Neural control of the kidney: functionally specific renal sympathetic nerve fibers

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DiBona, Gerald F. Neural control of the kidney: functionally specific renal sympathetic nerve fibers. Am J Physiol Regulatory Integrative Comp Physiol 279: R1517–R1524, 2000.—The sympathetic nervous system provides differentiated regulation of the functions of various organs. This differentiated regulation occurs via mechanisms that operate at multiple sites within the classic reflex arc: peripherally at the level of afferent input stimuli to various reflex pathways, centrally at the level of interconnections between various central neuron pools, and peripherally at the level of efferent fibers targeted to various effectors within the organ. In the kidney, increased renal sympathetic nerve activity regulates the functions of the intrarenal effectors: the tubules, the blood vessels, and the juxtaglomerular granular cells. This enables a physiologically appropriate coordination between the circulatory, filtration, reabsorptive, excretory, and renin secretory contributions to overall renal function. Anatomically, each of these effectors has a dual pattern of innervation consisting of a specific and selective innervation by unmyelinated slowly conducting C-type renal sympathetic nerve fibers in addition to an innervation that is shared among all the effectors. This arrangement permits the maximum flexibility in the coordination of physiologically appropriate responses of the tubules, the blood vessels, and the juxtaglomerular granular cells to a variety of homeostatic requirements.

THE UNDERSTANDING OF THE NEURAL control of renal function is an example of the importance of the relationship between structure and function. Before the early 1970s, although it was known that the renal arterial vasculature was innervated, neither the renal tubules nor the juxtaglomerular granular cells were thought to be innervated. Thus, in a study examining the influence of renal nerve stimulation on renin secretion, the unexpected observation that renal nerve stimulation decreased sodium excretion without changing renal perfusion pressure, renal blood flow, or glomerular filtration rate was dismissed as follows (20): “The renal nerves could have a direct action on tubular sodium reabsorption. This hypothesis seems unlikely, since the tubules are not thought to be innervated.”

The unambiguous demonstration that the renal tubules and the juxtaglomerular granular cells, in addition to the renal arterial vasculature, were innervated stimulated research into the functional significance of this intrinsic renal innervation. In regard to the tubules, the observation was straightforward (31): “Varicose regions of the axons, containing clusters of dense-cored vesicles (norepinephrine-containing), are in contact with the renal tubules. At the point of contact, only the basement membrane separates the nerve end-
ings from the tubule cells." The authors’ conclusion was prophetic (31): “These studies provide an anatomical basis for a direct action of the autonomic nervous system on renal tubular function.”

OVERVIEW OF NEURAL CONTROL OF RENAL FUNCTION

Although the role of the renal sympathetic nerves in control of renal function has been recently extensively reviewed (5, 6), a brief summary is relevant for introduction of the major focus of this review. Once the detailed intrinsic renal sympathetic innervation was more clearly understood, it was readily demonstrated in a variety of species that low-frequency renal sympathetic nerve stimulation, which did not affect renal blood flow or glomerular filtration rate, caused a reversible decrease in urinary sodium excretion. These results indicated that renal sympathetic nerves acting directly on the now known to be innervated renal tubules directly increased renal tubular sodium reabsorption. Subsequently, using combinations of free-flow micropuncture and continuous tubular microperfusion, it was shown that this effect occurred throughout the tubule: proximal convoluted tubule, ascending limb of Henle’s loop, distal convoluted tubule, and collecting duct. The magnitude of this effect was proportional to the density of the renal tubular innervation, being greatest in the ascending limb of Henle’s loop and least in the collecting duct. It was inhibited by antagonists specific for α1B-adrenoceptors, which are located on the peritubular basement membrane along the nephron. In vitro studies showed that the neurotransmitter in renal sympathetic nerve terminals, norepinephrine, increased Na⁺-K⁺-ATPase activity in cultured renal proximal tubular epithelial cells and that this was inhibited by α1B-adrenoceptor antagonists. Companion studies using renal denervation and either reflex increases or decreases in renal sympathetic nerve activity gave compatible results: when renal sympathetic nerve activity increased, renal tubular sodium reabsorption increased, resulting in antinatriuresis; when renal sympathetic nerve activity decreased, renal tubular sodium reabsorption decreased, resulting in natriuresis. These effects were easily demonstrable in conscious animals (27–30), serving to refute earlier claims that they were artifacts related to the stress of anesthesia and surgery (36, 37). Intact renal innervation was shown to contribute to homeostatic regulatory responses such as the normal renal responses to sodium depletion and sodium loading. When studies of the neural control of renal function were extended to pathophysiological states such as hypertension, further important observations were forthcoming. A variety of experimental animal models of hypertension are either completely prevented, reversed, or ameliorated by renal denervation; for example, the obesity model of hypertension in the dog is completely prevented by renal denervation in association with a 50% decrease in cumulative sodium retention (19). Approximately 30–40% of the renal sodium retention of edema-forming conditions, such as congestive heart failure (7), cirrhosis (7), and the nephrotic syndrome (17), are dependent on intact renal sympathetic innervation. On the afferent side, signals from renal sensory receptors coursing via afferent renal nerves to the neuraxis are involved in inhibitory renorenal reflexes and excitatory renosystemic reflexes, which, via peripheral sympathoexcitation, contribute to the hypertension of chronic renal disease (1, 2).

