-treated mice from 140 to 120 mmHg, and in bezafibrate-treated-mice from 140 to 125 mmHg. Diuresis values after volume expansion are shown in Figure 3. The urine flow in vehicle-treated DOCA-salt mice before sodium loading was 74.58 ± 7.4 μl/min per g kwt Baseline urine flow was 54.20 ± 6.02 and 60.57 ± 8.66 μl/min per g kwt, respectively, in lovastatin- and bezafibrate-treated DOCA-salt mice. After sodium loading in all groups, urine volume increased sharply. In vehicle-treated mice, the increased urine flow was greater than in the other two groups, which may depend on the slightly higher RPP of these mice. Changes in urine flow reached baseline values after 20 min in all of the groups. The peak values of percent increase in urine flow were 250 to 280% and did not differ between the groups. The same pattern as described for urine flows were found for sodium (UNaV) and potassium (UKV).

RBF (top panel) and RVR (bottom panel) values in response to acute volume expansion are shown in Figure 4. Acute volume expansion increased RBF in all groups. As in the protocol with step-by-step increases in RPP, RBF of lovastatin- and bezafibrate-treated DOCA-salt mice was higher at every time point than in vehicle-treated DOCA-salt mice ($P < 0.05$). Calculated as percentage from baseline values, RBF was increased to 120 to 130% 2 min after the saline infusion and did not differ between the groups. RBF decreased thereafter and reached baseline values after 20 min. The increase in RBF was caused by a sharp decrease in RVR, as observed in the bottom panel of Figure 4. Although the RVR of lovastatin- and bezafibrate-treated DOCA-salt mice was significantly lower than vehicle-treated DOCA-salt mice, the percent changes in RVR were similar.

Cortical and medullary blood flows as absolute and percent values after volume loading are shown in Figure 5. Absolute cortical flow signals of lovastatin-treated DOCA-salt mice
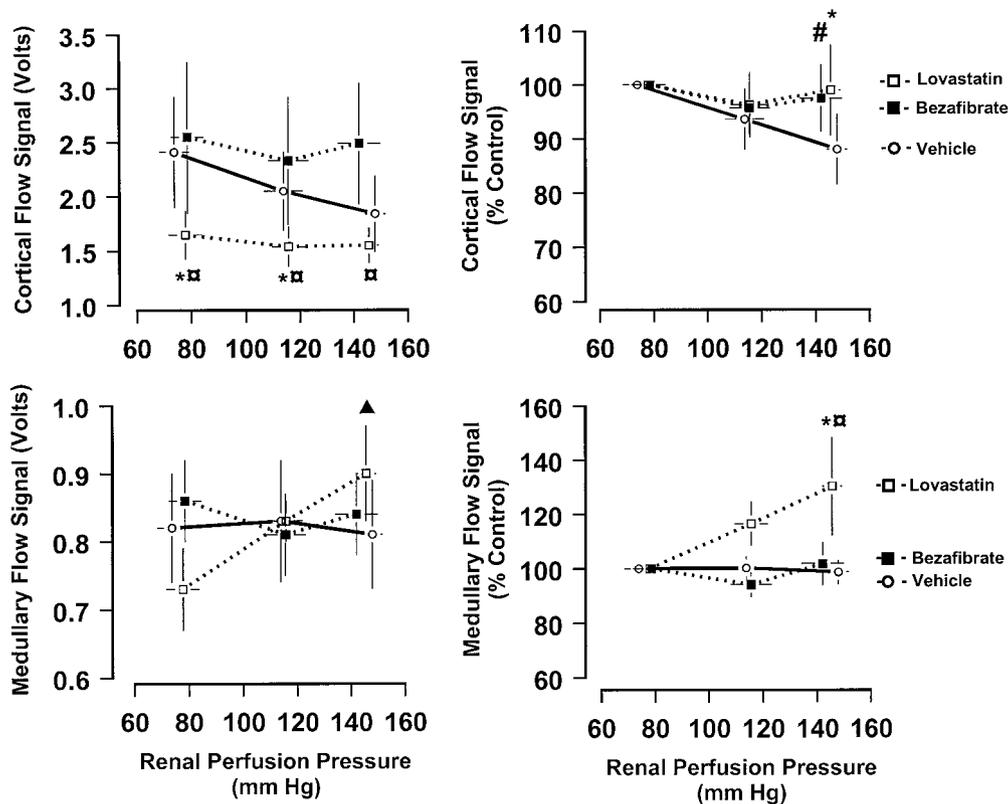
Figure 2. Relationship between RPP and cortical (top panels) and medullary (bottom panels) blood flow in lovastatin- (□), bezafibrate- (■), and vehicle- (○) treated DOCA-salt hypertensive mice. Absolute values in volts (left) and percent change from baseline (right). *$P < 0.05$, lovastatin *versus* vehicle; #$P < 0.05$, bezafibrate *versus* vehicle; ¤$P < 0.05$, lovastatin *versus* bezafibrate. Values are compared at equivalent RPP levels. Lovastatin: $P < 0.05$, 80 mmHg *versus* 140 mmHg. Cortical blood flow was well autoregulated in lovastatin- and bezafibrate-treated DOCA-salt mice and decreased slightly in vehicle-treated mice over the RPP range investigated. Step-by-step increase of RPP increased medullary flow signals either given as volts or given as percent from baseline values in lovastatin-treated DOCA mice compared with vehicle- and bezafibrate-treated DOCA-salt mice.

