Is the chronically denervated kidney supersensitive to catecholamines?

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Received 11 July 2001; accepted in final form 25 October 2001

A POPULAR METHOD for discerning the role of the renal sympathetic nerves in the regulation of renal function has been to chronically denervate one kidney. One concern with this approach is that increased renal responsiveness to plasma levels of norepinephrine may develop over time. This may reduce the apparent magnitude of the effect of the renal nerves or indeed completely mask their effect. In the present experiment, we used the rabbit unilateral denervated kidney model to examine the acute renal blood flow responses to phenylephrine to determine if there were differences between the responses in chronically denervated kidneys compared with either intact or acutely denervated kidneys. In addition, we examined the responses in rabbits that had been made hypertensive using a continuous infusion of ANG II for 7 wk. We found that chronic denervation did not result in increased renal responsiveness to phenylephrine compared with either the intact or acutely denervated kidney, suggesting that differences in renal function between renal nerve-intact and -denervated kidneys observed in previous studies are unlikely to be confounded by supersensitivity. These results suggest that the unilateral denervated kidney model is a valid model to study the role of the renal nerves in the regulation of renal function.

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epinephrine. If indeed this is the case, then the use of the unilateral denervated model to examine sympathetic responses in high-ANG II models of hypertension is questionable.

In the present experiment, we examined the acute RBF responses to phenylephrine to determine if there were differences between the responses in chronically denervated and either intact or acutely denervated kidneys. In addition, we examined the responses in rabbits that had been made hypertensive using a continuous infusion of ANG II for 7 wk.

METHODS

Animal preparation. Experiments were conducted on New Zealand White rabbits (mean wt = 2.7 ± 0.2 kg) and were approved by the University of Auckland Animal Ethics Committee. Throughout the study, the rabbits were fed daily (100 g standard rabbit pellets supplemented with hay, carrots, and apples) 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).

Animals were divided into two groups; one group was made hypertensive, and the other was a normotensive group. Baseline blood pressure (BP) measurements under conscious conditions were made in all animals via the central ear artery. All rabbits then underwent denervation of the right kidney. Under halothane anesthesia, the right kidney was approached via a retroperitoneal incision, and the renal artery and nerves were exposed. The renal sympathetic nerves were identified under a dissecting microscope. All the visible nerves were destroyed along the length of the artery. The artery was painted with isopropyl alcohol. In addition, during the same surgery, hypertension was induced in six rabbits using a continuous infusion of ANG II (50 ng·kg⁻¹·min⁻¹) via the jugular vein through an osmotic pump (model 2ML, Alzet).

BP measurement was subsequently repeated in both hypertensive and normotensive groups at 1, 2, and 6 wk after the osmotic pump was inserted. The BP measurements at weeks 1 and 2 were conducted to ensure that BP remained elevated with ANG II infusion. Because the osmotic pumps were patent for only 4 wk, the pumps were replaced after 4 wk. The BP measurement at 6 wk was conducted to ensure that change of the pump at 4 wk was successful. The change of pump was done under anesthesia induced by intravenous administration of pentobarbital sodium (90 mg/kg). The kidneys were then frozen in liquid nitrogen and stored at −80°C to allow norepinephrine concentrations to be determined. The norepinephrine concentrations were calculated by high-performance liquid chromatography. Briefly, the kidney samples (0.5 g) were homogenized with 0.4 M perchloric acid (with 5 mM reduced glutathione) (5 ml) and 3.5 μM dihydroxybenzylamine (100 μl) and centrifuged at 4,500 rpm at 4°C for 15 min. The supernatant was used to calculate the norepinephrine concentrations.

Data acquisition. BP and RBF to both left and right kidney signals were digitized at 500 Hz and continuously displayed on a computer, allowing continuous sampling of MAP (mmHg) and RBF (ml/min). Heart rate (HR; beats/min) was derived from the MAP waveform. During each experiment, data from each variable were saved continuously at 500 Hz.

Statistical analysis. Mean RBF, BP, and HR were calculated for the 1-min period before phenylephrine and the maximum response during each injection of phenylephrine. Comparisons between the intact and chronically denervated kidneys were done using an ANOVA. For comparisons with the acutely denervated kidney, results were compared using an ANOVA with a double repeated-measures design. The independent variable was the RBF after acute left renal denervation. The model used the RBF before acute left renal denervation as a covariant for the RBF after denervation. The left and right RBFs were assigned as repeated measures to account for both of them being measures in the same animal. The other factors were the group and the dose of phenylephrine. Data are presented as means ± SE. P values <0.05 were considered significant.

