Long-term control of renal blood flow: what is the role of the renal nerves?

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Received 16 August 2000; accepted in final form 4 January 2001

Barrett, Carolyn J., Michael A. Navakatikyan, and Simon C. Malpas. Long-term control of renal blood flow: what is the role of the renal nerves? Am J Physiol Regulatory Integrative Comp Physiol 280: R1534–R1545, 2001.—We have developed a system for long-term continuous monitoring of cardiovascular parameters in rabbits living in their home cage to assess what role renal sympathetic nerve activity (RSNA) has in regulating renal blood flow (RBF) in daily life. Blood pressure, heart rate, locomotor activity, RSNA, and RBF were recorded continuously for 4 wk. Beginning 4–5 days after surgery a circadian rhythm, dependent on feeding time, was observed. When averaged over all days RBF to the innervated and denervated kidneys was not significantly different. However, control of RBF around these mean levels was dependent on the presence of the renal sympathetic nerves. In particular we observed episodic elevations in heart rate and other parameters associated with activity. In the denervated kidney, during these episodic elevations, the increase in renal resistance was closely related to the increase in arterial pressure. In the innervated kidney the renal resistance response was significantly more variable, indicating an interaction of the sympathetic nervous system. These results indicate that whereas overall levels of RSNA do not set the mean level of RBF the renal vasculature is sensitive to episodic increases in sympathetic nerve activity.

conscious rabbit; sympathetic nervous system; renal denervation; circadian

The kidney receives a dense innervation of sympathetic nerves, and changes in the activity present in these nerves affects renal hemodynamics, sodium excretion, and renin release (9). Early studies, however, suggested that the vasculature was relatively insensitive to small changes in renal sympathetic nerve activity (RSNA). In past experiments RSNA was progressively increased, using either electrical stimulation of renal nerves in anesthetized cats or dogs (3, 10, 23) or reflex activation in conscious dogs (19, 21). It was found that at low RSNA levels only renin secretion was affected, then with slightly higher levels of activity changes in sodium excretion occurred, but only with even greater levels was renal blood flow (RBF) affected. This led to the concept that in daily life changes in RSNA around resting levels would have little impact on RBF (4). However, this concept has not been without challenge; Grady and Bullivant (8) measured RBF in conscious rats in their home cages during various activities ranging from sleep to active movement and found RBF decreased as the activity level of the rats increased, an effect negated by prior blocking of RSNA with local anesthetic. In the conscious rabbit a range of studies has suggested that a modest increase or decrease in RSNA does affect RBF. Air jet stress, noise stress, and hypoxia, each of which increased RSNA between 12 and 31%, produced RBF decreases of 8–12% from control levels (16). Conversely blood volume expansion that decreased RSNA 25% caused an increase in RBF of 17% (13). In each case the dynamic changes in RSNA with the onset of the stimuli were mirrored by changes in RBF and responses were absent in renally denervated animals.

One criticism of the study by Grady and Bullivant (8) was that because RSNA was not measured directly it was not possible to determine to what degree RSNA was altered during changes in the activity levels of the rats. This is important because the debate is not whether the renal nerves can affect blood flow but rather whether small physiological changes in RSNA around resting levels alter RBF. With regard to previous rabbit studies (13, 16), although experiments have been performed in conscious animals, the experiments have always been conducted in a laboratory environment in which it is likely baseline RSNA was higher than would be found in the home-cage environment. Thus, although resting RBF was higher in renally denervated rabbits compared with innervated animals (17), it could be argued that the baseline RSNA levels were high enough to impact the renal vasculature under the baseline conditions of the experiment. To fully test the hypothesis that physiological changes in RSNA play a role in regulating the resting level of RBF and counter the criticisms of previous studies, it is necessary to develop a new approach that allows continuous recording of RSNA and RBF in animals in their home-cage environment. It is proposed that knowledge of the degree of control of RBF by RSNA is...
fundamental in understanding how diseases such as hypertension and heart failure may involve the sympathetic nervous system in their pathogenesis. Because relatively long-term recordings of RSNA (up to 4 wk) in the rabbit are possible, this may provide the framework for investigating the link between sympathetic activity and the long-term control of arterial pressure (2).

