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Pathological effects of chronic myocardial infarction on peripheral neurons mediating cardiac neurotransmission

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ABSTRACT

Objective: To determine whether chronic myocardial infarction (MI) induces structural and neurochemical changes in neurons within afferent and efferent ganglia mediating cardiac neurotransmission.
Methods: Neuronal somata in i) right atrial (RAGP) and ii) ventral interventricular ganglionated plexi (VIVGP), iii) stellate ganglia (SG) and iv) T1–2 dorsal root ganglia (DRG) bilaterally derived from normal ($n = 8$) vs. chronic MI ($n = 8$) porcine subjects were studied. We examined whether the morphology and neuronal nitric oxide synthase (nNOS) expression in soma of RAGP, VIVGP, DRG and SG neurons were altered as a consequence of chronic MI. In DRG, we also examined immunoreactivity of calcitonin gene related peptide (CGRP), a marker of afferent neurons.
Chronic MI increased neuronal size and nNOS immunoreactivity in VIVGP (but not RAGP), as well as in the SG bilaterally. Across these ganglia, the increase in neuronal size was more pronounced in nNOS immunoreactive neurons. In the DRG, chronic MI also caused neuronal enlargement, and increased CGRP immunoreactivity. Further, DRG neurons expressing both nNOS and CGRP were increased in MI animals compared to controls, and represented a shift from double negative neurons.
Conclusions: Chronic MI impacts diverse elements within the peripheral cardiac neuraxis. That chronic MI imposes such widespread, diverse remodeling of the peripheral cardiac neuraxis must be taken into consideration when contemplating neuronal regulation of the ischemic heart.

1. Introduction

Sudden cardiac death (SCD) due to ventricular arrhythmias (VAs) is the leading cause of mortality in the developed world, resulting in an estimated four to five million deaths each year (Chugh et al., 2008). Autonomic neuronal dysfunction plays a crucial role in the genesis of cardiac arrhythmias and/or progression of the failing heart (Fukuda et al., 2015; Vaseghi and Shivkumar, 2008). The cardiac neuronal hierarchy is primarily focused on dynamic coordination of cardiac function to match whole body metabolic demands (Armour and Ardell, 2004 and includes neural networks located from the level of the heart (Ardell, 1994; Armour, 1991a, 1991b) and intra-thoracic extra-cardiac ganglia to the insular cortex (Oppenheimer and Hopkins, 1994).

At the organ level, the intrinsic cardiac nervous system (ICNS) comprises a distributed network of ganglia and interconnecting nerves (Ardell, 1994; Armour, 1991a, 1991b). Neurons within cardiac GPs interact in concert with neurons in intra-thoracic extra-cardiac (sympathetic) ganglia, nodose and dorsal root ganglia (DRG), and higher center neurons (including spinal neurons). Neurons throughout the cardiac neuraxis interact continuously to match cardiac output to whole body demands on a beat-to-beat basis (Ardell, 2004; Armour and Hopkins, 1990; Armour and Janes, 1988).

The ICNS contains all the neuronal elements necessary for cardiac reflex control independent of higher centers (Murphy et al., 2000), namely sensory neurons (Ardell et al., 1991; Armour et al., 1997; Cheng et al., 1997; Horackova et al., 1999; Yuan et al., 1994), adrenergic (Gebber et al., 1996; Lewis et al., 2001) and cholinergic (Gray et al., 2004a; Gray et al., 2004b) efferent postganglionic neurons, as well as interposed local circuit neurons (LCNs) (Beaumont et al., 2013a; Armour, 1991a, 1991b). The largest subpopulation, LCNs, accounts for the intra- and inter-ganglionic communication that occurs among neurons within the ICNS that sub-serves local information processing (Armour, 1991a, 1991b). Intra-thoracic extra-cardiac ganglia possess sensory and LCNs that target their sympathetic efferent postganglionic neurons therein. As such, collectively they are involved in intra-thoracic cardiocentric reflex control of cardiodynamics.