RESULTS

Normotensive animals (n = 9). Baseline RBF between the chronically denervated right kidney and the left kidney (initially renal nerve intact) was not significantly different (26 ± 5 and 25 ± 3 ml/min, respectively) for the normotensive group. The mean dose responses to phenylephrine infusion in the innervated and denervated kidneys are shown in Fig. 1. No significant difference in the RBF response to phenylephrine infusion was observed between the intact and chronically denervated kidneys.

The left renal nerve was then carefully sectioned distal to the flow probe. Baseline RBF in the left kidney was not altered by acute renal denervation (24 ± 2 ml/min after acute renal denervation).
Increasing doses of phenylephrine caused increasing reductions in RBF and an increase in BP. The response of one rabbit to 25 μg of phenylephrine is shown in Fig. 2. With the maximum dose of phenylephrine (75 μg), BP rose by 41 mmHg, HR decreased by 30 ± 16 beats/min, blood flow to the acutely denervated left kidney decreased to 11 ± 2 ml/min, and blood flow to the chronically denervated right kidney decreased to 10 ± 3 ml/min. The mean dose-response curves from all the normotensive rabbits (n = 9) are shown in Fig. 3. There were no significant differences in the RBF responses to phenylephrine infusions observed between the acutely denervated and the chronically denervated kidneys.

**Hypertensive animals (n = 6).** In animals that received continuous ANG II, BP increased from 77 ± 1 mmHg before ANG II to 104 ± 6 mmHg (conscious BP) after 6 wk of ANG II. There was no significant change in HR (214 ± 11 beats/min before ANG II and 198 ± 7 beats/min after 6 wk of ANG II). Baseline RBF between the chronically denervated right kidney and the intact left kidney was not significantly different (26 ± 6 and 28 ± 6 ml/min, respectively) in the hypertensive group. Following the same protocol as the normotensive animals, the blood flow responses to phenylephrine were measured in the two kidneys. Under anesthesia, the BP of the hypertensive animals fell to a mean of 82 ± 6 mmHg. The RBF responses of the innervated and the denervated kidneys to phenylephrine are shown in Fig. 4. Again, no significant difference was observed in the RBF responses to phenylephrine in the innervated and chronically denervated kidneys.

Baseline RBF in the left kidney was not significantly altered by acute renal denervation (30 ± 5 ml/min after denervation). When the RBF response between the

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**Fig. 1.** Blood pressure and renal blood flow (RBF) responses to increasing doses of phenylephrine in the normotensive animals (n = 6). ○, Left innervated kidney; ◦, right chronically denervated kidney. Values are means ± SE. When the RBF response between the innervated and the chronically denervated kidneys was compared, either as the change in absolute RBF or as the percentage change in RBF from control, no significant differences were observed between the kidneys.
acutely denervated and the chronically denervated kidneys to increasing doses of phenylephrine was compared in the hypertensive rabbits \((n = 6)\), no significant differences were observed between kidneys (Fig. 5).

Chronic renal denervation produced significant reductions in the norepinephrine content of the kidney in both normotensive and hypertensive rabbits. The norepinephrine concentration for the left kidney was \(1.2 \pm 0.09\) nmol/g of tissue. The highest concentration observed for the chronically denervated right kidney was \(0.12\) nmol/g of tissue. The norepinephrine contents were not significantly different in the hypertensive and normotensive rabbits.

**DISCUSSION**

In the present study, we found that chronic denervation did not result in increased renal responsiveness to phenylephrine compared with either the renal nerve-intact or acutely denervated kidneys, suggesting that differences in renal function between intact and denervated kidneys observed in previous studies are unlikely to be confounded by supersensitivity (1, 6). Previously, it has been shown that denervation results in increased expression of \(\alpha\)-receptors for norepinephrine in the rat kidney (14). Blockade of \(\alpha\)-receptors has also been shown to blunt the increased response to norepinephrine in sodium excretion of the denervated kidney compared with the innervated kidney in the dog (12). Thus it was logical to propose that renal denervation resulted in increased sensitivity to circulating norepinephrine. If indeed chronic denervation does lead to supersensitivity, differences between the innervated and denervated kidneys will be confounded by this supersensitivity. Given the number of research groups that have used chronic denervation (1) as a
means to assess the role of the renal nerves, this issue is potentially a major confounding variable.

