Regional responsiveness of renal perfusion to activation of the renal nerves

SARAH-JANE GUILD,1 GABRIELA A. EPPEL,2 SIMON C. MALPAS,1 NIWANTHI W. RAJAPAKSE,2 ALISTAIR STEWART,3 AND ROGER G. EVANS2

1Circulatory Control Laboratory, Departments of Physiology and Electrical and Electronic Engineering, and 3Biostatistics Unit, Division of Community Health, University of Auckland, Auckland, New Zealand; and 2Department of Physiology, Monash University, Melbourne, 3800 Australia

Received 25 February 2002; accepted in final form 15 July 2002

The renal medulla receives only a small fraction (~10%) of total renal blood flow, and flow per unit of tissue weight is only approximately 30–60% of that in the cortex (22, 23), yet its microcirculation appears to play an important role in the long-term maintenance of arterial pressure via its influence on sodium and water reabsorption (2, 4). The sympathetic nervous system clearly also plays an important role in arterial pressure regulation, and there is now much evidence implicating sympathetic overactivity as an important factor in the pathogenesis of essential hypertension (13). Given the role of the kidney in body fluid homeostasis, it is likely to be a key organ in the cascade of events linking sympathetic overactivity and hypertension (7, 8, 14, 16, 21). However, our understanding of the influence of the renal sympathetic nerves on the medullary microcirculation remains incomplete.

Our recent studies in anesthetized rabbits have provided evidence of differential neural control of cortical (CBF) and medullary perfusion (MBF). Thus MBF appears to be less sensitive to sympathetic nerve activity (SNA) than CBF, regardless of whether the nerves are activated by electrical stimulation (19) or reflexively (20). The relative insensitivity of the medullary microcirculation to the impact of SNA is particularly evident at relatively low stimulus intensities, as might occur under physiological conditions (19, 20). In our previous studies, MBF was measured by laser-Doppler flowmetry, with a standard flat probe on the cortical surface and a needle probe inserted into the kidney so that its tip was 10 mm below the kidney surface. Consequently, it cannot be assumed that these estimates of CBF and MBF were representative of flow throughout the renal cortex and medulla, respectively, because vascular organization is far from uniform throughout these kidney regions (22). For example, there are differences in the density and organization of vascular networks between the inner and outer medulla and even differences between the inner and outer stripes of the outer medulla (3). Indeed, Pallone et al. (22) suggest that the parallel arrangement of descending vasa recta in the vascular bundles of the medulla would theoretically allow preferential perfusion of the inner or outer medulla by constriction of the outer medullary portions of peripheral or central vessels of the bundle, respectively. However, because little is known about the relative density of the innervation in the various regions of these vascular bundles, the implications of

Address for reprint requests and other correspondence: R. Evans, Senior Research Fellow (NHMRC), Dept. of Physiology, Monash Univ., Victoria 3800, Australia (E-mail: Roger.Evans@med.monash.edu.au).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpregu.org 0363-6119/02 $5.00 Copyright © 2002 the American Physiological Society
this structural organization for the neural control of blood flow within the medulla remain unknown. Therefore, in the present study we examined whether renal SNA can differentially affect perfusion within both the renal cortex and medulla. To achieve this, we determined the responses to electrical stimulation of the renal nerves of laser-Doppler flux (LDF) at a range of depths below the kidney surface.

**METHODS**

Experiments were performed on male New Zealand White rabbits ($n = 13$, mean wt 2.71 ± 0.08 kg) and were approved in advance by the Monash University Department of Physiology/Central Animal Services Animal Ethics Committee. Animals were meal fed (9) and allowed water ad libitum until the experimental procedures began.

**Surgical Procedures**

These were similar to those used previously (19, 20) and thus will only be described briefly here. Induction of anesthesia was by intravenous administration of pentobarbital sodium (90–150 mg Nembutal; Rhone Merieux, Pinkenba, Queensland, Australia) and was immediately followed by endotracheal intubation and artificial respiration. Anesthesia was maintained throughout the surgery and experiment by pentobarbital sodium infusion (30–50 mg/h). During surgery 154 mmol/l NaCl solution was infused intravenously at a rate of 0.18 ml kg$^{-1}$ min$^{-1}$ to replace fluid losses. Body temperature was maintained at 36–38°C (20).