To characterize the effect of the renal nerves under daily life conditions we have undertaken continuous recordings of RSNA, arterial pressure, and RBF for a 4-wk period in rabbits. Because resting RBF can be quite different between animals, in each animal we have measured RBF to each kidney, one being innervated the other denervated. Thus the arterial pressure and circulating hormone levels seen in each kidney will be the same. We have assessed the spontaneous and circadian changes in each parameter in testing the hypothesis that the renal nerves play a role in regulating RBF in daily life.

METHODS

Animal preparation. Experiments were conducted in eight New Zealand White rabbits with initial weights of 2.4–3.5 kg and were approved by the University of Auckland Animal Ethics Committee. For all surgical procedures anesthesia was induced using intravenous administration of propofol (Diprivan, 10 mg/kg) followed by intubation and then maintenance with halothane. Arterial pressure was recorded throughout the study via a radiotelemetry transmitter (model PA-D70, Data Sciences International, St Paul, MN). This was implanted via an abdominal incision, and the area around the iliac bifurcation was exposed. The cannula of the transmitter was inserted into the left iliac artery and advanced so that the tip of the catheter lay in the abdominal aorta ~1 cm above the iliac bifurcation but well below the renal artery. The cannula was fixed in position with a drop of tissue glue, and the body of the transmitter was placed in the abdominal cavity. The incision was closed, and the animal was allowed to recover for 1 wk.

A second surgery was performed to insert renal flow probes, to denervate one kidney, and to place a recording electrode around the other renal nerve. Each renal artery and associated nerves were exposed via a retroperitoneal incision. A transit time flow probe (type 2SB Transonic Systems, Ithaca, NY) was placed around each renal artery, and a small sheet of silicone plastic was sewn around the flow probe to help maintain probe alignment. The renal sympathetic nerves were identified distal to the flow probes using a dissecting microscope. On one side all visible nerves were destroyed along the length of the artery (normally a 1-cm section of the nerves was destroyed), and the artery was painted with isopropyl alcohol. We have used this technique extensively in the past and have found the denervation to be effective, with the norepinephrine content of the denervated kidneys only 2% that of the innervated kidneys 3 wk after denervation (11). On the other side the intact nerve was fed through a coiled electrode, and the electrode and nerve were coated in Sil-Gel (Wacker-Chemie, Munich, Germany) to insulate it from surrounding tissue (5). To avoid movement artifacts affecting the RSNA signal, a purpose-built amplifier (10 × 5 × 7 mm, length × height × width) was implanted as close to the nerve site as possible. This amplifier had a fixed gain of 1,000. Because of the shortness of the right renal artery in rabbits, RSNA was recorded only from the left side, and thus animals in which the renal nerves to the right kidney were left innervated did not have their RSNA recorded. The other ends of the flow probes and nerve electrode were tunneled subcutaneously to the back of the neck where they were exteriorized using skin buttons.

After each surgery the rabbits were treated prophylactically with an antibiotic (enrofloxacin, Baytril, Bayer, New Zealand; 5 mg/kg sc daily for 5 days) and analgesic (ketoprofen, Ketofen, Rhone Merieux, Essex, UK; 2 mg/kg sc daily for 3 days). As soon as the rabbits regained consciousness they were returned to their home cages. A heating pad was placed in the cage for 24 h after the surgery. The rabbits were housed individually in cages (height 40 cm, width 35 cm, and depth 55 cm) with a telemetry receiver (model RLA2000, Data Sciences), positioned on the ceiling inside each cage. The rabbits were fed daily (100 g standard rabbit pellets, supplemented with hay, carrot, and apple) at 0900, and water was available ad libitum. The room was kept at a constant temperature (18°C) and dark-light cycle (lights on from 0600 to 1800).