were within 2.12 to 2.23 V, which was slightly lower than in the other two groups in which the readings ranged between 2.70 and 2.20 V. Calculated as percentage from the baseline values in vehicle-treated DOCA-salt mice, cortical blood flow was approximately 95% of the baseline values after acute volume expansion. In contrast, lovastatin-treated DOCA-salt mice exhibited increased cortical blood flow values after volume loading and measured 105% of the baseline values. These reactions were significantly different. The corresponding cortical blood flow signals of bezafibrate DOCA-salt mice resided between the values of these two groups. Acute volume expansion resulted in a sharper increase in medullary blood flow (Figure 5, bottom panel) in lovastatin-treated DOCA-salt mice, compared with vehicle- or bezafibrate-treated DOCA-salt mice ($P < 0.05$). In lovastatin DOCA-salt mice, medullary blood flow increased after volume loading from 0.86 ± 0.08 to 1.06 ± 0.12 V, whereas the corresponding values of vehicle and bezafibrate DOCA-salt mice achieved only an increase 0.81 ± 0.08 to 0.90 ± 0.09, or from 0.86 ± 0.08 to 0.93 ± 0.08 V. Given in percentage from baseline readings, the values for medullary blood flow after saline loading were 123% in lovastatin-treated DOCA-salt mice, compared with 112 and 108% in vehicle- or bezafibrate-treated mice. Beginning with

the sixth to eighth minute after saline loading, medullary blood flow signals were no longer significantly different between the groups.

Light microscopy, as shown in Figure 6A, revealed a marked hemostasis in the vasa recta, predominantly in the outer stripe of the medulla in vehicle-treated DOCA-salt mice. As a sign of a disturbed medullary circulation, these vessels were distended with red blood cells, whereas in lovastatin-treated DOCA-salt mice the kidneys showed no pathologic changes (bottom panel). In bezafibrate-treated DOCA-salt mice, the morphologic changes of the medulla were not as prominent as in vehicle-treated DOCA-salt mice. However, the renal morphology was not entirely normal (section not shown). In vehicle- and lovastatin-treated mice, the hematocrit values at the end of the experiments were 49.98 ± 1.95 and 50.76 ± 1.28%. In bezafibrate-treated mice, the hematocrit was 41.71 ± 2.19%.

