Remodeling of stellate ganglion neurons after spatially targeted myocardial infarction: Neuropeptide and morphologic changes

Olujimi A. Ajijola, MD, PhD,[*†] Daigo Yagishita, MD,[*] Naveen K. Reddy, BS,[*] Kentaro Yamakawa, MD,[‡] Marmar Vaseghi, MD, MS, FHRS,[*†] Anthony M. Downs, BS,[§] Donald B. Hoover, PhD,[§] Jeffrey L. Ardell, PhD,[*†] Kalyanam Shivkumar, MD, PhD[*†]

From the [*]UCLA Cardiac Arrhythmia Center, University of California–Los Angeles, Los Angeles, California, [†]Neurocardiology Research Center of Excellence, University of California–Los Angeles, Los Angeles, California, [‡]Department of Cardiac Anesthesia, University of California–Los Angeles, Los Angeles, California, and [§]Department of Biomedical Sciences, Center for Inflammation, Infectious Disease, and Immunity, Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee.

BACKGROUND Myocardial infarction (MI) induces remodeling in stellate ganglion neurons (SGNs).

OBJECTIVE We investigated whether infarct site has any impact on the laterality of morphologic changes or neuropeptide expression in stellate ganglia.

METHODS Yorkshire pigs underwent left circumflex coronary artery (LCX; n = 6) or right coronary artery (RCA; n = 6) occlusion to create left- and right-sided MI, respectively (control: n = 10). At 5 ± 1 weeks after MI, left and right stellate ganglia (LSG and RSG, respectively) were collected to determine neuronal size, as well as tyrosine hydroxylase (TH) and neuropeptide Y immunoreactivity.

RESULTS Compared with control, LCX and RCA MIs increased mean neuronal size in the LSG (451 ± 25 vs 650 ± 34 vs 577 ± 55 μm^2, respectively; $P = .0012$) and RSG (433 ± 22 vs 646 ± 42 vs 530 ± 41 μm^2, respectively; $P = .002$). TH immunoreactivity was present in the majority of SGNs. Both LCX and RCA MIs were associated with significant decreases in the percentage of TH-negative SGNs, from 2.58% ± 0.2% in controls to 1.26% ± 0.3% and 0.7% ± 0.3% in animals with LCX and RCA MI, respectively, for LSG ($P = .001$) and from 3.02% ± 0.4% in controls to 1.36% ± 0.3% and 0.68% ± 0.2% in LCX and RCA MI, respectively, for RSG ($P = .002$). Both TH-negative and TH-positive neurons increased in size after LCX and RCA MI. Neuropeptide Y immunoreactivity was also increased significantly by LCX and RCA MI in both ganglia.

CONCLUSION Left- and right-sided MIs equally induced morphologic and neurochemical changes in LSG and RSG neurons, independent of infarct site. These data indicate that afferent signals transduced after MI result in bilateral changes and provide a rationale for bilateral interventions targeting the sympathetic chain for arrhythmia modulation.

KEYWORDS Neuropeptide remodeling; Myocardial infarction; Autonomic nervous system; Sympathetic ganglia; Neuronal remodeling

ABBREVIATIONS ANOVA = analysis of variance; **ANS** = autonomic nervous system; **ChAT** = choline acetyltransferase; **gp130** = glycoprotein 130; **HF** = heart failure; **ICN** = intrinsic cardiac network; **LCX** = left circumflex coronary artery; **LSG** = left stellate ganglion; **MI** = myocardial infarction; **NPY** = neuropeptide Y; **RCA** = right coronary artery; **RSG** = right stellate ganglion; **SGN** = stellate ganglion neuron(s); **TH** = tyrosine hydroxylase

(Heart Rhythm 2015;12:1027–1035)

Introduction

Alterations in autonomic nervous system (ANS) function have been linked to ventricular and atrial arrhythmias.[1–3] Modulation of elements within the cardiac neural hierarchy has been used in the management of arrhythmias and in the study of ANS function in humans and in animal models.[4–7] Morphologic changes in neurons located in ganglia within and extrinsic to the heart have been reported in association with ischemic and nonischemic injury to the heart. Neuronal enlargement, nerve sprouting, and enhancement in neural signals have been reported in stellate ganglia in a myocardial infarction (MI) model in canines and rabbits, as well as in humans.[8–10]

In addition, altered neurochemical expression patterns were shown in stellate ganglion neurons (SGNs) in a number

This study was supported by an A.P. Giannini postdoctoral award to Dr. Ajijola, the National Institutes of Health (GM107949 to Dr. Hoover), and the National Heart, Lung, and Blood Institute (R01HL084261 to Dr. Shivkumar). **Address reprint requests and correspondence:** Dr. Kalyanam Shivkumar, UCLA Cardiac Arrhythmia Center, UCLA Health System, David Geffen School of Medicine at UCLA, Suite 660, Westwood Boulevard, Los Angeles, CA 90095-1679. E-mail address: kshivkumar@mednet.ucla.edu.