Data collection. Once rabbits were returned to their home cages, the flow probe and RSNA recording wires were immediately connected to the relevant amplifiers via a swivel system (Airflyte Electronics, Bayonne, NJ). This system allowed rabbits a complete range of movement. Initially locomotor activity was recorded by displacement...
of the blood pressure transmitter located in the rabbit’s abdomen relative to the blood pressure receiver. In preliminary experiments we found this system recorded only large movements of the rabbit. Therefore we later developed a purpose-built activity monitor that used an infrared detector (Optex RX-40QZ, Otsu, Japan) to detect all animal movements. Data collection began within 3 h of completion of surgery. RSNA was further amplified outside the animal between 20 and 200 times to give a final gain between 20,000 and 200,000, filtered between 50 and 2,000 Hz, full-wave rectified and integrated using a low-pass filter with a time constant of 20 ms. With chronic continuous recording conditions it is not possible to experimentally determine a “zero” baseline RSNA level, e.g., using ganglionic blockade. However, the approach of rectification and integration of the original RSNA signal allowed us to automatically subtract the baseline noise. The integration of RSNA over 20 ms reflects the bursts of RSNA as a series of waves or peaks, and between these bursts there are silent periods where the integrated level drops to baseline. Our data acquisition program automatically set the background noise, which is of a constant magnitude, to zero. This integrated RSNA signal, the RBF waveforms via transit time flowmetry (Transonic Systems), and arterial pressure were sampled at 500 Hz using an analog-to-digital data acquisition card (AT-MIO64E-3 National In-
All subsequent data collection and analysis were performed using a purpose-written data acquisition program developed using the LabVIEW graphical programming language (National Instruments). In brief, calibrated signals were continuously displayed on a computer screen both as the pulsatile waveforms and as averaged data. Previously collected data could be viewed online from minutes to days. Although data was sampled at 500 Hz, the length of the experiment (28 days) did not permit continuous saving of all of these data. Two-second averages of all parameters were saved continuously to disk. In addition 10-min periods of the 500-Hz data were automatically saved every 2 h to record the quality of the RSNA and RBF signals. Because absolute microvolt levels of RSNA cannot be compared between animals, we converted RSNA to a percentage of the average microvolt values obtained from a 4- to 10-day period at least 11 days after surgery. The mean microvolt value for this period was defined as 100%. We regularly validated our RSNA signal by triggering off the systolic pressure and constructing averaged RSNA for 1-s periods (Fig. 1). In general this cardiac-related signal was apparent for at least 21 days postoperatively (maximum 32 days).

**Statistical analysis.** Data are presented as mean ± SE. Unless otherwise stated data were compared using paired two-tailed *t*-tests. To examine relationships between different parameters a coefficient of linear correlation was calculated. To compare the regression lines between intact and denervated kidneys a linear mixed model was used because it allows the simultaneous modeling of the fixed effects (differences between slopes or intercepts) and random effects (estimates of variances). In this case the fixed factor is either the innervated or the denervated kidney, while the random factor is the rabbit. The subroutine PROC MIXED in SAS version 8 was used to fit the models. Preliminary visual examination of the data showed consistent heterogeneity of residual variances between regression lines for the innervated and denervated RBF and renal resistance within rabbits. This was accommodated by allowing separate error variances for innervated and denervated kidneys. A signifi-
RESULTS

Circadian profile. In each rabbit blood pressure, heart rate activity, RSNA, and RBF to one innervated and one denervated kidney were recorded continuously for up to 32 days. In six of the eight rabbits the heart rate and blood pressure gradually fell over the first 5–6 days of recording in each rabbit eliminated, is shown in Fig. 2). In every rabbit a circadian profile was characterized by an abrupt shift in the circadian profile in all parameters with the increase now occurring at 1500 (Fig. 4).

Comparison between RBF to innervated and denervated kidneys. When looking at the group data, we found that the RBF to innervated and denervated kidneys was not significantly different (Table 1). Immediately after feeding, an increase in both RBF and MAP was observed, and at the same time renal resistance decreased. A decrease in resistance at the same time as an increase in pressure suggests that the increase in RBF must have been proportionally greater than the increase in MAP. Surprisingly, this decrease in resistance coincided with the increase in RSNA (Fig. 3). Renal resistance in the innervated kidney and the denervated kidney was not significantly different (Fig. 5).

Although overall levels of RBF and renal resistance were not different between innervated and denervated kidneys, this value was derived from data collected over many days of recording. This averaging process tended to minimize the effect of transient changes in any parameter. We hypothesized that it may be during transient changes that the control of RBF is different between kidneys, i.e., rather than the mean level of RBF being different, it is the ability to control transient changes about the mean level that is different in the presence of RSNA. As examples of such transient changes we observed spontaneous events most distinguishable by an abrupt increase in heart rate, but also...
associated with rises in MAP, RSNA, and renal resistance, which we termed episodic elevations. These episodic elevations were observed in every rabbit (Fig. 6) and generally were associated with locomotor activity.