Discussion

The important findings in this study were that lovastatin and bezafibrate increased RBF and decreased RVR in DOCA-salt hypertensive mice. Furthermore, in lovastatin-treated DOCA-salt mice, the morphology of the medullary circulation was normalized and medullary blood flow increased as RPP was
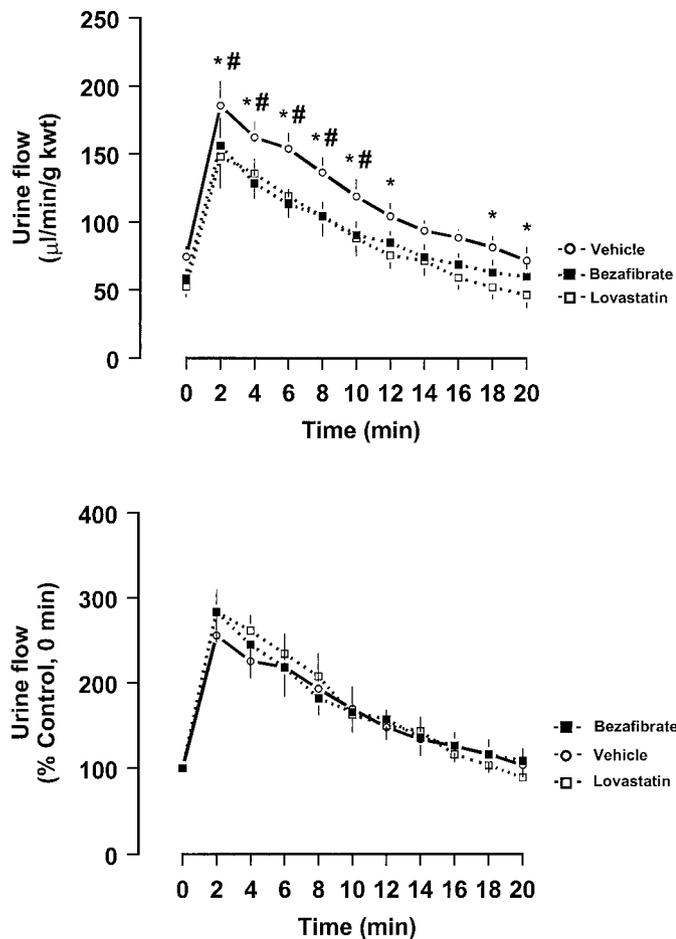
Figure 3. Diuresis (absolute values, top) and percent changes (bottom) in lovastatin- (□), bezafibrate- (■), and vehicle- (○) treated DOCA-salt hypertensive mice after volume loading (0.9% saline, intravenously, 1 ml/min). *$P < 0.05$, lovastatin *versus* vehicle; #$P < 0.05$, bezafibrate *versus* vehicle. Absolute diuresis of vehicle-treated DOCA-salt mice was greater than in lovastatin- or bezafibrate-treated DOCA-salt mice after volume loading. Percent changes in diuresis were not different between the groups.
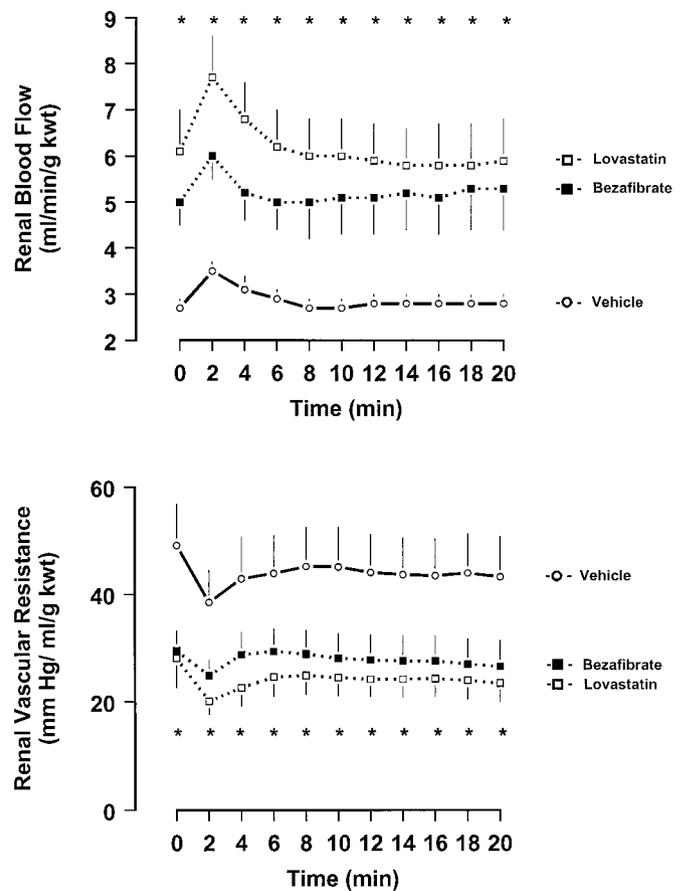
Figure 4. Flow probe-measured RBF (top) and RVR (bottom) in lovastatin- (□), bezafibrate- (■), and vehicle- (○) treated DOCA-salt hypertensive mice after volume loading (0.9% saline, intravenously, 1 ml/min). *$P < 0.05$, lovastatin/bezafibrate *versus* vehicle. Before and after sodium loading, RBF values were higher in lovastatin- and bezafibrate-treated DOCA-salt mice, depending on a lower RVR compared with controls (vehicle-treated DOCA-salt mice). The differences in RBF between lovastatin- and bezafibrate-treated mice were not significant.

increased or extracellular volume was expanded. In bezafibrate-treated DOCA-salt mice, pressure- and volume-dependent medullary blood flow changes were similar to responses seen in vehicle-treated DOCA-salt mice. These effects were accompanied in bezafibrate-treated DOCA-salt mice with a slight, and in lovastatin-treated DOCA-salt mice with a significant, reduction of systemic BP.