A catheter was inserted into the central ear artery for monitoring arterial pressure. The left kidney was approached via a retroperitoneal incision, and the renal artery and nerves were carefully exposed. The kidney was then freed from the peritoneal lining and surrounding fat and nerves were carefully exposed. The kidney was then freed from the peritoneal lining and surrounding fat and placed in a stable cup. A transit-time ultrasound flow probe (type 2SB Transonic Systems, Ithaca, NY) was placed around the renal artery for measuring renal blood flow (RBF). The major renal nerve trunks were then identified using a surgical microscope and placed across a pair of hooked stimulating electrodes. It is therefore likely that the electrodes were in contact with most, but not all, sympathetic nerves innervating the kidney. The nerves were then sectioned proximal to the stimulating electrodes. A mixture of paraffin oil and petroleum jelly was applied to the nerves throughout the experiment to prevent dehydration (19).

**Protocol 1 (n = 8)**

Regional kidney perfusion was estimated by laser-Doppler flowmetry, using two 26-gauge needle-type laser-Doppler flow probes (DP4s, Moor Instruments, Millwey, Devon, UK) that had been precalibrated using a motility standard provided by the manufacturer (Flux Standard, Moor Instruments). The probes were held in a stainless steel holder so that the tip of one probe was 4 mm forward of, and 10 mm lateral to, the tip of the other. The holder was directed toward the midpoint of the lateral aspect of the kidney by means of a micromanipulator (Narashige, Tokyo, Japan). Small holes were made in the renal capsule, ~5 mm from either side of the vertical midpoint of the lateral aspect of the kidney, to allow for insertion of the probes into the renal parenchyma. The probes were then inserted into the kidney so that the tips of the two probes were either 1 and 5, 3 and 7, 9 and 13, or 11 and 15 mm, respectively, below the surface of the kidney. Ten to 20 min were allowed for perfusion to stabilize before electrical stimulation commenced (see below). At the completion of the stimulation sequence, the probes were moved so that their tips were positioned at one of the other pairs of probe depths, and after a 10- to 20-min stabilization period, the electrical stimulation sequence was repeated. This whole process was repeated until all four pairs of depths had been tested, in a random order, giving a total of eight sets of measurements in each rabbit.

Because of the close proximity of these probes, it was necessary to determine whether there was interference between them. For example, light emitted from one probe might have been received by the other and interpreted as reflected light of its own emission. To test this, the probes were alternately disconnected from the laser source, and we tested whether there were any changes in the variables measured by the other probe. This test was performed both in the calibrating solution supplied by the manufacturer (Flux Standard, Moor Instruments) and in the kidney tissue during each experiment. In both cases there was no indication of interference, as assessed by changes in LDF, the laser-Doppler concentration signal [a measure of the amount of Doppler-shifted light detected by the probe, which varies with the number of moving erythrocytes or other sources of scattered light (31)], or backscatter [a measure of the amount of the transmitted light that is detected by the probe (31)].

At the conclusion of the experiment the rabbit was killed with an intravenous overdose of pentobarbital sodium (300 mg), and the left kidney was excised for postmortem measurements (see below). In our previous studies (19, 20) we corrected all LDF measurements for the background level measured after the rabbit was dead but still being artificially ventilated (which introduces a small movement artifact). Because the laser-Doppler probes had been moved to different positions during protocol 1, this was not possible. We did not attempt to estimate this in protocol 1 by, for example, periodic aortic occlusion, because we were concerned that repeated renal ischemia might damage the kidney and thus confound our observations. Therefore, a second study (protocol 2) was undertaken to determine whether this limitation of protocol 1 confounded our observations. The design of protocol 2 also allowed us to test whether tissue trauma associated with insertion and withdrawal of the needle probes affects baseline LDF or responses of LDF to renal nerve stimulation.

**Protocol 2 (n = 5)**

The experimental preparation was identical to that in protocol 1, except that a large-bore catheter was placed in the abdominal aorta below the level of the renal arteries for measurement of renal artery pressure and a snare was placed around the aorta above the level of the renal arteries so that renal artery pressure could be reduced to zero when required. Small holes were made in the renal capsule as for protocol 1, and both laser-Doppler needle probes were inserted into the kidney so that their tips were positioned 2 mm below the cortical surface. After a 20-min stabilization period, resting LDF was measured for 5 min and then renal artery pressure was reduced to zero for 60 s. During the final 20 s of this period, artificial ventilation was suspended to allow estimation of the contribution of respiratory movement artifact to the LDF signal. Once the hyperemic response to reperfusion concluded (~10 min), the needle probes were advanced so that their tips were positioned 7 mm below the cortical surface. After a 20-min stabilization period, responses to renal nerve stimulation were tested (see below), and then resting LDF and LDF during aortic occlusion (as above) were determined. The probes were then advanced to a depth of 12 mm, and after a 20-min stabilization period,
baseline LDF and LDF during aortic occlusion were again determined. The probes were then withdrawn to a depth of 7 mm again, and after a 20-min stabilization period, responses to nerve stimulation, resting LDF, and LDF during aortic occlusion were again determined. The rabbit was then killed with an overdose of pentobarbital sodium, and background LDF (at 7 mm, with and without artificial ventilation) was determined.

