Control of ventricular excitability by neurons of the dorsal motor nucleus of the vagus nerve

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BACKGROUND The central nervous origins of functional parasympathetic innervation of cardiac ventricles remain controversial.

OBJECTIVE This study aimed to identify a population of vagal preganglionic neurons that contribute to the control of ventricular excitability. An animal model of synuclein pathology relevant to Parkinson's disease was used to determine whether age-related loss of the activity of the identified group of neurons is associated with changes in ventricular electrophysiology.

METHODS In vivo cardiac electrophysiology was performed in anesthetized rats in conditions of selective inhibition of the dorsal vagal motor nucleus (DVMN) neurons by pharmacogenetic approach and in mice with global genetic deletion of all family members of the synuclein protein.

RESULTS In rats anesthetized with urethane (in conditions of systemic beta-adrenoceptor blockade), muscarinic and neuronal nitric oxide synthase blockade confirmed the existence of a tonic parasympathetic control of cardiac excitability mediated by the actions of acetylcholine and nitric oxide. Acute DVMN silencing led to shortening of the ventricular effective refractory period (vERP), a lowering of the threshold for triggered ventricular tachycardia, and prolongation of the corrected QT (QTc) interval. Lower resting activity of the DVMN neurons in aging synuclein-deficient mice was found to be associated with vERP shortening and QTc interval prolongation.

CONCLUSION Activity of the DVMN vagal preganglionic neurons is responsible for tonic parasympathetic control of ventricular excitability, likely to be mediated by nitric oxide. These findings provide the first insight into the central nervous substrate that underlies functional parasympathetic innervation of the ventricles and highlight its vulnerability in neurodegenerative diseases.

KEYWORDS Arrhythmia; Atrioventricular; Brain; Cardiac electrophysiology; Nitric oxide; parasympathetic; Parkinson's disease; Vagus nerve; Ventricle

ABBREVIATIONS **7-NI** = 7-Nitroindazole; **AlstR** = allatostatin receptor; **AVNERP** = atrioventricular node effective recovery period; **DVMN** = dorsal vagal motor nucleus; **eGFP** = enhanced green fluorescent protein; **LVV** = lentiviral vector; **NO** = nitric oxide; **nNOS** = neuronal nitric oxide synthase; **QTc** = corrected QT interval; **SNRT** = sinus node recovery time; **vERP** = ventricular effective refractory period; **VT** = ventricular tachycardia; **WT** = wild type

Introduction

Sudden cardiac death is devastating for the family affected and represents a significant public health burden.[1] Sudden circulatory collapse is often attributable to malignant arrhythmias, such as ventricular tachycardia (VT). Predictors of sudden cardiac death include conventional coronary risk factors and conditions such as congestive heart failure, as well as markers of parasympathetic (vagal) dysfunction that include reduced heart rate variability and reduced baroreflex sensitivity.[2,3] It is not surprising that vagus nerve stimulation is being explored as a therapeutic option in heart failure, and

This study was supported by the British Heart Foundation and The Wellcome Trust. MB PhD funding for A. Machhada was provided by the Medical Research Council and The Rosetrees Trust. Dr. Ackland is an Academy of Medical Sciences/Health Foundation Clinician-Scientist. Dr. Trapp is supported by the Diabetes UK. Dr. Gourine is a Wellcome Trust Senior Research Fellow. This work was facilitated by the National Institute for Health Research Barts Cardiovascular Biomedical Research Unit. **Address reprint requests and correspondence:** Alexander V. Gourine, Neuroscience, Physiology & Pharmacology, University College London, Gower Street, London WC1E 6BT, United Kingdom; e-mail address: a.gourine@ucl.ac.uk or Andrew Tinker, William Harvey Heart Centre, Barts & The London School of Medicine and Dentistry, London EC1M 6BQ, United Kingdom. E-mail address: a.tinker@qmul.ac.uk.

http://dx.doi.org/10.1016/j.hrthm.2015.06.005

one component of its potential benefit may be an antiarrhythmic effect.[4,5]

Experimental studies in animal models have demonstrated profound antiarrhythmic effects of vagus nerve stimulation.[6–8] It effectively reduces the restitution slope, prevents alternans, and increases the ventricular effective refractory period (vERP).[6] Vagus nerve stimulation also decreases the incidence of ventricular arrhythmia associated with heightened sympathetic activity during myocardial infarction.[8] There is clinical evidence that vagal tone suppresses an accelerated ventricular rhythm,[9] and experimental data show that the vagal influence on ventricular refractoriness and arrhythmia threshold is tonic and could be abolished by bilateral vagotomy or systemic muscarinic receptor blockade.[10] Studies from one of our laboratories demonstrated that in mice, global genetic deletion of the inhibitory G protein $G\alpha_{i2}$ (which mediates muscarinic influences on the heart) is associated with a reduced vERP, a prolonged corrected QT interval (QTc), and a predisposition to VT.[11] There is also evidence that the protective vagal influence on cardiac electrical stability might be mediated by nitric oxide (NO), produced by neuronal NO synthase (nNOS).[12,13]