In vehicle-treated DOCA-salt mice, RBF values were similar to those observed in our earlier studies (11,13), at about 3.5 ml/min per g kwt. Lovastatin treatment increased RBF to approximately 5.5 ml/min per kg kwt. The effect of lovastatin on RBF in DOCA-salt mice is in agreement with observations in normal Wistar rats (14), or in Wistar rats after 5/6 nephrectomy (15). Despite increased flowprobe-determined total RBF in lovastatin-treated mice, the absolute baseline values of laser-Doppler cortical and medullary flow readings were lower than in vehicle- or bezafibrate-treated mice. We have no immediate explanation why the absolute voltage signals in the lovastatin-

treated group was lower than in the other groups. The laser-Doppler technique has limitations in terms of quantification, which caused us to show the results both as absolute and percent values.

The cortical blood flow in vehicle-treated DOCA-salt mice was not as stable, with increasing RPP or extracellular volume increases as we observed in an earlier study (11). The reason for this difference is not clear, especially since RBF was well autoregulated. In rats, a strong correlation between total RBF and cortical blood flow has been reported (16). The increased RBF in lovastatin-treated DOCA-salt mice was paralleled by an increase in medullary blood flow to the same extent as we found earlier in normotensive control mice (11) when RPP was increased or when extracellular volume was expanded. These medullary blood flow reactions were accompanied by a histologic normalization of the medullary circulation. The venous hyperemia and stasis in the vasa recta vessels, which could be the result of increased postglomerular vascular resistance in
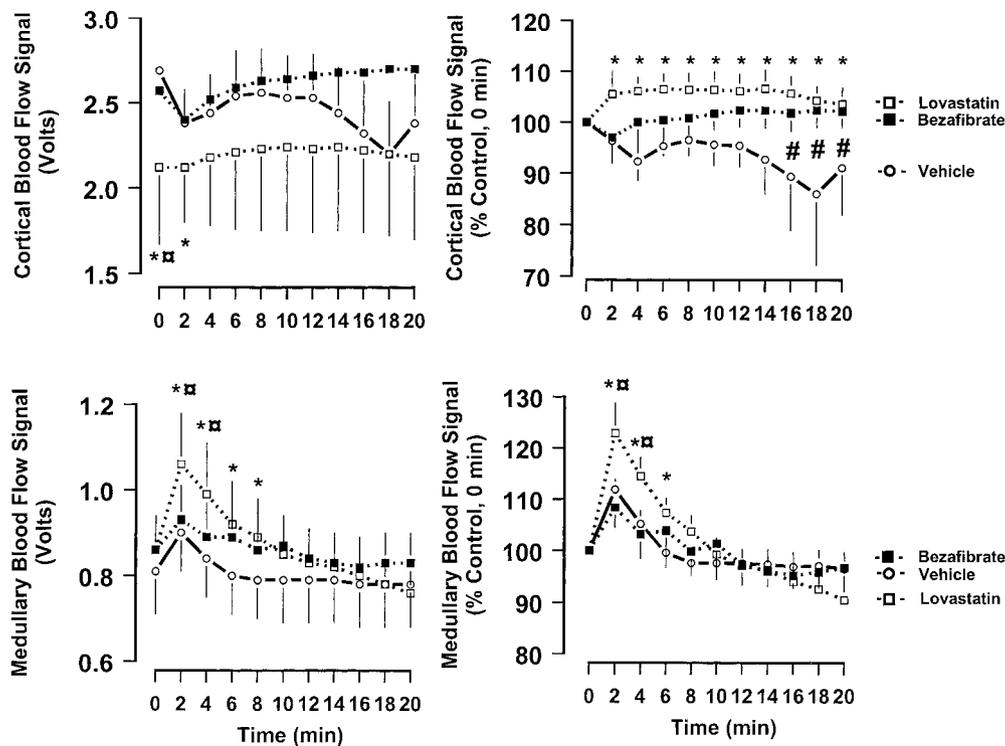
Figure 5. Changes in cortical (top panels) and medullary (bottom panels) blood flows measured by laser-Doppler flowmetry in lovastatin- (□), bezafibrate- (■), and vehicle- (○) treated DOCA-salt hypertensive mice after volume loading (0.9% saline, intravenously, 1 ml/min). Absolute values in volts (left) and percent change from baseline (right). *$P < 0.05$, lovastatin *versus* vehicle; #$P < 0.05$, bezafibrate *versus* vehicle; ¤$P < 0.05$, lovastatin *versus* bezafibrate. After volume loading, cortical blood flow increased slightly in lovastatin-treated and decreased slightly in vehicle-treated DOCA-salt mice. Medullary blood flow increased either given as volts or given as percent from baseline values to a greater degree in lovastatin- than in bezafibrate- or vehicle-treated DOCA-salt mice after volume loading.