In summary, as shown in Fig. 1, increases in renal sympathetic nerve activity produce increases in renin secretion rate, decreases in urinary sodium excretion by increasing renal tubular sodium reabsorption, and decreasing renal blood flow (and glomerular filtration rate). Using graded frequency renal sympathetic nerve stimulation (Fig. 2), it was found that the frequency response curve for renin secretion rate was to the left of that for decreases in urinary sodium excretion, which, in turn, was to the left of that for decreases in renal blood flow. In practical terms, this means that an increase in renin secretion rate can be achieved without antinatriuresis or renal vasoconstriction, that antinatriuresis can be achieved without renal vasoconstriction but will be accompanied by increased renin...
secretion rate, and that renal vasoconstriction will be associated with an increase in renin secretion rate and antinatriuresis.

**FUNCTIONALLY SPECIFIC RENAL SYMPATHETIC NERVE FIBERS**

It was not known whether these three effector target structures (juxtaglomerular granular cells, arterioles, tubules) in the kidney are each supplied by a functionally distinct group of renal sympathetic postganglionic neurons that could be the prerequisite for an independent neuronal influence on each of them. Although Muller and Barajas (31) noted that the same renal sympathetic nerve fiber could synapse "en passant" with all three structural elements, there were also examples of renal sympathetic nerve fibers synapsing with only one of them. As shown in Fig. 3, left, there are renal sympathetic nerve fibers that make sequential contact with each of the three intrarenal effectors, usually starting with a vascular element. As shown in Fig. 3, right, there are also unique renal sympathetic nerve fibers that only make contact with one of the three intrarenal effectors and not any of the others.

The situation where a renal sympathetic nerve fiber makes sequential contact with multiple effectors is consistent with each effector having a different response threshold (e.g., exhibiting frequency dependence at supramaximal amplitude). However, it is also possible that each effector could respond by virtue of effector-specific information being encoded in the renal sympathetic nerve discharge pattern (e.g., variations in frequency, amplitude, duration; regular vs. irregular). This suggests that each effector may possess unique response characteristics in either the time or frequency domain. The situation where unique renal sympathetic nerve fibers specifically and selectively innervate arterioles, tubules, and juxtaglomerular granular cells suggests that they might be coupled to separate central neuron pools with specific and selective afferent reflex inputs.

Additional evidence for a differentiated innervation of effectors comes from the work of Luff et al. (21, 22), who noted two different types of sympathetic axons innervating the juxtaglomerular arterioles and the interlobular artery of the rabbit and rat kidney that were structurally different from those supplying other arteries.

Evidence for the existence of functionally specific sympathetic nerve fibers was obtained by Folkow and colleagues (16). They showed that differences in stimulation threshold of preganglionic fibers were associated with the activation of functionally differentiated effectors. As shown by stimulation of the abdominal sympathetic trunk of the cat, the fibers to the cutaneous arteriovenous anastomoses, the nictitating membrane, and the pupil had a lower threshold than the constrictor fibers to the ordinary vessels in the skin and tongue, whereas the sympathetic vasodilator fibers to the skeletal muscles had the highest threshold. By analogy with somatic nerves (16), this was explained by an inverse relationship between stimulation threshold and fiber diameters.