Abbreviations: ANS, autonomic nervous system; MI, myocardial infarction; ICNS, intrinsic cardiac nervous system; LCN, local circuit neuron; ECG, electrocardiogram; LAD, left anterior descending coronary artery; IV, intravenous; VIV GP, ventral interventricular ganglionated plexus; DRG, dorsal root ganglion; LV, left ventricle/ventricular; RVOT, right ventricular outflow tract; MRI, magnetic resonance imaging; SG, stellate ganglion.

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Cardiac disease, such as MI, adversely affects the myocardium (Cao et al., 2000; Vracko et al., 1991). How it affects neurons throughout the cardiac neuraxis, remains unknown – particularly with respect to how its varied thoracic components are affected (Ajijola et al., 2012, 2015). Neural remodeling within the cardiac nervous system post-MI contributes to autonomic imbalances (Kember et al., 2013) and the potential for SCD (Fukuda et al., 2015). It is known that human IC neurons undergo structural remodeling in the presence of chronic obstruction to their regional arterial blood supply (Hopkins et al., 2000b). Such neurons display enlarged somata that are replete with vacuoles, in addition to displaying degenerative changes in their dendrites and axons (Hopkins et al., 2000a). In the porcine model, chronic ventricular infarction is accompanied by similar changes in somata of the stellate ganglia (SG) (Ajijola et al., 2015). In agreement with such histological evidence, in vitro intracellular studies of IC neurons derived from chronic MI animals have demonstrated enhanced neuronal excitability, altered synaptic efficacy, and adaptive reorganization of neurochemical phenotypes and neuromodulation within the intrinsic cardiac nervous system (Hardwick et al., 2014; Hardwick et al., 2008). In fact, it is becoming evident that the ICNS, as the final integrator of cardiac neuraxial control, can undergo significant reorganization – both anatomical and functional – during the evolution of chronic MI (Rajendran et al., 2016).

Thus, by histologic means, we sought to establish whether chronic (regional) left ventricular infarction remodels select neuronal elements within the peripheral cardiac neuraxis.

2. Materials and methods

Yorkshire pigs (35 ± 15 kg) of either gender with normal hearts ($n = 8$) and those with chronic left antero-apical infarctions ($n = 8$) were studied. All experiments were performed in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* and approved by the University of California - Los Angeles Chancellor's Animal Research Committee.

2.1. Creation of chronic myocardial infarction

Pigs were sedated with telazol (8 mg/kg, intramuscular (IM)), intubated and then ventilated. General endotracheal anesthesia (isoflurane 1–2%, inhalation) was instituted. A 6-lead electrocardiogram (ECG) and right femoral arterial pressure were monitored. An 8-French sheath was placed in the left or right femoral artery and an Amplatz-type catheter was guided over a wire into the left main coronary artery under fluoroscopy. Using an over-the-wire technique, an angioplasty balloon catheter (3 mm, Abbot Vascular, FoxCross PTA Catheter, Temecula, CA, USA) was then advanced and inflated in the mid-left anterior coronary descending (LAD) artery. Thirty seconds after balloon inflation, a five mL suspension of saline containing one mL polystyrene microspheres (Polybead 90 μm diameter, Polysciences Inc., Warrington, PA, USA) was injected distally into that artery. The MI was confirmed by the presence of ST-segment elevations in lead II of the ECG. Following this, sedation was gradually weaned, and animals extubated. Close monitoring for arrhythmias was performed for two hours after extubation, and ventricular arrhythmias treated with esmolol (5 mg boluses) and lidocaine (10–20 mg boluses). Following full recovery (i.e. the animal is upright and clinically stable), the animal was returned to its pen and monitored daily.