Electrical Stimulation

Electrical stimulation of the renal nerves was produced using purpose-written software in the LabVIEW graphical programming language (National Instruments, Austin, TX) coupled to a LabPC+ data-acquisition board (National Instruments). Initially, we determined the stimulation voltage that gave the maximum decrease in RBF in each rabbit, by giving short periods (30 s) of 8-Hz stimuli at various voltages to the renal nerves and recording the RBF response. This voltage (5–10 V) was used as the stimulating voltage for each subsequent stimulus sequence. Each stimulation series was applied using a pulse width of 2 ms at constant voltage, at five different frequencies in random order: 0.5, 1.0, 2.0, 4.0, and 8.0 Hz. The stimulation was of 3-min duration per step with a 5-min recovery period before delivering the next stimulus.

Data Acquisition

The ear artery catheter was connected to a pressure transducer (Cobe, Arvada, CO), the transit-time ultrasound flow probe was connected to a compatible flowmeter (TI08, Transonic Systems), and the laser-Doppler flow probes were connected to a laser-Doppler flowmeter (DRT4, Moor Instruments). These analog signals were digitized at 500 Hz and continuously displayed by a data-acquisition program (Universal Acquisition, University of Auckland, Auckland, New Zealand), allowing continuous sampling of mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min, derived from the MAP waveform), RBF (ml/min), and LDF (perfusion units; equivalent to the instrument output in mV10). In protocol 2 we also continuously sampled the laser-Doppler concentration signal (U). During each experiment, data were saved continuously as 2-s averages of each variable and as a 500-Hz binary file.

Statistical Analysis

The responses to stimulation were calculated from the average values of each variable during the minute before the stimulus began (control) and that during the final minute of stimulation. They are presented as means ± SE of either percent or absolute changes from control. P values ≤ 0.05 were considered to be statistically significant.

Protocol 1. We first determined whether resting levels of hemodynamic variables (MAP, HR, and RBF) changed across the course of the four stimulation series, using time (1st to 4th stimulation sequence) from a repeated-measures ANOVA. Using a mixed-modeling procedure to perform repeated-measures analysis, we then determined whether electrical stimulation of the renal nerves affected each variable in a frequency-dependent manner. The model used allowed the response of each rabbit (at each depth) to have its own regression slope and related the value of MAP, HR, or RBF during stimulation as the dependent variable to the baseline value before stimulation, the frequency of stimulation, and the depth of measurement. The effect of time on the response of each variable to electrical stimulation was also investigated using a similar analysis with time (1st to 4th stimulation sequence) as a factor.

To determine whether the effects of electrical stimulation of the renal nerves on LDF differed at various depths within the kidney, a mixed-modeling procedure was again used. A similar model to that above related LDF during stimulation as the dependent variable to the baseline perfusion before stimulation, the frequency of stimulation, and the depth of measurement. First, the difference in response between the cortex and medulla was investigated with an additional categorical variable, region, to separate the measurements made in the cortex (1 and 3 mm) from those made in the medulla (5–15 mm). Each region was then analyzed separately to assess the effect of depth within the cortex and medulla.

Protocol 2. ANOVAs were partitioned to make specific contrasts of resting LDF and the laser-Doppler concentration signal between various stages of the experiment. Student’s paired t-test was used to determine whether artificial ventilation affected the magnitude of background LDF. ANOVAs were used to test whether responses to renal nerve stimulation at 7 mm differed between the two stimulation series.

To test whether the results of protocol 1 were confounded by our inability to correct LDF for background, we applied the mean background levels obtained during aortic occlusion in protocol 2 (31, 31, and 44 LDF U, respectively, in the cortex, outer medulla, and inner medulla) to the raw data from protocol 1 and subjected these corrected data to an identical analytic procedure to that described above for the raw data. The validity of this approach is supported by our finding of little between-rabbit variation in these background levels (coefficient of variation <20%) and little variation between studies in our laboratory using these needle probes (see background LDF in Refs. 19 and 20).