During the episodic elevations it appeared that heart rate and RBF were shifting between certain levels. We plotted the frequency of coinciding heart rate and blood flow values to further examine whether “preferred” values were occurring with the elevations. In each animal there seemed to be preferred states with distinct peaks in the data (Fig. 7). In the example presented, the relationship between blood flow to the innervated kidney and heart rate in one rabbit over a 24-h period is shown (42,793 points) and clearly illustrates four distinct peaks. Two peaks occurred during the nighttime period: the baseline, where heart rate was ~220 beats/min and RBF 45 ml/min, and a peak that corresponded to the episodic elevations where heart rate was 280 beats/min and RBF 47 ml/min. During the day a peak at 250 beats/min and 59 ml/min occurred between the episodic elevations, and a second, even greater peak at about 300 beats/min and 58 ml/min occurred during episodic elevations.

To further characterize the episodic elevations we used the heart rate increases to automatically detect them; a moving average over 200 s was performed and any increase or decrease in heart rate above 10 beats/min within the 200 s was identified as the start or end of an elevation. The 200-s period immediately before and after the elevation was taken as the baseline, and
mean values for each parameter during the elevation were calculated. The frequency of elevations was evenly spread across the day and night, with 2.2 elevations per hour and between 34 and 81 elevations per 24 h across all eight rabbits. The duration of the elevations averaged 11 min (range 1–81 min), but they were consistently longer during the day than the night, with an average 27 min of each hour occupied by the elevation during the daytime hours compared with 21 min during the night ($P < 0.05$). The increase in heart rate at the beginning of each episodic elevation generally occurred within 1 min (the time constant for heart rate to reach 63% of its steady-state value during the elevation had a median of 17 s, mean 27 ± 4 s). The average increase in heart rate for all rabbits was 25 ± 1 beats/min (Table 2).

During each elevation there was a strong positive correlation between the change in heart rate and both the change in MAP and in resistance in each kidney (Table 2). There also tended to be a correlation between the change in heart rate and the change in RSNA, although this did not reach significance ($n = 4$). A negative correlation existed between the change in RBF to the denervated kidney and the elevations in heart rate (7 of 8 rabbits, $P < 0.01$). No correlation was seen with the flow to the innervated kidney (4 rabbits had slight positive correlations and 4 negative).

The change in renal resistance that occurred with the episodic elevations were small but significant. Although the mean change in flow or resistance that occurred with the elevations was not different between the innervated and denervated kidney (Table 2), in each animal there was a clear difference in the distribution of the responses in the two kidneys ($P < 0.001$ using Brandt-Snedecor $\chi^2$ test for comparison of arbitrary distributions, Fig. 8). The change in flow to the denervated kidney with each elevation showed a narrow distribution, whereas the response in the innervated kidney was more variable. Similarly, the fall in RBF and increase in renal resistance that occurred with the episodic elevations were small but significant. Although the mean change in flow or resistance that occurred with the elevations was not different between the innervated and denervated kidney (Table 2), in each animal there was a clear difference in the distribution of the responses in the two kidneys ($P < 0.001$ using Brandt-Snedecor $\chi^2$ test for comparison of arbitrary distributions, Fig. 8). The change in flow to the denervated kidney with each elevation showed a narrow distribution, whereas the response in the innervated kidney was more variable. Similarly, the change in renal resistance that occurred in the innervated kidney with each episodic elevation also appeared to be less dependent on the change in blood pressure than that in the denervated kidney. There was a significant positive correlation between the change in blood pressure and the change in renal resistance that occurred with each episodic elevation in both the innervated and denervated kidneys (Fig. 9). However, in every rabbit the correlation was significantly stronger in the denervated kidney ($r = 0.897 ± 0.012$ denervated vs. $0.788 ± 0.036$ innervated, $P < 0.01$). The regression lines were further examined using linear mixed model analysis. The renal hemodynamics for the intact and denervated kidney, both in terms of change in resistance and RBF, were related to the changes in heart rate and blood pressure that occurred with the episodic elevations. Neither the intercepts nor slopes of any of the regression lines showed any remarkable differences between the innervated and denervated kidneys. However, in all cases there was a significantly larger variance around the regression line for the innervated kidney than the denervated kidney (i.e., the standard deviation of the regression line for the innervated kidney was significantly greater than that for the denervated kidney in all cases, $P < 0.0001$, Table 3).