1547-5271/$-see front matter http://dx.doi.org/10.1016/j.hrthm.2015.01.045

of cardiovascular conditions. In a rat nonischemic model of heart failure (HF), SGNs were shown to undergo transdifferentiation from an adrenergic to a cholinergic phenotype, manifested by an increase in tyrosine hydroxylase (TH)–negative (cholinergic) neurons, mediated by glycoprotein 130 (gp130) inflammatory cytokines.[11] In addition, low-level vagal stimulation induced an increase in the percentage of TH-negative neurons in canine stellate ganglia,[12] the vast majority of which stained positive for choline acetyltransferase (ChAT). These data suggest that under conditions of nonischemic HF, or during chronic vagal stimulation, there is an adrenergic to cholinergic phenotypic switch. Whether ischemic injury to the heart induces a similar phenotypic switch is not known.

The pattern of ventricular innervation from the left and right stellate ganglia (LSG and RSG, respectively) has been shown in electrical mapping studies to predominate on the posterior and leftward aspect of the ventricles for LSG and the anterior and rightward aspect of the ventricles for RSG.[13,14] Whether neuronal remodeling after infarction is greater in the ganglion (LSG vs RSG) predominantly innervating the damaged myocardial bed remains unknown. This issue is important because it may guide the laterality of interventional strategies aimed at modulating cardiac sympathetic tone, such as cardiac sympathetic denervation after MI.[7]

The aim of the present study was 2-fold: (1) to determine whether MI is associated with altered SGN expression of adrenergic phenotypes and neuropeptides; and (2) to assess whether stellate ganglion neural remodeling is greatest in the ganglion (LSG vs RSG) ipsilateral to the area infarcted.

Materials and methods

Animal model and infarct induction

Animal experimentation was performed with approval from and in accordance with guidelines set by the University of California Institutional Animal Care and Use Committee and the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*. A total of 22 animals were studied (12 infarcted and 10 controls).

MI was induced as described previously.[14] Briefly, animals weighing 77 ± 10 lb (n = 12) were sedated with tiletamine/zolazepam (8–10 mg/kg intramuscularly) and fentanyl (50–100 μg intravenously). After intubation, general endotracheal anesthesia was maintained by inhaled isoflurane (0.8%–1.5%), and analgesia was maintained by hourly boluses of fentanyl (50–100 μg intravenously). Next, a balloon-tipped coronary angioplasty catheter was advanced over a guide wire to the right coronary artery (RCA; n = 6) or left circumflex artery (LCX; n = 6). The balloon was inflated to subocclusive pressures, and a 10- to 20-mL suspension containing radio-opaque contrast, sterile saline, and 5 to 7.5 mL of polystyrene microspheres (Polybead 90 μm, Polysciences Inc, Warrington, PA) was slowly injected over 3 to 5 minutes via the angioplasty catheter. ST-segment elevation in inferior or left-sided electrocardiogram leads confirmed immediate myocardial injury. Ex vivo contrast-enhanced magnetic resonance imaging was performed in some animals at 6 weeks after infarction.

Histologic and immunohistochemical studies

Tissue handling

Stellate ganglia were removed before animals were euthanized and were then rinsed and immediately transferred to cold 10% phosphate-buffered formalin (Fisher Scientific, Pittsburgh, PA) for 5 to 7 days and then to cold 70% ethanol (Sigma-Aldrich, St Louis, MO) until embedding (< 1 week).

Histologic stains

Neuronal size was determined from thionin staining (Fisher Scientific) by use of computerized morphometry analysis (Tissue Studio, Definiens Inc, Parsippany, NJ) as published previously.[9]

Immunohistochemical stains

Adrenergic phenotype was quantified with anti-TH antibody (1:200 dilution; catalog No. ab112, Abcam, Cambridge, MA) and cholinergic phenotype by anti-ChAT antibody (1:100 dilution; Abcam, catalog No. ab18736), both detected by diaminobenzidine (Life Technologies, Green Island, NY) per the manufacturer's recommended protocol. All neurons present in the slides were counted by use of computerized image analysis (Aperio ImageScope, Leica Biosystems Nussloch GmbH, Nussloch, Germany) and considered adrenergic or cholinergic if they demonstrated any level of TH or ChAT immunoreactivity, respectively. Staining and quantification of the groups were performed in a blinded fashion. Neuropeptide Y (NPY) immunoreactivity was quantified with anti-NPY antibodies (1:500 dilution; catalog No. 22940, ImmunoStar, Hudson, WI) and developed with ImmPACT VIP peroxidase substrate kit (Vector Laboratories, Burlingame, CA) for a constant time of 4 minutes to ensure uniform staining intensity. All slides were digitally scanned, and the electronic images were stored for analysis (Aperio ImageScope, Leica Biosystems Nussloch GmbH).[9] NPY immunoreactivity was assessed differently from the other stains. Quantifications were performed in 5 high-power fields (40×) at the top, bottom, left, right, and center aspects of the slide. Neurons were either labeled darkly or faintly or were completely negative. All neurons were counted and analyzed in these high-power fields; however, only those neurons that stained darkly were considered NPY immunoreactive and were quantified as a percentage (of darkly stained neurons per slide). Staining and quantification of the groups were performed in a blinded fashion.

Statistical analyses

Data are reported as mean ± standard error. Normality and homoscedasticity were assessed for each dataset. Multigroup comparisons were performed by 2-way analysis of variance