Postmortem Measurements and Supplementary Experiments

At postmortem in each protocol, the left kidney was bi- sected transversely at the level of insertion of the needle probes, and the distances from the surface to the corticomedullary junction (3.9 ± 0.2 mm; range 3–5 mm) and the tip of the papilla (17.9 ± 0.6 mm; range 14–21 mm) were measured. For the five rabbits used in protocol 2, we also estimated the depth of the inner margin of the inner stripe of the outer medulla (7.0 ± 0.3 mm; range 6–8 mm). Thus all perfusion measurements at depths of ≥5 mm were considered to be made within the medulla, while those of ≤3 mm were considered to be made within the cortex. Measurements at depths from ~4–7 mm most likely represent the outer medulla.

We performed an additional experiment to determine the volume of tissue from which laser-Doppler measurements are derived. The two needle probes were set up to face each other at a 180° angle and were advanced toward each other in steps using micromanipulators, both in air and in kidney tissue excised at postmortem. In kidney tissue, we found that the backscatter (DC) signal [which is proportional to the amount of laser-derived light detected by the probe (31)] was uniformly low (5–11% of its maximum detectable range) until the distance between the probes was <1.5 mm. At 1.0-, 0.5-, and 0-mm separation, the signal increased to be 23, 54, and 100% of its maximum detectable level, respectively (Fig. 1A). Provided that the optical transmittance of the kidney was not greatly affected by the conditions of this experiment (e.g., the tissue was at room temperature and was not perfused), these observations indicate that virtually all of the LDF signal.
arises from within 1.5 mm of the probe tip. When this experiment was repeated in air, backscatter signals were detected when the probes were as far away as 10 mm, demonstrating the dependence of the estimated volume of LDF measurement on the physical properties of the medium within which measurements are made.

To determine the linearity of our laser-Doppler flowmetry system, the needle probes were positioned on the outside of a length of polyvinyl chloride tubing (OD 1.52 mm, ID 0.86 mm) through which human blood diluted to a hematocrit of 8% with 154 mM NaCl was infused at known rates. The relationship between the flow of dilute human blood (hematocrit = 8%) through polyvinyl chloride tubing (OD 1.52 mm, ID 0.86 mm) and laser-Doppler flux (LDF). The relationship between LDF and blood flow remained linear until calculated erythrocyte velocity was $\sim 4$ mm/s (0.14 ml/min). Erythrocyte velocity in renal capillaries is <1 mm/s (27, 29).

**RESULTS**

**Protocol 1**

Baseline levels (levels before each period of nerve stimulation, averaged over the 5 frequencies) of total RBF (24 ± 2 ml/min), MAP (69 ± 2 mmHg), and HR (244 ± 6 beats/min) were similar to those we have observed previously (19). With the exception of RBF, these remained stable across the course of the experiment ($P_{time} > 0.1$ for HR and MAP). Baseline RBF did not change significantly across the course of the experiment ($P_{time} = 0.03$); it was on average 2.8 ± 0.5 ml/min greater during the first set of nerve stimuli than during the other three (Fig. 2). The baseline levels of LDF in the cortex (measured at 1 and 3 mm) were significantly greater than in the medulla (measured at 5–15 mm) ($P_{region} = 0.003$) (Fig. 3). Within both the cortex and medulla, baseline LDF was not significantly affected by the depth of measurement ($P_{depth} = 0.85$ and $P_{depth} = 0.11$, respectively).

Electrical stimulation of the renal nerves caused frequency-dependent decreases in RBF (by 19 ± 2

![Fig. 1. Results of bench-top experiments testing the characteristics of the laser-Doppler flowmetry system used in these studies. A: backscatter (DC) values as the 2 needle probes (facing each other at 180°) were advanced toward each other in air and in kidney tissue. Values are mean values for both probes. B: relationship (for 1 of the probes) between the flow of dilute human blood (hematocrit = 8%) through polyvinyl chloride tubing (OD 1.52 mm, ID 0.86 mm) and laser-Doppler flux (LDF). The relationship between LDF and blood flow remained linear until calculated erythrocyte velocity was 4 mm/s (0.14 ml/min). Erythrocyte velocity in renal capillaries is <1 mm/s (27, 29).](http://ajpregu.physiology.org/)