vehicle-treated DOCA-salt mice, was not seen in lovastatin-treated mice. This pattern was not related to peculiarities of our mouse strain or our model, since SHR chronically treated with lovastatin also exhibited an increased medullary blood flow and an attenuation of hypertension-dependent morphologic changes in the kidneys (9).

The increase in total RBF and medullary blood flow may have improved the ability of lovastatin-treated mice to excrete sodium and water by a parallel increase in renal interstitial hydrostatic pressure and loss of medullary osmotic gradient (17). Infusion of captopril into the renal medullary interstitium increased renal medullary perfusion and lowered arterial pressure in SHR (18). On the other hand, chronic infusion of N^G-nitro-L-arginine methyl ester led to a decrease in renal medullary blood flow, sodium and water retention, and the development of hypertension (19). These experiments underscore the importance of medullary perfusion for BP regulation and the development of hypertension. The small, but significantly decreased, BP in conscious, lovastatin-treated DOCA-salt mice may therefore also be a consequence of the improved medullary circulation in these mice. A decrease in arterial BP was described both for lovastatin-treated SHR (9) and lovastatin-treated Dahl salt-sensitive rats fed 4% NaCl chow (20).

In contrast, Roullet *et al.* (6–8) found that lovastatin increased BP in SHR and WKY, which was accompanied by an increase in intracellular Ca^{2+} and an enhanced vascular re-

sponsiveness to vasoconstrictor substances. The reason(s) for these disparate results is not clear. We did not find any noteworthy changes in circulating lipid concentrations. Our results are similar to those reported for HMG-CoA reductase inhibitors by other investigators (21,22). We speculate that the lovastatin-related effects on RBF and the medullary circulation are related to characteristics (23,24) that are independent of plasma lipids or cholesterol synthesis. Although the pathogenesis of DOCA-salt hypertension remains unclear, alterations in the nitric oxide (NO) system have been implicated as a mechanism (25–27). NO serves as an important endogenous vasodilator system, and NO activity is especially expressed in the medulla (28,29), so that lovastatin-induced changes in the NO system (30) could be responsible for improvement of medullary flows in these mice. Since NO synthase inhibition is followed by a decrease in total RBF (31), the increase in RBF by lovastatin, which was also reported in other animal models (14,15), could be also due to lovastatin-induced changes in the NO system.