Despite this evidence, there has been no attempt to study the central nervous mechanisms underlying parasympathetic antiarrhythmic influences. In this study, we aimed to identify a population of vagal preganglionic neurons that provide functional parasympathetic innervation of the ventricles and control ventricular excitability. Vagal preganglionic neurons projecting to cardiac ganglia are located in the brainstem. Neuronal tracing experiments in different species[14,15] have identified the dorsal vagal motor nucleus (DVMN) as one such population of neurons with long-latency C-fiber axons converging on cardiac plexuses.[14] Because the activities of DVMN neurons appear to protect ventricular cardiomyocytes against acute ischemia/reperfusion injury,[16] we hypothesized that functional ventricular innervation is provided by DVMN neuronal projections.

Autonomic dysfunction has emerging clinical importance contributing to the pathogenesis of Parkinson's disease.[17,18] Parkinson's disease is the second most common neurodegenerative disorder[19] characterized by loss of dopaminergic neurons, with consequent motor impairment as well as DVMN dysfunction, which results in a host of autonomic abnormalities.[18,20] Aggregation of α-synuclein is a major molecular event in the development of the disease because of the toxicity of certain intermediate products of this process. Age-dependent decline of substantia nigra pars compacta synaptic function has been reported in α-synuclein–deficient mice.[21] These changes appeared to be even more pronounced in triple-synuclein-null ($\alpha\beta\gamma^{-/-}$) mice that have been generated to limit functional compensation by other members of the synuclein family.[22] In this study, we used $\alpha\beta\gamma^{-/-}$ mice to determine whether synuclein deficiency leads to a reduction in the activity of DVMN neurons and has an impact on the electrophysiological properties of the ventricle.

Methods

All experiments were performed in accordance with European Commission Directive 2010/63/EU (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes) and the UK Home Office (Scientific Procedures) Act (1986) with project approval from the respective institutional animal care and use committee.

Cardiac electrophysiology

Cardiac pacing with extrastimulation was performed with a Grass S88 stimulator (Grass Instruments/Natus Neurology, Warwick, RI) as described in detail previously.[11] Adult male Sprague-Dawley rats (weight 380–450 g), wild-type (WT) mice, and $\alpha\beta\gamma^{-/-}$ mice were anesthetized with urethane (1.3 g/kg IP), and an octapolar electrophysiology catheter (1.6F for rats and 1.1F for mice) was positioned in the right atrium and the right ventricle via the jugular vein or in the left ventricle via the right common carotid artery. For assessment of vERP, 10 paced beats (10 × S1) were applied with a cycle length of 85 ms in mice and 100 ms in rats, followed by a gradually shortened extra single paced beat (S2) until failure of ventricular capture. The maximum S1-S2 coupling interval was measured as the vERP. VT threshold (in rats only) was evaluated by application of 15 paced beats (15 × S1), with the cycle length shortened from 100 ms to 20 ms in 4ms increments (10–60 Hz burst pacing). One episode of VT was defined as at least 10 consecutive broad complex systoles (all of which were noted to undergo self-cardioversion). The definition of VT was similar to that in recent publications.[11]

For assessment of the atrioventricular node effective refractory period (AVNERP) in rats, 10 paced beats (10 × S1) with a cycle length of 141 ms were applied, followed by a gradually shortened extra single paced beat (S2) until failure of ventricular capture. The Wenckebach point was determined by shortening the cycle length (from 150 ms) until 2:1 block was observed. Sinus node recovery time (SNRT) was measured as the period between the last paced beat and the onset of the first P wave after 30 seconds of atrial pacing (cycle length 141 ms).

Pharmacological study

To determine the presence of a tonic parasympathetic influence on cardiac electrical stability in the main experimental model used in the present study (rat anesthetized with urethane), systemic beta-adrenoceptor blockade was applied (atenolol 2 mg · kg^{-1} · h^{-1} IV), and the effects of sequential systemic muscarinic (atropine methyl nitrate 2 mg^{-1} · kg^{-1} · h^{-1} IV) and nNOS (7-nitroindazole [7-NI]; 20 mg/kg IP) blockade on AVNERP, SNRP, Wenckebach point, left and right vERP, and left and right VT were determined.

Targeting vagal preganglionic neurons in the DVMN

Cholinergic vagal preganglionic neurons of the DVMN characteristically express the transcriptional factor Phox2