![Fig. 2. Mean baseline levels (levels before each period of nerve stimulation, averaged over the 5 frequencies) of total renal blood flow (A), mean arterial pressure (B), and heart rate (C) for the 1st, 2nd, 3rd, and 4th stimulation sequences in protocol 1. The mean levels of mean arterial pressure and heart rate remained stable across the experiment. Total renal blood flow was on average 2.8 ± 0.5 ml/min greater during the first set of nerve stimuli than in the other 3 (*Statistical significance, $P_{time} = 0.03$).](http://ajpregu.physiology.org/)
Cessation of artificial ventilation reduced the LDF signal during aortic occlusion by 19 ± 3% across all three depths. At a depth of 7 mm, baseline LDF and laser-Doppler concentration and LDF during aortic occlusion were similar when these were initially tested compared with measurements made after the probes had been inserted to, and subsequently withdrawn from, 12 mm (Table 1).

As in protocol 1, electrical stimulation of the renal nerves caused frequency-dependent reductions in RBF and medullary LDF (at 7 mm and corrected for background during aortic occlusion). When the stimulation

ml/min at 8 Hz; \( P_{\text{freq}} = 0.0001; \) Fig. 4), which were similar during each of the four stimulation sequences (\( P_{\text{time}} = 0.47 \)). These were accompanied by small but significant increases in MAP (4 ± 1 mmHg at 8 Hz; \( P_{\text{freq}} = 0.0001 \)) and reductions in HR (2 ± 1 beats/min at 8 Hz; \( P_{\text{freq}} = 0.01 \)) (Fig. 4).

Electrical stimulation of the renal nerves also caused frequency-dependent decreases in LDF at each of the eight depths at which it was measured (\( P_{\text{freq}} \) always ≤ 0.001). Marked differences were observed in the LDF responses to electrical stimulation between cortical and medullary regions. For example, 4-Hz stimulation reduced LDF measured at a depth of 1 mm by 54 ± 11% but only reduced LDF at 9 mm by 19 ± 6% (Fig. 5). Our statistical analysis demonstrated that reductions in LDF in the cortex (measured at 1 and 3 mm) were systematically greater than those in the medulla (measured at 5–15 mm) (\( P_{\text{freq} \times \text{region}} < 0.001 \)) but that there were relatively uniform responses within both the cortex (\( P_{\text{depth}} = 0.80 \)) and medulla (\( P_{\text{depth}} = 0.97 \)) (Figs. 5 and 6).

**Protocol 2**

Baseline LDF 2 mm below the cortical surface was 311 ± 87% of that at 7 mm (\( P = 0.003 \)), but LDF at 7 and 12 mm was not significantly different (Table 1). The laser-Doppler concentration signal was similar at 2 and 7 mm but was 24 ± 8% greater at 12 mm. LDF during aortic occlusion was similar at 2 and 7 mm (31 ± 4 U) but was slightly greater (44 ± 5 U) at 12 mm. There was little between-rabbit variation in these background LDF levels. Furthermore, they were similar to those we have observed postmortem, using these needle probes at a depth of 10 mm, in two previous studies [41 ± 8 U (19) and 35 ± 5 U (20), respectively].

**Fig. 4.** Response of renal blood flow (A), mean arterial pressure (B), and heart rate (C) to electrical stimulation of the renal nerves (supramaximal voltage (5–10 V) and 2-ms pulse duration), at frequencies of 0.5, 1, 2, 4, and 8 Hz in protocol 1. The mean responses from all sets of stimulation for all rabbits are shown. There were frequency-dependent changes in all 3 variables during the periods of electrical stimulation (\( P_{\text{freq}} < 0.01 \)).
sequence was repeated after the needle probes had been inserted to 12 mm, and then withdrawn back to 7 mm, the responses of both RBF and medullary LDF were reduced, but to a similar extent ($P = 0.97$; comparison of RBF and LDF by ANOVA). For example, at 8 Hz the percent reductions in RBF and medullary LDF during the second stimulation sequence were 7 ± 3 and 12 ± 8%, respectively, less than during the first stimulation sequence (Fig. 7). Electrical stimulation of the renal nerves also reduced medullary laser-Doppler concentration, at least at 4 Hz (10 ± 7%) and 8 Hz (23 ± 7%) during the initial stimulation sequence. These reductions in concentration were blunted during the second stimulation sequence (Fig. 7).

Correction of LDF for estimates of biological zero or background resulted in increases in calculated percentage changes in MBF, particularly at higher stimulation frequencies (Fig. 8). For example, when no corrections for background were made, 1- and 8-Hz stimulation reduced estimated MBF by 10 ± 2 and 67 ± 6%, respectively, yet the corresponding values were 12 ± 4 and 83 ± 11%, respectively, when the LDF signals were corrected for the value observed during aortic occlusion.