**Anatomic features of renal nerve fibers.** When characterized as to myelination and size, the renal nerves appear to be rather homogeneous (11). Approximately 96% are unmyelinated fibers with a size range of 0.4–2.5 μm. However, when the distribution of fiber diameters was examined, it was found to be bimodal with a primary mode at 1.1 μm and a secondary mode at 1.6 μm. This suggested at least two populations of renal sympathetic nerve fibers, possibly subserving different functions (i.e., functional nonuniformity).

**Neurophysiological features of renal nerve fibers.** The conduction velocity averaged 2.10 ± 0.10 m/s, consistent with unmyelinated C-type fibers (11). Strength-duration analysis (at constant frequency) indicated that, for any stimulus duration, a lower stimulus strength (volts) was needed to elicit an antidiuretic response than a vasoconstrictor response. These results indicated that the renal sympathetic nerve fibers producing the effect on the renal tubules (increased sodium and water reabsorption) were not the same as those producing the effect on the intrarenal arterioles (constriction). By analogy with somatic nerves where stimulation threshold is inversely related to fiber diameter, this suggests that the diameters of the nerve fibers involved in the antidiuretic response are greater than the diameters of the nerve fibers involved in the vasoconstrictor response. In addition, these results confirmed the earlier observations, shown in Fig. 2, demonstrating that during graded renal nerve stimulation, the response of the renal tubules occurs at a lower level of stimulation than that of the renal arterial vasculature.

**Reflex responses in single units.** Heterogeneity (i.e., functional nonuniformity) of renal sympathetic nerve fibers was more firmly demonstrated by recording the responses of single postganglionic renal sympathetic nerve units to a variety of reflex stimuli (11). The majority of single units had spontaneous activity and exhibited the expected responses to stimulation of ar-
arterial baroreceptors and central and peripheral chemoreceptors and peripheral thermal receptors. However, there was a minority population of single units that lacked spontaneous activity. These silent single units were shown to be renal by virtue of their response to stimulation of the splanchnic nerve, which contains the preganglionic input to postganglionic renal sympathetic nerves. These single units did not respond to stimulation of arterial baroreceptors and central and peripheral chemoreceptors but responded robustly to stimulation of peripheral thermal receptors. If the postganglionic renal sympathetic nerve fibers were homogeneous (i.e., functional uniformity), a consistent response pattern to all reflex stimuli would be expected. The observed different response patterns to the reflex stimuli indicate the existence of at least two populations of renal sympathetic nerve fibers, likely with functional specificity. Although evidence for homogeneity in renal single-unit responses was found in the rabbit by Dorward et al. (13) and in the cat by Stein and Weaver (38), Riedel and Peter (35) found two populations of renal single units in the rabbit that could be separated based on differences in their spike amplitude and their responses to norepinephrine and angiotensin II.

Peripheral thermal receptor stimulation. The increase in total integrated voltage of renal sympathetic nerve activity that occurs with peripheral thermal receptor stimulation (heat) decreases renal blood flow, and the renal vasoconstriction is prevented by prior renal denervation (34). As it was this stimulus that identified a unique subset of single renal sympathetic nerve fibers, we sought to determine quantitative aspects of the renal sympathetic neural discharge seen in multifiber recordings that were produced by peripheral thermal receptor stimulation. Postganglionic multifiber renal sympathetic nerve activity occurs in synchronized sympathetic discharges (bursts, peaks) with distinct coupling to the cardiac cycle. These synchronized renal sympathetic peaks may be characterized by their amplitude, duration, and frequency. Total integrated voltage encompasses the product of voltage under the curve of each peak (governed largely by peak amplitude as peak duration changes little) and peak frequency. Therefore, changes in total integrated voltage of renal sympathetic nerve activity can be due to changes in peak amplitude, peak frequency, or both. With the development of a method to quantitatively analyze these components of synchronized renal sympathetic discharge (25), interest in their significance has increased (23, 24). Peak amplitude is influenced by the number of active renal sympathetic nerve fibers wherein increased peak amplitude reflects the recruitment of previously silent fibers to fire and decreased peak amplitude reflects the silencing of previously firing fibers. Peak duration is influenced by the firing synchrony and the dispersion of the mass discharge due to different conduction velocities of the multiple renal nerve fibers. Peak frequency is a reflection of the rhythm of central oscillators and is subject to baroreceptor modulation. Using this approach, it was observed that peripheral thermal receptor stimulation increased total integrated voltage of renal sympathetic nerve activity solely by increase in peak amplitude with little to no change in peak frequency (3). When expressed as percent control, 95% of the increase in total integrated voltage of renal sympathetic nerve activity could be accounted for by an increase in peak amplitude. Therefore, peripheral thermal receptor stimulation elicits activity in previously silent renal nerve fibers, which likely explains the unmasking of a unique subgroup of single renal sympathetic nerve fibers by this stimulus. Similarly, intravenous volume loading, which decreased total integrated voltage of renal sympathetic nerve activity, did so predominantly by decreasing peak amplitude (84%) with a far lesser decrease in peak frequency. Therefore, intravenous volume loading causes previously discharging single sympathetic nerve fibers to become silent. In addition to efficiency in regulating total integrated voltage of renal sympathetic nerve activity, this regulatory system enables the selective activation and deactivation of functionally specific renal sympathetic nerve fiber groups.

