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ORIGINAL INVESTIGATIONS

Anatomic Assessment of Sympathetic Peri-Arterial Renal Nerves in Man

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ABSTRACT

BACKGROUND Although renal sympathetic denervation therapy has shown promising results in patients with resistant hypertension, the human anatomy of peri-arterial renal nerves is poorly understood.

OBJECTIVES The aim of our study was to investigate the anatomic distribution of peri-arterial sympathetic nerves around human renal arteries.

METHODS Bilateral renal arteries were collected from human autopsy subjects, and peri-arterial renal nerve anatomy was examined by using morphometric software. The ratio of afferent to efferent nerve fibers was investigated by dual immunofluorescence staining using antibodies targeted for anti-tyrosine hydroxylase and anti-calcitonin gene-related peptide.

RESULTS A total of 10,329 nerves were identified from 20 (12 hypertensive and 8 nonhypertensive) patients. The mean individual number of nerves in the proximal and middle segments was similar (39.6 $\pm$ 16.7 per section and 39.9 $\pm$ 1 3.9 per section), whereas the distal segment showed fewer nerves (33.6 $\pm$ 13.1 per section) ($p = 0.01$). Mean subject-specific nerve distance to arterial lumen was greatest in proximal segments (3.40 $\pm$ 0.78 mm), followed by middle segments (3.10 $\pm$ 0.69 mm), and least in distal segments (2.60 $\pm$ 0.77 mm) ($p < 0.001$). The mean number of nerves in the ventral region (11.0 $\pm$ 3.5 per section) was greater compared with the dorsal region (6.2 $\pm$ 3.0 per section) ($p < 0.001$). Efferent nerve fibers were predominant (tyrosine hydroxylase/calcitonin gene-related peptide ratio 25.1 $\pm$ 33.4; $p < 0.0001$). Nerve anatomy in hypertensive patients was not considerably different compared with nonhypertensive patients.

CONCLUSIONS The density of peri-arterial renal sympathetic nerve fibers is lower in distal segments and dorsal locations. There is a clear predominance of efferent nerve fibers, with decreasing prevalence of afferent nerves from proximal to distal peri-arterial and renal parenchyma. Understanding these anatomic patterns is important for refinement of renal denervation procedures. (J Am Coll Cardiol 2014;64:635-43) © 2014 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

BP = blood pressure

CGRP = calcitonin gene-related peptide

H&E = hematoxylin and eosin

NFP = neurofilament protein

TH = tyrosine hydroxylase

Renal sympathetic denervation is a promising new therapy for patients with resistant hypertension, which is defined as failure to achieve control of blood pressure (BP) despite treatment with optimal doses of ≥3 antihypertensive medications (1). Catheter-based radiofrequency renal denervation has demonstrated both safety and efficacy for the treatment of resistant hypertension, with 93% of patients having a reduction in office-based systolic BP of ≥10 mm Hg at 3 years (2). Furthermore, other denervation technologies, such as catheter-based ultrasound, externally applied focused ultrasound, or catheter-based microinfusion of neurotoxic drugs, have been developed (3). Although all renal denervation technologies target renal sympathetic nerves around the renal artery, our understanding of human anatomy of peri-arterial renal nerves remains limited.

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The aim of the present study was: 1) to examine the morphological characteristics of nerve fibers with respect to density, size, and distance from renal artery lumen; 2) to investigate the influence of hypertension on peri-arterial renal nerve distribution; and 3) to determine the proportion of efferent and afferent fibers along the peri-arterial neuronal network of renal arteries and within the kidney.

METHODS

Bilateral renal arteries with attached abdominal aorta and kidneys were collected from 25 autopsy subjects. The first 20 cases were used for the investigation of peri-arterial nerve anatomy, and the other 5 cases were used to investigate the ratio of efferent and afferent nerve fibers within nerve fascicles. Identification of hypertension was on the basis of patient histories and histological examination of kidney sections (4). In the first 20 cases, renal arteries were perfusion-fixed ex vivo under physiological pressure (80 to 100 mm Hg) with 10% neutral-buffered formalin. The use of dyes demarcated the ventral, dorsal, superior, and inferior regions around the renal artery. Each artery with surrounding soft tissue was sectioned at 4- to 5-mm intervals and equally divided into proximal, middle, and distal segments. At least 2 sections distal to the arterial bifurcation also were submitted. Each segment was dehydrated, embedded in paraffin, cut at 5 μm thickness, and stained with hematoxylin and eosin (H&E) and Movat pentachrome. To minimize the effect of autolysis on immunohistochemistry (5), the last 5 cases were collected from subjects within 24 hours of death. After cutting, each section was fixed in paraformaldehyde (4%), followed by microwave fixation.

Digital images from H&E-stained histological sections were acquired at 1.25× magnification. The images were divided into 4 quadrants on the basis of the dye labeling and analyzed with image analysis software (IP Lab for Mac OS X, Scanalytics, Rockville, Maryland). Measurements of the distance from the luminal surface of the renal arteries to each nerve were performed in each quadrant around the renal artery. Details of the methods used for immunohistochemistry are described in the Online Appendix.

STATISTICAL ANALYSIS. Results for continuous variables are expressed as mean ± SD. The Shapiro-Wilk test was used to statistically assess violations of the normal distribution assumption. Each individual distance from lumen to nerve was used in the tables for whole distribution or figures for cumulative percentile of nerves, whereas mean values per renal artery were used for statistical comparison. For statistical comparison of spatial nerve distribution, mean values of nerve counts were derived for proximal, middle, and distal regions, as well as for ventral, dorsal, superior, and inferior location, and matched comparisons were performed by using paired Student *t* tests or repeated measures analysis of variance for normally distributed parameters. For skewed data distribution, a matched comparison using the Wilcoxon signed rank or Friedman test was applied. Comparisons between hypertensive and non-hypertensive subjects were performed by using the independent Student *t* test or Wilcoxon rank sum test. Categorical data were analyzed by using the chi-square test or the Fisher exact test. The Spearman correlation coefficient was calculated to assess the correlation between nerve counts identified by using H&E staining and those identified by using neurofilament protein (NFP) staining.

All analyses were performed by using SPSS version 19 (IBM SPSS Statistics, IBM Corporation, Armonk, New York) and JMP 5 (SAS Institute, Inc., Cary, North Carolina). All reported p values were determined by 2-sided analysis, and values <0.05 were regarded as statistically significant.

RESULTS

A total of 40 renal arteries from the first 20 patients were allocated for histological evaluation to determine the renal artery anatomy and nerve distribution. Patient characteristics and renal arterial anatomy are shown in Table 1. Mean individual lumen diameter between the proximal, middle, and distal segments