Analysis of Responses to Nerve Stimulation (Protocol 1) Using Estimates of Background from Protocol 2

As estimates of background LDF were not obtained at each depth in protocol 1, we applied the mean background levels obtained during aortic occlusion in protocol 2. We found that, as with the raw LDF data, there was a significant difference between the cortex (1–3 mm) and medulla (5–15 mm) in both baseline LDF ($P_{region} < 0.001$) and responses to nerve stimulation ($P_{freq \times region} = 0.03$). However, within the cortex and medulla, responses to nerve stimulation were relatively uniform (Fig. 6; $P_{depth} > 0.2$).

DISCUSSION

In this study we electrically stimulated the renal nerves at a supramaximal voltage and at a range of frequencies so as to produce graded renal vasoconstriction. We simultaneously recorded tissue perfusion by laser-Doppler flowmetry at a range of depths below the kidney surface. Consistent with previous findings (17–20), medullary LDF (5–15 mm below the kidney surface) was less sensitive to neural activation than was cortical LDF (1–3 mm below the kidney surface). Our new finding was that responses of LDF to nerve stimulation within each of these vascular territories were relatively uniform.

The mechanisms underlying the relative insensitivity of MBF to the impact of the renal nerves remain to be definitively determined, as do the precise vascular elements that regulate MBF in vivo. We can be confident that this is not an artifact due to differential autoregulation of CBF and MBF, because medullary LDF is efficiently autoregulated in anesthetized rabbits between at least 50 and 90 mmHg (6) and MAP...
increased by 4 mmHg at most during renal nerve stimulation. The medullary microcirculation arises from the efferent arterioles of juxtamedullary glomeruli, so although it is in series with this anatomically specific subset of the cortical circulation, it can also be considered to be in parallel to the bulk of the cortical circulation. Thus structural and functional differences between juxtamedullary arterioles and those in other regions of the cortex would contribute to the differential neural regulation of CBF and MBF. Furthermore, the sympathetic innervation extends to the outer medullary descending vasa rectae, which likely also play some role in the regulation of MBF (2, 22, 23). Thus regional differences in the density of sympathetic innervation, postjunctional responsiveness to norepinephrine and other sympathetic neurotransmitters, or structural differences in arteriolar diameter and smooth muscle content might contribute, as we have discussed previously (19, 20). Regional differences in local counterregulatory vasodilator mechanisms might also play some role. One interesting possibility is that norepinephrine may activate \( \alpha_2 \)-adrenoceptors in the medullary microcirculation to release nitric oxide, which can act to blunt \( \alpha_1 \)-mediated vasoconstriction (25, 32). Another possible mechanism relates to regional differences in the geometry of glomerular arterioles. Juxtamedullary (particularly efferent) arterioles have greater diameters than their counterparts in other cortical regions, so Poussiel's relationship predicts that equivalent vasoconstriction should increase resistance to a lesser extent in juxtamedullary arterioles, which are in series with the medullary microvasculature, compared with those in cortical vascular territories in parallel with the medullary microvasculature. This likely explains in part why renal arterial infusion of ANG II in rabbits can reduce CBF but not MBF (25), even though it reduces the diameters of juxtamedullary arterioles to a similar extent to those in the outer and midcortex (5). This mechanism may also contribute to the relative insensitivity of MBF to the impact of renal SNA.

There is a number of previous studies, using a range of techniques, that have provided data at odds with our present and previous (19, 20) observations. Some early studies used \(^{85}\)Kr autoradiography (24) or \(^{86}\)Rb uptake (12), methods that allow some distinctions to be made across anatomic regions. These studies suggested variously that MBF is more sensitive than CBF to neural activation (12) or that renal nerve stimulation can increase MBF (24). Regardless, these studies should be interpreted with great care, because the validity of their methodology has been questioned (11, 23).

More recent studies have used more valid methods, such as local hydrogen gas clearance (1) and laser-Doppler flowmetry (17–20, 28), but which provided data from only small anatomic regions in the outer medulla (1), inner medulla (17–20), and papillary tip (28). Taken together, the results of these previous studies suggest that, compared with CBF (or total RBF), perfusion of the outer medulla is similarly sensitive (1), perfusion of the inner medulla is less sensitive (19, 20), and perfusion at the tip of the papilla is almost completely insensitive (28), to the effects of neural activation. That is, given the caveat that these experiments were performed in different species and under different experimental conditions, they provide indirect evidence supporting the hypothesis that a gradient of sensitivity to neural stimulation exists from the outer medulla to papillary tip. In the present study we directly tested this hypothesis, and our data provide evidence for its rejection.