In an effort to learn more about the nature of the information conveyed to the kidney by this unique group of renal sympathetic nerve fibers activated by peripheral thermal receptor stimulation, we compared neural and renal hemodynamic responses to peripheral thermal receptor stimulation (heat) and another somatic stimulus, tail compression (pinch) (9). The stimuli were adjusted so as to increase total integrated voltage of renal sympathetic nerve activity to the same magnitude (Fig. 4). Under these circumstances, the decrease in renal blood flow and the increase in renal vascular resistance were substantially greater with heat than with pinch. These results suggested that the different renal sympathetic nerve fiber groups being activated by the two stimuli resulted in different types of information being encoded in the resulting renal sympathetic nerve activity signal. Power spectral and transfer function analysis of the renal sympathetic nerve activity (total integrated voltage) and the renal blood flow signals disclosed important differences between heat and pinch. With pinch there was a single coherent oscillation of both renal sympathetic nerve activity and renal blood flow at 0.3–0.4 Hz. With heat there were two coherent oscillations of both renal sympathetic nerve activity and renal blood flow at 0.2 and 0.3–0.4 Hz. Renal denervation eliminated the oscillations in renal blood flow at both 0.2 and 0.3–0.4 Hz, indicating their transfer from the renal sympathetic nerve activity signal into the renal blood flow signal. Therefore, despite similar increases in the total integrated voltage of renal sympathetic nerve activity, heat resulted in a greater renal vasoconstrictor response than pinch because of the activation of a unique population of renal sympathetic nerve fibers with different frequency-response characteristics of the renal vasculature. These results further emphasize the significance of the nature of the information encoded in the renal sympathetic nerve activity signal (beyond total
integrated voltage) as an important determinant of the overall renal functional response.

**Pattern of renal sympathetic nerve activation.** These results served to focus our attention on the morphological differences between the renal sympathetic nerve burst pattern recorded from multifiber preparations in vivo and the pattern commonly employed in studies where the renal sympathetic nerves are electrically stimulated. The in vivo pattern of a multispike burst is diamond shaped, with the amplitude of the initial and terminal spikes being less than those in the middle of the burst; as noted above, these bursts vary in their amplitude, duration, and frequency. When studying the influence of renal sympathetic nerve activity on renal function, investigators have generally used constant voltage electrical stimulation to apply stimuli that were square wave in nature to a multifiber renal sympathetic nerve preparation, consisting of single pulses of fixed amplitude, duration, and frequency. By means of this approach, considerable information has been acquired concerning the renal sympathetic neural control of renal function. Results using direct electrical renal sympathetic nerve stimulation have been in qualitative agreement with those using reflex stimuli to physiologically activate renal sympathetic nerve fibers with regard to the control of the renal circulation, renal tubular sodium and water reabsorption, and renin secretion rate. This qualitative agreement has supported the validity of using results from artificial square wave stimulation in understanding the physiological effects of renal sympathetic nerve activity on renal function. However, quantitative comparison has been difficult because of substantial differences in experimental design involved in these two quite different approaches.