Our results agree with the previous studies by Lohmeier et al. (9), who used the unilaterally denervated kidney preparation in the dog. Sodium excretion was measured in the intact and denervated kidneys in response to continuous norepinephrine infusions. While there were transient changes in sodium excretion between the two kidneys, there were no sustained differences in the sodium excretions between the two kidneys in response to high physiological levels of norepinephrine. The transient increases in sodium excretion by the innervated kidney were attributed to sympatheoinhibition. These authors compared the denervated kidney with the innervated kidney. In addition to supersensitivity of the chronically denervated kidney, another difference between the two kidneys under this condition is the presence of changes in renal sympathetic nerve activity during changes in BP. Baroreflex-induced changes in RBF resulting from changes in BP are a potential confounding factor in examining the supersensitivity of the chronically denervated kidney. For this reason, in the present study we compared the sensitivity of the chronically denervated kidney to the renal nerve-intact and acutely denervated condition. We found no difference in the response to phenylephrine infusion in the chronically denervated kidney compared with either the intact or acutely denervated contralateral kidney. Our results suggest that the presence of the renal nerves does not confound the results when comparing the sensitivity of intact and chronically denervated kidneys.

Our finding of a lack of denervation supersensitivity is in contrast to the conclusions reached by Kline and Mercer (2) and Krayacich and co-workers (3, 4), who found that the chronically denervated kidney had a greater decrease in blood flow with norepinephrine infusions. It must be mentioned that the above authors used a continuous infusion of norepinephrine, whereas we used boluses of phenylephrine. The above authors...
also used ganglionic blockade to eliminate reflex control of renal function in the innervated kidney. We chose to physically denervate the left kidney to eliminate the decrease in pressure that would occur with ganglionic blockade. Unlike ganglionic blockade, physical denervation of the nerves to the left kidney did not result in a fall in BP.

In previous studies, the issue of supersensitivity has been examined generally up to 15 days after denervation of the kidney (2–4, 9). One possibility is that reinnervation of the kidney can occur after extended time periods, e.g., 2–3 mo. While evidence suggests regeneration of the adrenergic nerves does occur, there is no evidence of regeneration even after 3 mo in the dog (10). Previously, we have used chronic renal denervation for 4 wk and measured the RBF differences between intact and denervated kidneys (1). Besides denervation supersensitivity, one potential criticism is that the renal nerves may reinnervate during this time. However, in the present study we showed significant reductions in the norepinephrine content of the chronically denervated kidney, suggesting reinnervation was not a confounding issue.

Renal denervation has been shown to increase ANG II receptors in the glomeruli of the denervated kidney (13). This is a potentially confounding factor if chronic denervation is a method used in studies involving ANG II. However, in our study, chronic ANG II infusion did not result in increased sensitivity of the chronically denervated kidney to phenylephrine. The finding of comparable RBFs in both the innervated and denervated kidneys during ANG II infusion in both the present study in rabbits, and as has previously been described in dogs (6), also argues against an exaggerated response of the denervated kidney to ANG II.

Limitations. In the present study, we used infusions of phenylephrine and recorded the RBF responses. The RBF responses we recorded are comparable to that
observed using electrical stimulation of the renal nerves in the rabbit (5). Furthermore, we have observed similar decreases in RBF under reflex activation of sympathetic activity using hypoxia, suggesting the responses to phenylephrine were well within the physiological range.

It should also be remembered that adrenergic stimulation does not only cause vascular responses but also leads to release of renin via the juxtaglomerular cells. We have shown that the vascular response of the chronically denervated kidney to phenylephrine is not exaggerated. However, it is conceivable that chronic denervation may lead to increased sensitivity of the juxtaglomerular cells in one kidney compared with the other. The increased renin release by one kidney and subsequent changes in ANG II will similarly affect both kidneys. Thus, because we did not measure renin release, we cannot rule out that the juxtaglomerular apparatus of the chronically denervated kidney might be sensitized.

**Perspectives**

The inherent difficulties in making long-term direct recordings of sympathetic nerve activity have hindered researchers interested in neural control. Indirect assessment of sympathetic influences such as chronic denervation and measurement of some functional parameter were developed to try and circumvent this limitation. However, a persistent concern was always that the model used may itself have produced compensatory changes. It is therefore with some relief that we have shown that the chronically denervated kidney of the rabbit is not supersensitive to phenylephrine compared with either the intact or acutely denervated kidney. These results suggest that the unilateral de-
nervated kidney model is a valid model to study the role of the renal nerves in the regulation of renal function both under normotensive and hypertensive situations.

We acknowledge the help of Dr. A. Stewart with the analysis of the data and the help of Dr. T. Yandle with the norepinephrine concentration measurements. We also acknowledge the assistance of Dr. M. Navakatikyan.

This research was funded by the Marsden Fund of New Zealand, the Auckland Medical Research Foundation, the Maurice and Phyllis Paykel Trust, and the University of Auckland.

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