Bezafibrate also decreased RVR and increased total RBF, but did so independently of medullary blood flow. Because the decrease in systemic BP in these mice was small and not significant compared with lovastatin-treated DOCA-salt mice, the changes in the medullary circulation could have predominantly influenced BP behavior. In an acute study, bolus injection of bezafibrate increased total RBF and cortical blood flow in SHR and WKY, whereas medullary blood flow was not
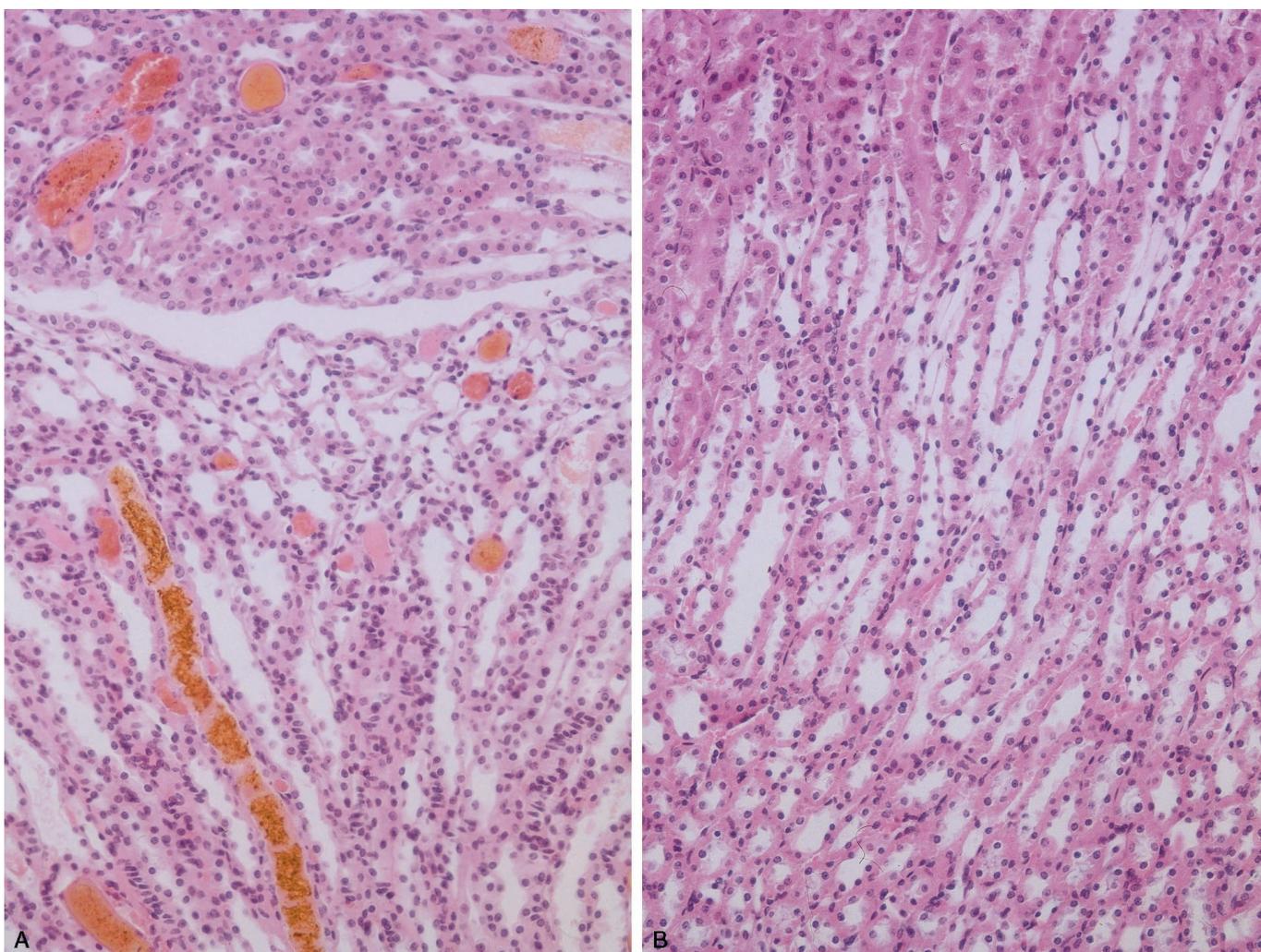
Figure 6. Kidney sections (hematoxylin and eosin) from vehicle- (A) or lovastatin- (B) treated DOCA-salt mice. Vehicle-treated DOCA-salt mice showed hyperemia and hemostasis predominantly in the outer medullary stripe (venous vasa recta vessels). In lovastatin-treated DOCA-salt mice, these stasis changes in the medullary circulation were not seen. Magnification, ×250.

affected (32). To our knowledge, long-term effects of fibrates on RBF and cortical and medullary blood flow have not been published elsewhere. Since blood lipids were decreased by bezafibrate, bezafibrate-induced effects on RBF could depend on direct or indirect vascular reactions.

Bezafibrate belongs to a group of compounds known to stimulate the peroxisome proliferator-activated receptor and to induce the expression of the P4504A genes in the liver and kidney (33,34), which may play a role in cardiovascular and renal function; however, the mechanisms by which these effects occur are unclear (35,36) and controversial. The inhibition of renal 20-HETE formation produced hypertension in Lewis rats fed a high salt diet (37). By contrast, in SHR renal expression of the P4504A2 gene and production of 20-HETE is elevated, and a pharmacologically induced decrease in 20-HETE production prevented the development of hypertension in SHR (38,39). In addition, the genes of the P450 family induced by fibrates in mice have not been characterized. Hemodilution is one factor mediating changes in renal hemodynamics and is claimed to be responsible for an increase in RBF

after volume expansion (40). In bezafibrate-treated DOCA-salt mice, the hematocrit values were lower than in lovastatin, or vehicle-treated DOCA-salt mice. Another factor influencing RBF could be the changed fibrinogen concentration described after bezafibrate (5), which also could have decreased blood viscosity in these mice and thereby increased RBF. In addition and further complicating the interpretation of the mechanism of bezafibrate effects, is the fact that similar to lovastatin, fibrates may reduce HMG-CoA reductase activity (41).