There is also evidence from previous studies that renal sympathetic drive can differentially affect blood flow within the renal cortex. Using radioactive microspheres in isolated and blood-perfused dog kidneys, Gotshall and Itskovitz (10) found that electrical stimulation of the renal nerves reduced blood flow in the inner cortex more than that in the outer cortex. Our present results indicate that a similar phenomenon does not occur in vivo. On the other hand, because all blood flow to the renal medulla arises from juxtamedullary glomeruli, we would expect that perfusion of these glomeruli should be less sensitive to the effects of the renal nerves than those in other cortical regions. Our present data are not necessarily at odds with this concept, because our methodology did not provide suf-
sufficient anatomic resolution for us to specifically monitor perfusion within the juxtamedullary cortex.

The above conclusions, however, must be limited to the effects of electrical stimulation, where all individual nerves in the nerve bundle are stimulated. We cannot as yet exclude the possibility that reflex increases in renal sympathetic nerve activity might involve functional recruitment of neurons and subsequent redistribution of blood flow within the renal medulla and cortex.

A criticism of previous studies (17–20, 28) using laser-Doppler flowmetry to examine the impact of renal SNA on regional kidney blood flow is that LDF measurements were made in small areas of tissue that may not be representative of the entire vascular territory in question. Our present results suggest that these previous observations have not been confounded by this limitation of the laser-Doppler technique. Furthermore, although the laser-Doppler technique measures red blood cell flux (LDF) in arbitrary units rather than an absolute value of flow (ml/min), our supplementary study confirmed the linear relationship between these variables. The presentation of results as a percentage of prestimulus control therefore allows a direct comparison between responses in different regions of the kidney, regardless of the resting level of perfusion.

Because our present study used a within-animal design, our observations are not confounded by between-animal differences in the impact of renal nerve stimulation on the renal circulation. These could arise from variations in the sensitivity of the renal vasculature to SNA or variations in the experimental preparation, resulting in greater or lesser proportions of the total number of renal sympathetic nerve fibers being stimulated. This latter factor may account for the fact that RBF was more profoundly reduced by renal nerve stimulation in protocol 2 compared with protocol 1. Regardless, such variability between protocols does not confound the interpretation of our experiment because all comparisons were made in a within-animal fashion.

A limitation of our initial experiment (protocol 1) was that we were unable to determine the background or biological zero signal (15) at each depth below the cortical surface and thereby correct the raw LDF measurements for this systematic error. Therefore, the percentage changes in LDF we calculated underestimated the true magnitude of the effect of renal nerve stimulation on perfusion, particularly in the medulla, where baseline LDF is lower than in the cortex. This does not undermine the notion that MBF is less sensitive than CBF to the impact of the renal nerves, because this systematic error was accounted for in our

Fig. 7. Changes in renal blood flow (A), medullary LDF (B), and medullary laser-Doppler concentration (C) in response to 2 sequences of electrical stimulation of the renal nerves in protocol 2. For calculation of changes in medullary LDF, raw LDF was corrected for its background level during aortic occlusion (see Table 1). Symbols and error bars represent means ± SE of percent changes in each variable (n = 5). The first stimulation sequence was applied after insertion of the laser-Doppler probes at a depth of 7 mm below the cortical surface (●). The probe was then advanced to 12 mm for 20 min and subsequently withdrawn back to 7 mm (○). P values are the outcomes of ANOVAs, testing whether responses differed between the 2 stimulation sequences.

Fig. 8. Percent changes in medullary LDF from protocol 2 (7-mm depth), calculated from raw LDF units (●) or from LDF units from which estimates of biological zero (background) have been subtracted (○). Symbols and error bars represent means ± SE of levels averaged across the 2 stimulation sequences shown in Fig. 7.
previous studies (19, 20). However, it could potentially undermine our present conclusion regarding the sensitivities of perfusion within these regions to neural activity, particularly if large differences in resting or background LDF are encountered. However, this seems unlikely for four reasons. First, we quantified this systematic error in protocol 2. The error becomes greater as stimulus intensity increases, so at 1 and 8 Hz, differences between corrected and uncorrected percent changes in outer medullary LDF (7 mm) were only 2 and 16% of resting LDF, respectively, well within the range of variation in responses observed in protocol 1. Second, we found that background LDF was similar in the outer medulla (7 mm) and the cortex (2 mm), although it was slightly but statistically significantly greater in the inner medulla (12 mm). Third, in neither of the experimental protocols could we detect statistically significant differences in baseline LDF between inner and outer medullary sites. Fourth, application of the mean background LDF in the cortex (31 U), outer medulla (31 U), and inner medulla (44 U) determined in protocol 2 to the data obtained in protocol 1 had little impact on the outcomes of our statistical analyses. Nevertheless, our results do not allow us to completely exclude the possibility of regional differences in the responses of perfusion to renal SNA within the cortex and medulla but do show that they must at least be markedly less than those between these vascular territories.