We used digital methods to construct an external stimulus pattern that faithfully reproduces that recorded in vivo from a multifiber renal sympathetic nerve preparation (10). The effect of this diamond wave pattern was compared with the effect of a square wave pattern, which was matched for total integrated voltage (amplitude × duration). When delivered at the same total integrated voltage, the diamond wave pattern produced a greater renal vasoconstrictor response than the square wave pattern (Fig. 5A). Within the diamond wave pattern, increasing total integrated voltage by increasing amplitude produced twofold greater renal vasoconstrictor responses than by increasing duration. Given the mathematics of the calculation of the area for a square versus a diamond wave pattern, for the same total integrated voltage either the amplitude or the duration of the diamond wave pattern will be twice that of the square wave pattern. Thus it could be argued that the greater renal vasoconstrictor response to the diamond wave pattern, for the same total integrated voltage, either the amplitude or the duration of the diamond wave pattern will be twice that of the square wave pattern. However, when diamond and square wave patterns were matched for amplitude, the diamond wave pattern still produced a greater renal vasoconstrictor response than the square wave pattern (Fig. 5B).

With similar total integrated voltages that were subthreshold for renal vasoconstriction, neither diamond nor square wave patterns altered glomerular filtration rate, whereas diamond but not square wave patterns reversibly decreased urinary sodium excretion by 25 ± 3%. This suggests that the diamond wave pattern exhibits a selectivity for tubular compared with vascular responses.
At the same number of pulses per second, intermittent stimulation produced faster and greater renal vasoconstriction than continuous stimulation. At the same number of pulses per second, increases in rest period duration during intermittent stimulation proportionally augmented the renal vasoconstrictor response compared with that observed with continuous stimulation; the maximum augmentation of 55% occurred at a rest period of 500 ms (Fig. 6). This suggests that increasing rest time may enhance the opportunity for repletion of neurotransmitter within the renal sympathetic nerve terminal, serving to offset exhaustion of neurotransmitter release mechanisms.

Overall, these results indicate that the pattern of renal sympathetic nerve stimulation (activity) significantly influences the rapidity, magnitude, and selectivity of the renal vascular and tubular responses.

As noted above, analyzing the components of the synchronized renal sympathetic discharge response to peripheral thermal receptor stimulation showed an approximate doubling (100% increase) in total integrated voltage, which was virtually fully accounted for by a similar doubling (nearly 100% increase) in peak amplitude with no significant changes in peak duration or frequency. This renal sympathetic response produced a $32 \pm 4\%$ increase in renal vascular resistance (calculated as area under the curve to incorporate changes in magnitude over the 2-min time interval).

We used the data on the change in the components of the synchronized renal sympathetic nerve discharge response to peripheral thermal receptor stimulation to digitally construct pairs of diamond and square wave patterns of similar magnitude. Two diamond wave patterns were constructed so that the total integrated voltage of one was twice that of the other solely by virtue of a doubling in peak amplitude; a pair of square wave patterns was constructed with the same quantitative relationships. The hypothesis tested was that the renal vasoconstrictor response seen with peripheral thermal receptor stimulation would be more closely reproduced by renal sympathetic nerve stimulation with the diamond than with the square wave patterns. When applying these stimuli to the decentralized renal sympathetic nerve bundle in anesthetized rats, the increase in renal vascular resistance (calculated as area under the curve over the 2-min time interval) was $24 \pm 3\%$ for the diamond wave pattern.

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Fig. 5. A: effect of diamond vs. square wave stimuli at identical steps of integrated voltage (voltage $\times$ time, $V \times$ ms) delivered on RBF. $n = 11$. B: effect of diamond vs. square wave stimuli at identical steps of amplitude (voltage, $V$) on RBF. $n = 10$. For both panels, stimuli were 150 ms in duration, delivered at a frequency of 1 Hz; renal nerves were stimulated for 30 s at each step. ■, square wave pattern; ●, diamond wave pattern. Data are means ± SE. In each panel, the curves are significantly different from each other at $P < 0.05$. Adapted from Ref. 7.