In summary, our study indicates that chronic treatment with lovastatin and bezafibrate causes renal hemodynamic effects and influences systemic BP in DOCA-salt hypertensive mice. Both lovastatin and bezafibrate increased total RBF and decreased RVR. In addition, lovastatin normalized medullary hemostasis and increased medullary blood flow as RPP or extracellular volume were increased to levels observed in normotensive mice. These changes in RBF after lovastatin were associated with a decreased systemic BP. In bezafibrate-treated mice, RBF was increased; however, the decreases in systemic BP were smaller and not statistically significant.

Acknowledgments

References

1. Luft FC: Treatment and prevention of hypertension: Where have we been and were are we going? *Kidney Int* 48[Suppl 50]: S14–S18, 1995

2. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ: For the west Scotland coronary prevention study group: Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 333: 1301–1307, 1995

3. Pedersen TR: For the Scandinavian simvastatin survival study group: Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian simvastatin survival study (4S). *Lancet* 344: 1383–1389, 1994

4. Treasure HB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW: Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *N Engl J Med* 332: 481–487, 1995

5. Goa KL, Barradell LB, Plosker GL: Bezafibrate: An update of its pharmacology and use in the management of dyslipidemia. *Drugs* 52: 725–753, 1996

6. Roullet JB, Xue H, Pappu AS, Roulet C, Holcomb S, McCarron DA: Mevalonate availability and cardiovascular function. *Proc Natl Acad Sci USA* 90: 11728–11732, 1993

7. Roullet JB, Xue H, Roullet CM, Fletcher WS, Cipolla MJ, Harker CT, McCarron DA: Mevalonat availability affects human and rat resistant vessel function. *J Clin Invest* 96: 239–244, 1995

8. Roullet JB, Xue H, Chapman J, McDougal P, Roulett CM, McCarron DA: Farnesyl analogues inhibit vasoconstriction in animal and human arteries. *J Clin Invest* 97: 2384–2390, 1996

9. Jiang J, Roman RJ: Lovastatin prevents development of hypertension in spontaneously hypertensive rats. *Hypertension* 30: 968–974, 1997

10. Roman RJ, Ma YH, Frohlich B, Markham B: Clofibrate prevents the development of hypertension in Dahl salt-sensitive rats. *Hypertension* 21: 985–988, 1993

11. Gross V, Lippoldt A, Bohlender J, Bader M, Hansson A, Luft FC: Cortical and medullary hemodynamics in deoxycorticosterone acetate-salt hypertensive mice. *J Am Soc Nephrol* 9: 346–354, 1998

12. Krege JH, Hodgin JB, Hagaman JR, Smithies O: A noninvasive computerized tail-cuff system for measuring blood pressure in mice. *Hypertension* 25: 1111–1115, 1995

13. Gross V, Lippoldt A, Luft FC: Pressure diuresis and natriuresis in DOCA-salt mice. *Kidney Int* 52: 1364–1368, 1997

14. Stowe NT, Inman SR, Tapolyai M, Brouhard BH, Hodge EE, Novick AC: Lovastatin has direct renal hemodynamic effects in a rodent model. *J Urol* 156: 249–252, 1996

15. Hafez KS, Inman SR, Stowe NT, Novick AC: Renal hemodynamic effects of lovastatin in a renal ablation model. *Urology* 48: 862–867, 1996

16. Mattson DL, Lu S, Roman RJ, Cowley AW Jr: Relationship between renal perfusion pressure and blood flow in different regions of the kidney. *Am J Physiol* 264: R578–R583, 1993

17. Cowley AW Jr, Mattson DL, Lu S, Roman RJ: The renal medulla and hypertension. *Hypertension* 25: 663–673, 1995

18. Lu S, Mattson DL, Cowley AW Jr: Renal medullary captopril delivery lowers blood pressure in spontaneously hypertensive rats. *Hypertension* 23: 337–345, 1994

19. Nakanishi K, Mattson DL, Cowley AW Jr: Role of renal medullary blood flow in the development of L-NAME hypertension in rats. *Am J Physiol* 268: R317–R323, 1995

20. O'Donnell MP, Kasiske BL, Katz SA, Schmitz PG, Keane WF: Lovastatin but not enalapril reduces glomerular injury in Dahl salt-sensitive rats. *Hypertension* 20: 651–658, 1992

21. Endo A, Tsujita Y, Kuroda M, Tanzawa K: Effects of ML-236B on cholesterol metabolism in mice and rat: Lack of hypocholesterolemic activity in normal animals. *Biochem Biophys Acta* 575: 266–276, 1979