DISCUSSION

We have developed a system for the long-term continuous monitoring of cardiovascular parameters in rabbits living in home cages that allowed us to test the hypothesis that the renal nerves play a role in regulating RBF in daily life. By comparing RBF to an innervated and denervated kidney in the same rabbit we found that mean levels of RBF and renal resistance were not different but that the control around this mean level was significantly different. In particular we observed episodic elevations in renal resistance, heart
rate, and MAP. In the denervated kidney the increase in renal resistance that occurred with these episodic elevations closely followed the increase in arterial pressure. In contrast the renal resistance to the innervated kidney displayed a more variable response, indicating an interaction of the sympathetic nervous system.

In previous experiments in which we have recorded RBF in conscious rabbits in a laboratory setting, we have observed a significant difference in the baseline blood flows to innervated and denervated kidneys, although in these studies within-animal comparisons were not made (16, 17). In those studies we presented a case for the resting levels of RSNA modulating the level of RBF. Although RSNA was recorded directly and changes in RSNA and RBF during a variety of stimuli reported, it was not possible to ascertain whether the resting level of RSNA was higher than would be found in the home-cage environment. In the present study RBF to the innervated kidney was higher than previously reported (16), especially during daytime hours and there was no difference in RBF to the innervated and denervated side. These results suggest that in previous studies the “resting” RSNA levels were indeed higher than that found in the home-cage environment. Furthermore, the similar RBF to the innervated and denervated kidneys under resting conditions in this study suggests that baseline RSNA is usually very low.

We believe an important strength of our experimental design was recording RBF bilaterally with one kidney having the renal nerves intact and the other denervated. As we observed sizable differences in mean RBF between animals (range 23–57 ml/min) a between-animal design may have failed to detect any
effect of RSNA on the control of RBF. This within-animal design ensured that each kidney was subjected to the same perfusion pressure and circulating levels of hormones, thus any difference in RBF or renal resistance between the kidneys was likely due to the action of the renal nerves.

We observed a decrease in renal resistance after feeding, although both RBF and arterial pressure increased at this time. Thus the increase in RBF was proportionally greater than the increase in arterial pressure. Interestingly this time was also when the maximum RSNA was recorded. Because increased RSNA would be expected to be associated with increased renal resistance, doesn’t this suggest that the renal nerves were not affecting RBF? We suggest that RSNA is simply one of a host of factors that regulate RBF and that other factors such as circulating hormonal and metabolite levels clearly dominate in controlling mean RBF at this time. For example, it has been shown that the hormone secretin, released in the small intestine in response to an increase in acidity, can cause renal vasodilation (18). It might be suggested that because we did not find differences in mean RBF between kidneys, RSNA was not affecting the renal vasculature but may have been selectively affecting renin secretion or sodium excretion. Again we do not believe that this is so. It must be remembered that the calculation of the circadian rhythm in all parameters was determined from averaging data collected over many days. Thus, unless RSNA was playing a major role in setting the mean level of RBF, we would be unlikely to observe its effect in the circadian analysis because of the range of additional factors e.g., circulating hormones, that also affect RBF. Rather to discern the effect of RSNA we believe that it is necessary to examine the control around the mean level.

In each rabbit we observed episodic elevations in a number of parameters, most noticeably heart rate. These elevations occurred throughout the day and night and were associated with movement. With re-