2.2. Characterization of chronic myocardial infarction

Cardiac magnetic resonance imaging (MRI) was performed using a 3 Tesla scanner (Magnetom Trio, A Tim System, Siemens, Munich, Germany) to confirm the location and extent of scar tissue (Fig. 1A & B) (Nakahara et al., 2011). Fifteen minutes after injecting the contrast agent gadopentetate dimeglumine (0.2 mm/kg, IV; Magnevist, Bayer, Whipanny, NJ, USA), animals were humanely euthanized and their hearts excised. Thereafter, cardiac MRI images were acquired using a three-dimensional T1-weighted gradient echo sequence. A series of parallel short axis images of the entire heart were then acquired (bandwidth: 200 Hz/pixel; spatial resolution: 0.33 × 0.33 × 0.50 mm). Areas that showed hyper enhancement were considered to represent scar tissue. A left ventricular mask was first generated by manually cropping the atria and the right ventricle using Osirix image processing software (Pixmeo, Geneva, Switzerland) and applying a threshold based mask to remove background. An infarct mask was quantified by voxels with signal intensities >6 standard deviations above the mean signal in the remote myocardium (as measured in a user-defined ROI). LV and infarct volumes were calculated by multiplying the number of voxels in the respective masks by the image voxel volume.

2.3. Terminal study

Chronic MI animals, studied six weeks post-MI, and age-matched control animals were sedated with telazol (8 mg/kg, IM), intubated and ventilated as described above. General endotracheal anesthesia was maintained with isoflurane (1–2%, INH). Depth of anesthesia was determined by monitoring hemodynamic indices, jaw tone and pedal withdrawal reflex; anesthesia was adjusted as necessary. Right femoral venous access was obtained for fluid replacement and the right femoral artery was access for monitoring arterial pressure. Body temperature was monitored continuously and maintained via heating pads. Acid-base status was evaluated hourly; respiratory rate and tidal volume were adjusted and bicarbonate was infused as necessary to maintain blood gas homeostasis. The following tissues were collected: bilateral stellate ganglia, ventral inter-ventricular and right atrial ganglionated plexi, and bilateral dorsal root ganglia at the first–second thoracic level (T1–T2). At the completion of the tissue collection, animals were euthanized under deep anesthesia using sodium pentobarbital (100 mg/kg, IV).

2.4. Histological and immunohistochemical analyses

2.4.1. Tissue fixation

Under general anesthesia, tissues were dissected and rapidly excised, rinsed in cold saline for 1–2 s, and transferred immediately to cooled phosphate-buffered formalin (10%, Fisher, Pittsburgh, PA, USA) for 3–4 days. Afterwards, tissues were rinsed in cooled saline for 1–2 s and transferred to cold 70% ethanol for paraffin embedding. Sections cut four 4 μm thick were placed on charged slides.

2.4.2. Histology and immunohistochemistry

Hematoxylin and eosin staining was used to characterize neuronal morphology, and distribution. Adrenergic neurons were identified using anti-tyrosine hydroxylase antibody (1:2000 dilution, #ab112, Abcam, Cambridge, MA, USA). nNOS immunoreactivity was detected using anti-NOS1 (1:500, sc648, Santa Cruz Biotechnology, Dallas, TX, USA). CGRP immunoreactivity was assessed using anti-CGRP (1:500, ab36001, Abcam). Prior to immunohistochemistry, slides with paraffin sections were placed in xylene to remove paraffin, and washed with ethanol. Antigen retrieval was performed by incubation for 10 min in EDTA Solution pH 8 for TH and nNOS (Invitrogen Corporation, Ref# 005,501) at 95 °C; and in Citrate solution pH 6 for CGRP using a steamer. Incubation with primary antibody was performed for one hour, and 30 min for secondary antibody incubation. Detection was performed using diaminobenzidine (Life Technologies, Green Island, NY, USA) at manufacturer's recommendation for all stains.

2.4.3. Quantification

All slides were digitally scanned at 20–40× magnification and electronically stored for analyses (Aperio Imagescope, Leica Systems, Buffalo Grove, IL, USA). Automated quantifications were done by image analysis software in a blinded fashion using Tissue Studio, Definiens

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