22. Amin P, Rutledge RZ, Needle SJ, Hele DJ, Neuenswander K, Bush RC, Bilder GE, Perrone MH: RPR 101821, a new potent cholesterol-lowering agent: Inhibition of squalene synthase and 7-dehydrocholesterol reductase. *Naunyn Schmiedebergs Arch Pharmacol* 353: 233–240, 1996

23. Vaughan CJ, Murphy MB, Buckley BM: Statins do more than just lower cholesterol. *Lancet* 348: 1079–1082, 1996

24. Massy ZA, Keane WF, Kasiske BL: Inhibition of mevalonat pathway: Benefits beyond cholesterol reduction? *Lancet* 347: 102–103, 1996

25. Lippoldt A, Gross V, Schneider K, Hannson A, Nadaud S, Schneider W, Bader M, Yagil C, Yagil Y, Luft FC: NO synthetase and renin angiotensin system gene expression in salt-sensitive and salt-resistant Sabra rats. *Hypertension* 30: 409–415, 1997

26. Hirata Y, Hayakawa H, Kakogi M, Tojo A, Suzuki E, Kimura K, Goto A, Kikuchi K, Nagano T, Hirobe N, Omata M: Nitric oxide release from kidneys of hypertensive rats treated with imidapril. *Hypertension* 27: 672–678, 1996

27. Hirata Y, Hayakawa H, Suzuki E, Kimura K, Kikuchi K, Nagano T, Hirobe M, Omata M: Direct measurement of endothelium-derived nitric oxide release by stimulation of endothelin receptors in rat kidney and its alteration in salt-induced hypertension. *Circulation* 91: 1229–1235, 1995

28. Chin SY, Wang CT, Majid DAS, Navar LG: Renoprotective effects of nitric oxide in angiotensin II-induced hypertension in the rat. *Am J Physiol* 274: F876–F882, 1998

29. Mattson DL, Roman RJ, Cowley AW Jr: Role of nitric oxide in renal papillary blood flow and sodium excretion. *Hypertension* 19: 766–769, 1992

30. Laufs U, Fata LV, Plutzky J, Liao JK: Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 97: 1129–1135, 1998

31. Guarasci GR, Kline RL: Pressure natriuresis following acute and chronic inhibition of nitric oxide synthase in rats. *Am J Physiol* 270: R469–R478, 1996

32. Agrawal B, Kopecky J, Kränzlin B, Rohmeiss P, Pill J, Gretz N: Acute effects of bezafibrate on blood pressure and renal haemodynamics in SHR and WKY rats. *Nephrol Dial Transplant* 13: 333–339, 1998

33. Johnson EF, Palmer CNA, Griffin KJ, Hsu MH: Role of peroxisome proliferator-activated receptor in cytochrome P450 4A gene regulation. *FASEB J* 10: 1241–1248, 1996

34. Simpson AECM: The cytochrome P 450 4 (CYP4) family. *Gen Pharmacol* 28: 351–359, 1997

35. Rahman M, Wright JT Jr, Douglas JG: The role of cytochrome P450-dependent metabolites of arachidonic acid in blood pres-

sure regulation and renal function: A review. *Am J Hypertens* 10: 356–365, 1997

36. Macita K, Falck JR, Capdevila JH: Cytochrome P450, the arachidonic acid cascade, and hypertension: New vistas for an old enzyme. *FASEB J* 10: 1456–1463, 1996

37. Roman RJ, Alonso-Galicia M, Wilson TW: Renal P450 metabolites of arachidonic acid and the development of hypertension in Dahl salt-sensitive rats. *Am J Hypertens* 10: 63S–67S, 1997

38. Omata K, Abraham NG, Schwartzman ML: Renal cytochrome P450-dependent arachidonic acid metabolism: Intrarenal localization and hormonal regulation in spontaneously hypertensive rats. *Am J Physiol* 262: F591–F599, 1992

39. Escalanta B, Sacerdoti D, Davidian MM, Laniado-Schwartzman MC, McGiff JC: Chronic treatment with tin normalized pressure in spontaneously hypertensive rats. *Hypertension* 17: 776–779, 1991

40. Franchini KG: Hemodilution mediates changes in renal hemodynamics after acute volume expansion in rats. *Am J Physiol* 274: R1670–R1676, 1998

41. Stegmeier K, Stork H, Lenz H, Leuschner F, Liede V: Pharmacology and mode of action of bezafibrate in animals. In: *Lipoproteins and Coronary Heart Disease*, edited by Greten H, Lang PD, Schettler G, New York, Baden-Baden, Cologne, Gerhardt Witzstock Publishing House, 1980, pp 76–82