Table 2. Cardiovascular parameters during episodic elevations

<table>
<thead>
<tr>
<th>Change in Variable</th>
<th>Coefficient of Correlation With Change in HR</th>
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<tr>
<td>HR</td>
<td>25 ± 1 beats/min*</td>
</tr>
<tr>
<td>MAP</td>
<td>6 ± 1 mmHg*</td>
</tr>
<tr>
<td>Integrated renal sympathetic activity</td>
<td>40 ± 10%†</td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>51 ± 8%*</td>
</tr>
<tr>
<td>RBF to innervated kidney</td>
<td>−1.3 ± 0.5 ml/min‡</td>
</tr>
<tr>
<td>RBF to denervated kidney</td>
<td>−1.3 ± 0.2 ml/min§</td>
</tr>
<tr>
<td>Renal resistance in innervated kidney</td>
<td>0.22 ± 0.04 mmHg·ml⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Renal resistance in denervated kidney</td>
<td>0.21 ± 0.02 mmHg·ml⁻¹·min⁻¹</td>
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Values are means ± SE; n = 8 for all parameters except for RSNA, where n = 4. In each rabbit the same 4–11 day period used for the circadian analysis was evaluated for episodic elevations. The change in variable values represents the mean difference between the 200 s prior to and immediately after the elevation and the average value obtained during the elevation. RSNA, renal sympathetic nerve activity. Average RSNA and locomotor activity for the whole day were taken as 100%: *P < 0.001, †P < 0.01, ‡P < 0.05.
gard to heart rate we observed almost two states, low and high, with rapid shifts between these levels. These episodic elevations appeared similar to the cardiovascular changes occurring with exercise; that is, heart rate, blood pressure, and sympathetic activity all increased. Although we did not measure cardiac output we suggest it is likely that the increase in blood pressure was predominantly due to an increase in cardiac output. Why heart rate should “jump” between such states is unknown. It would appear that these episodic elevations are not unique to the rabbit as there are reports of similar spontaneous changes in RBF in the conscious dog (24).

The episodic elevations allowed us to examine neural control over the renal vasculature under spontaneous conditions. We believe that the data collected under such conditions strengthen our hypothesis that changes in RSNA from resting levels do regulate RBF. Although RSNA can be measured during a range of stimuli, it is always referred to as a percentage change. This makes it difficult to reference the level of activation obtained during any stimuli to the home-cage environment and the changes that occur in daily life. Analysis of RBF control during the episodic elevations indicated that RSNA was affecting RBF under these conditions. In particular in the presence of RSNA the RBF response was more variable than that in the denervated side during the episodic elevations. This larger variability indicates the presence of an extra input in the control of RBF (17). Thus we suggest that the renal nerves are actively regulating the renal vasculature in daily life. It will be noted that the effect of RSNA on RBF is small. However, we do not believe that the magnitude of the change in RBF is the important aspect but rather that we were actually able to observe any effect of RSNA. Previously it had been proposed that the renal vasculature was relatively insensitive to changes in RSNA (4). Electrical stimulation of the nerves in anesthetized dogs (10, 23) or reflex activation using hemorrhage in conscious dogs failed to see any change in RBF during low levels of sympathetic activation (21). We believe our observations indicate that with any increase in RSNA levels, the renal vasculature will be affected. Such information is important when considering that a number of disease states are associated with increased levels of sympathetic nerve activity (1, 6, 7, 12, 20) and may be impor-

![Fig. 8. Change in RBF to the innervated and denervated kidney during episodic elevations. Data obtained from the innervated kidney (○) and the denervated kidney (●). Data includes all elevations for a 6-day-period in a single animal, n = 321 elevations. A: change in flow vs. the change in arterial pressure during each elevation. B: distribution of the change in flow in response to each episodic elevation. The change in flow to the denervated kidney showed a significantly narrower distribution than the change in flow to the innervated kidney (P < 0.001 using Brandt-Snedecor χ² test for comparison of arbitrary distributions). This same pattern was seen in all 8 animals. Thus in response to an episodic elevation the change in RBF in the presence of RSNA was more variable than without RSNA.](http://ajpregu.physiology.org/)
tant in understanding the pathogenesis of altered renal function in these conditions.

It is likely that not only the home-cage environment but also the time for recovery after surgery is important when examining resting RSNA. In the present experiment we observed elevated RSNA and heart rate over the first 4- to 5-day-period following surgery; a similar time was also required before the circadian rhythm became evident. These results suggest that in rabbits any experiments performed immediately after surgery are not representative of the resting condition and tend to overestimate RSNA and heart rate.