The LDF signal is the product of the mean velocity of moving objects that reflect the laser signal (chiefly erythrocytes) and the amount of Doppler-shifted light detected by the probe (the concentration signal, which reflects the volume density of moving erythrocytes) (31). The concentration parameter was continuously recorded in protocol 2, but not protocol 1, so we do not have information regarding the impact of renal SNA on the concentration signals at different sites within the kidney. In protocol 2, we found that the basal concentration signal was similar in the cortex and outer medulla (although it was slightly greater in the inner medulla), consistent with previous studies showing little regional heterogeneity in the volume density of erythrocytes in the kidney (26, 30), even though capillary hematocrit is considerably less in the medulla (particularly the inner medulla) than the cortex (23). We also found that the concentration signal in the outer medulla was reduced during renal nerve stimulation, but only at the highest frequencies. This suggests that reductions in both red blood cell velocity and the volume density of erythrocytes (reflecting the number of perfused vasa recta) probably contribute to reduced MBF during renal nerve stimulation.

A strength of our experimental approach in protocol 1 was its randomized, within-animal design. Randomization by depth ensured that changes in the state of the preparation over the course of the experiment would not confound our observations. As it was, resting hemodynamic variables and their responses to electrical stimulation of the renal nerves were remarkably stable in protocol 1. The disadvantage of this experimental approach was that, between stimulation sequences, the probe tips were either advanced further into the renal parenchyma toward the tip of the papilla or withdrawn toward the cortical surface. Although these probes have a diameter of only 0.46 mm, this must have produced some damage and subsequent hemorrhage. However, postmortem examination of the tissue revealed little macroscopic damage, and the results of protocol 2 showed that insertion and subsequent withdrawal of the probes had no detectable effects on baseline or background LDF or baseline concentration. There were statistically significant reductions in both the RBF and LDF responses to nerve stimulation, but these were similar and so likely reflect time-dependent changes in the impact of the nerves on renal hemodynamics in this protocol (but not protocol 1), rather than a technical problem associated with our LDF measurements.

Our experiment using postmortem kidney tissue suggested that LDF signals in kidney arise almost exclusively from within 1.5 mm of the probe tip. This suggests that the 2-mm intervals between the eight sites of LDF measurements used in protocol 1 were sufficient to ensure no overlap between the measurement sites. The size of the rabbit kidney (~18 mm to the tip of the papilla) lends itself well to this type of study. Similar studies in smaller species such as the rat (~9 mm to the tip of the papilla) would provide less spatial resolution than that achieved herein in rabbits. Ideally one would have performed the measurements at anatomically equivalent positions in each rabbit, but this was not possible in an intact kidney. Nevertheless, our postmortem measurements of gross kidney morphology indicate that the various depths of measurement can be roughly equated with the cortex (1–4 mm), outer medulla (5–7 mm), and inner medulla (8–15 mm).

In conclusion, the results of the present study indicate that while the vascular elements controlling MBF are less sensitive to the effects of electrical stimulation of the renal nerves than are those controlling CBF, sensitivity within these vascular territories appears to be relatively homogeneous. This information is important for two reasons. First, these data do not support the notion that the renal nerves can differentially affect perfusion at different levels within the medulla. Furthermore, it allows us to interpret our previous studies (19, 20), in which MBF and CBF were monitored at single sites, with greater confidence. Importantly, it appears that provided the probe is advanced past the corticomedullary junction, the relative response of LDF to electrical stimulation of the renal nerves is representative of the response within the entire medulla.

This work was supported by grants from the National Health and Medical Research Council of Australia (143603, 143785), the National Heart Foundation of Australia (G 98M 0125), the Ramaciotti Foundations (A 6370, RA 159/98), and the Marsden Fund of New Zealand. N. Rajapakse is a recipient of a Commonwealth Postgraduate Scholarship (Australia).