Fig. 6. Effect of variations in rest time on RBF responses to intermittent stimulation compared with continuous stimulation. Square wave stimulation at 5 pulses/s. Data are means ± SE, $n = 14$. Data are presented as % change in area under the curve for RBF (AUC RBF) for intermittent stimulation vs. continuous stimulation with number of pulses being constant at 5/s (C5). All points are significantly different ($P < 0.05$) from no (0%) change. Adapted from Ref. 7.
and was 18 ± 2% for the square wave pattern. Thus when stimulus patterns were constructed so as to match the quantitative aspects of total integrated voltage and peak amplitude as observed in the experimentally obtained synchronized renal sympathetic discharge signal, the experimentally observed renal vasoconstrictor response to peripheral thermal receptor stimulation was closer to the response obtained with the diamond than with the square wave pattern. However, these results should be interpreted with caution because the response to peripheral thermal receptor stimulation involves, in addition to a decrease in renal blood flow, an increase in arterial pressure that is incorporated into the calculation of renal vascular resistance. In contrast, the response to stimulation of the decentralized renal nerve bundle consists of a decrease in renal blood flow with little to no change in arterial pressure.

Nonlinear dynamic analysis of renal sympathetic nerve activity. In pathophysiological states, such as the later stages of decompensated congestive heart failure, renal sympathetic nerve activity is increased, often associated with impairment in central and/or peripheral portions of arterial and/or cardiac baroreflex control mechanisms (8). This is manifest as increases in peak amplitude of synchronized renal sympathetic bursts, reflecting an increase in the number of active fibers by virtue of recruitment of previously silent fibers to fire. In addition, there are more cardiac cycles that have a defined burst (i.e., fewer cardiac cycles are burst free), and, with increases in heart rate, this gives the appearance of continuous bursting with a reduction in the length of silent diastolic periods (15). It is intuitive as to how this shift in activity pattern would lead to greater renal sympathetic neural input to the intrarenal effectors resulting in the well-documented reductions in renal blood flow and glomerular filtration rate, increased fractional renal tubular sodium reabsorption with antinatriuresis, and increased renin secretion rate that characterize the later stages of decompensated congestive heart failure (14).

In addition, in congestive heart failure, there are changes in the nonlinear dynamic properties of renal sympathetic nerve activity (4) and cardiovascular variables that are heavily influenced by sympathetic nervous system activity such as arterial pressure and heart rate (33). Using methods of nonlinear dynamic and chaos analysis (18, 32, 33, 39), the transition from the normal state to congestive heart failure is characterized by a decrease in both the correlation dimension (an index of “complexity”) and the greatest Lyapunov exponent (an index of chaotic behavior). This represents an overall decrease in chaotic behavior. These changes may be described as a shift from a more complex, less predictable state under normal conditions to a less complex, more predictable state in congestive heart failure. Congestive heart failure is characterized by impaired arterial and cardiac baroreflex function. It is of interest that, in terms of nonlinear dynamic properties, the changes produced by arterial and cardiac baroreceptor denervation in normal rats and dogs are similar to those seen in rats and humans with congestive heart failure (4, 33, 39). In both situations, there are decreases in both correlation dimension and Lyapunov exponent, reflecting an overall reduction in chaotic behavior. It may be that a less complex, more predictable and stable system results, at least in terms of synchronized renal sympathetic discharge, in greater recruitment of an increased number of previously silent fibers to fire. It is possible that some of these newly recruited renal sympathetic nerve fibers have functional specificity that may contribute to the abnormal renal functional profile associated with congestive heart failure.

Perspectives

The renal sympathetic nerves serve as important regulators of the function of the renal effects, the vasculature, the tubules, and the juxtaglomerular granular cells. In this way, they produce alterations in renal hemodynamics, tubular reabsorption, and renin secretion rate that are important contributions to renal adaption and compensation during normal physiological conditions. With dysregulation of renal sympathetic nerve activity, the renal functional effects contribute importantly to the underlying pathophysiology, e.g., in congestive heart failure or hypertension.

Similar to sympathetic fiber groups elsewhere, the renal sympathetic nerves are likely functionally differentiated. The concept of functionally specific renal sympathetic nerve fibers provides an additional dimension of control and regulation of the individual effectors within the kidney. A more complete knowledge of these specific and selective neuroeffector relationships would greatly improve our understanding of how the kidney responds to various stimuli in both health and disease.

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