Limitations. We used renal denervation to remove the effect of RSNA on the kidney. Previously we have shown that 3 wk after denervation the norepinephrine content of the denervated kidneys was only 2% that of the innervated kidneys (11). Furthermore the process of implanting the flow probe and nerve recording electrode did not change norepinephrine content compared with nonexperimented animals. Thus we suggest that it is unlikely for functional reinnervation of the kidney to have occurred during the experiment. We cannot discount the possibility that after denervation the kidney became supersensitive to circulating norepinephrine levels, although other research has indicated that this does not occur (15). If this was a factor, it would tend to have reduced our ability to determine the effect of the renal nerves, and the true effect on the renal vasculature might be larger than actually recorded. As we outlined previously we believe that our unilateral renal denervation preparation was the most appropriate control because this meant that each kidney saw the same perfusion pressure and circulating hormonal levels. However, it should be acknowledged that other factors may have further reduced the differences in RBF between the innervated and denervated kidneys. These factors include the neurally mediated renin release from the innervated kidney and the ensuing angiotensin II level, which could have altered RBF in the denervated kidney. Thus the effect of the renal nerves may indeed be larger than we have shown. It should also be noted that in denervating a kidney, not only have we removed the effferent sympathetic nerve fibers but also any afferent nerves fibers. Although it is clear that renal afferents play a role in sensing the composition and volume of urine (4), it is unclear as to their effect for the long-term control of RBF around the

Table 3. Regression analysis of the renal hemodynamic responses in the innervated and denervated kidneys during the episodic elevations

<table>
<thead>
<tr>
<th>Change in Variables With Episodic Elevations</th>
<th>Slope ± SE</th>
<th>Innervated Kidney Intercepts ± SE</th>
<th>Residual SD</th>
<th>Denervated Kidney Intercepts ± SE</th>
<th>Residual SD</th>
</tr>
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<tbody>
<tr>
<td>MAP</td>
<td>0.029 ± 0.004</td>
<td>0.061 ± 0.025</td>
<td>0.124</td>
<td>0.044 ± 0.024</td>
<td>0.150*</td>
</tr>
<tr>
<td>HR</td>
<td>0.008 ± 0.002</td>
<td>0.024 ± 0.041</td>
<td>0.166</td>
<td>0.008 ± 0.041</td>
<td>0.061</td>
</tr>
<tr>
<td>Blood flow vs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>−0.074 ± 0.024</td>
<td>−0.849 ± 0.297</td>
<td>1.86</td>
<td>−0.938 ± 0.294</td>
<td>1.35*</td>
</tr>
<tr>
<td>HR</td>
<td>−0.026 ± 0.009</td>
<td>−0.658 ± 0.307</td>
<td>1.88</td>
<td>−0.437 ± 0.299</td>
<td>1.34*</td>
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Linear mixed-model analysis was used to compare the regression lines describing the relationship between the change in blood flow or resistance compared to either the change in HR or MAP during the episodic elevations for the innervated and denervated kidneys. These results show that in all cases the variability of the response (as indicated by the error of variance) was greater in the innervated kidney than the denervated one. Innervated vs. denervated: *P < 0.0001, †P < 0.05.
resting levels and thus they cannot be ignored at this stage.

In the present study we describe episodic elevations that seem to be associated with locomotor activity. However, the precursor of the changes in hemodynamics cannot be definitively stated because there was no independently controlled variable. The aim of this study was to study the rabbits in their home-cage environment, minimizing the stressors associated with laboratory manipulations. Although the introduction of external stimuli would have been useful in determining the cause of the changes in renal function it was beyond the scope of this study.

Perspective

The ability to make long-term recordings of sympathetic activity has been proposed to be a critical hurdle to overcome in assessing the long-term neural control of blood pressure (2). Our aim in the current study was to develop such technologies and to test the role of RSNA in the regulation of RBF. Our results indicate that RSNA does exert control over RBF, but the combination of a number of hormonal and intrinsic factors dominate in setting the mean level of RBF. Thus pathologies where RSNA is increased may initially lead not to changes in the mean level of RBF but to the ability to control that mean level in response to everyday stimuli. How such altered control could be translated into chronic changes in renal function remains to be established.

We are grateful to Bridget Leonard and Sarah-Jane Guild for helpful comments and assistance, Rekha Wilks for technical assistance, Richard Biddle and Shaun O’Donnell for the development of the implantable RSNA amplifier, and Associate Prof. Brian McCrindle for help with the statistical analysis.

Work in the authors’ laboratory is supported by the Auckland Medical Research Foundation, the Marsden Fund, the Wellcome Trust, and the Lottery Grants Board of New Zealand.

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