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PRE-CLINICAL RESEARCH

Carotid Chemoreceptor Ablation Improves Survival in Heart Failure

Rescuing Autonomic Control of Cardiorespiratory Function

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Objectives This study sought to investigate whether selective ablation of the carotid body (CB) chemoreceptors improves cardiorespiratory control and survival during heart failure.

Background Chronic heart failure (CHF) is a recognized health problem worldwide, and novel treatments are needed to better improve life quality and decrease mortality. Enhanced carotid chemoreflex drive from the CB is thought to contribute significantly to autonomic dysfunction, abnormal breathing patterns, and increased mortality in heart failure.

Methods Chronic heart failure was induced by coronary ligation in rats. Selective CB denervation was performed to remove carotid chemoreflex drive in the CHF state (16 weeks post-myocardial infarction). Indexes of autonomic and respiratory function were assessed in CB intact and CB denervated animals. CB denervation at 2 weeks post-myocardial infarction was performed to evaluate whether early targeted CB ablation decreases the progression of left ventricular dysfunction, cardiac remodeling, and arrhythmic episodes and improves survival.

Results The CHF rats developed increased CB chemoreflex drive and chronic central pre-sympathetic neuronal activation, increased indexes of elevated sympathetic outflow, increased breathing variability and apnea incidence, and desensitization of the baroreflex. Selective CB ablation reduced the central pre-sympathetic neuronal activation by 40%, normalized indexes of sympathetic outflow and baroreflex sensitivity, and reduced the incidence of apneas in CHF animals from 16.8 ± 1.8 events/h to 8.0 ± 1.4 events/h. Remarkably, when CB ablation was performed early, cardiac remodeling, deterioration of left ventricle ejection fraction, and cardiac arrhythmias were reduced. Most importantly, the rats that underwent early CB ablation exhibited an 85% survival rate compared with 45% survival in CHF rats without the intervention.

Conclusions Carotid chemoreceptors play a seminal role in the pathogenesis of heart failure, and their targeted ablation might be of therapeutic value to reduce cardiorespiratory dysfunction and improve survival during CHF. (J Am Coll Cardiol 2013;62:2422–30) © 2013 by the American College of Cardiology Foundation

Elevated sympathetic outflow and breathing disorders are 2 hallmarks of chronic heart failure (CHF), and both have been strongly related to decreased quality of life, poor prognosis, and increased mortality (1,2). Enhanced sympathetic drive and breathing instability during CHF have been associated with alterations in peripheral and central neural pathways that regulate autonomic function and breathing control (3,4). Despas et al. (5) showed that CHF patients with high peripheral chemosensitivity displayed higher sympathetic outflow compared with CHF patients with normal chemosensitivity. In addition, impaired baroreflex function observed in CHF has been associated with an augmented peripheral chemosensitivity in patients with CHF (6). Previous studies from our laboratory have demonstrated an augmented afferent input from the carotid body (CB) chemoreceptors in pacing-induced CHF rabbits

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and myocardial-infarcted CHF rats and have shown that reducing chemoreflex afferent traffic results in reductions in the sympathetic drive in CHF animals (7–9). Also, transient inhibition of the CB chemoreflex with brief hyperoxic stimulation in CHF patients results in a decreased sympathetic tone (5) and an improvement in the baroreflex function (6).

Patients with CHF exhibit a high incidence (up to 60%) of breathing disorders characterized by apnea/hypopneas

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and higher breathing variability (10,11) that have been related to the progression of the disease (12). Exaggerated CB-mediated ventilatory responses to apneas or hypopneas could contribute to respiratory instability. We have previously shown, in support of this notion, that reductions in CB afferent activity during CHF decrease breathing variability and apnea/hypopnea incidence in rats (13).

Together these findings suggest that the CB chemoreflex sustains the cardiorespiratory dysfunction observed in CHF. Indeed, evidence showing higher mortality rates in CHF patients with high chemosensitivity compared with patients with normal chemosensitivity (3) suggests a crucial role for the CB chemoreflex in exacerbating the pathogenesis of CHF. Although a causal link between exaggerated CB chemoreflex drive and high mortality risk during CHF has not been proven, sympathoexcitation and the apnea-related hypoxemia exacerbated by the augmented chemoreflex could lead to an increased arrhythmogenesis and deterioration of cardiac function associated with a high mortality risk (2,14).

Our prior work has shown that the exaggerated chemoreflex in CHF emanates from elevated tonic afferent nerve traffic from the CB (7–9). Recently, it has been proposed that targeting the CB by denervation of the afferent inputs might be beneficial in cardiovascular diseases exacerbated by sympathetic hyperactivity (15). The impact of carotid body denervation (CBD) on autonomic function and survival during CHF has not been studied before and could represent a novel strategy to slow the progression of cardiac deterioration and lower mortality rates in CHF. In this study, we asked whether CBD improves autonomic balance and breathing regularity during CHF and whether CBD in the early stage of cardiac dysfunction reduces cardiac remodeling and arrhythmia incidence and increases survival of myocardial-infarcted CHF rats.

Methods

Induction of heart failure. Seventy-one (2-month-old) male Sprague-Dawley rats, weighing between 430 and 560 g, were studied. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and were carried out under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996). Chronic heart failure (CHF) was produced by coronary artery ligation (CAL) as previously described (13).

Selective CB denervation. At 16 weeks (Protocol 1) or 2 weeks (Protocol 2) post-CAL surgery, the rats underwent cryogenic destruction of the CBs (16). Graphic timelines of the protocols are provided in Online Figure 1. We found that this surgical approach allows the elimination of the CB chemosensory, but not the carotid baroreceptor afferents (Online Figs. 2 and 3). The CBD did not affect water consumption or daily food intake (Online Fig. 4). The sham CBD surgery performed in sham and CHF rats showed no cardiorespiratory effects (Online Figs. 5 to 11).

Abbreviations and Acronyms

- **CAL** = coronary artery ligation
- **CB** = carotid body
- **CBD** = carotid body denervation
- **CHF** = chronic heart failure
- **eCBD** = early carotid body denervation
- **EF** = ejection fraction
- **Fra-1** = Fos-related antigen 1
- **HRV** = heart rate variability
- **IVS** = interventricular septum
- **LV** = left ventricle/ventricular
- **RVLM** = rostral ventrolateral medulla

Echocardiography. Cardiac function was determined by echocardiography (Vevo 770, Visualsonics, Inc., Toronto, Ontario, Canada) as previously described (13,17). M-mode tracings were recorded through the anterior and posterior left ventricular (LV) walls, and anterior and posterior wall thicknesses (end-diastolic and end-systolic) and LV internal dimensions were measured. Rats with ejection fractions (EFs) of <45% were considered to be in CHF (13,17).

Radiotelemetric monitoring of arterial blood pressure and heart rate. At 14 weeks, rats were chronically implanted with a radio-telemetry pressure transducer (TA11PA-C40, DSI, St. Paul, Minnesota) with a catheter directed into the abdominal aorta. Blood pressure (BP) and heart rate were acquired in conscious resting state.

Autonomic balance. Heart rate variability (HRV) and the low-frequency component of the systolic blood pressure variability (LF-SBPV) were assessed as indirect measures of autonomic balance (18) with power spectral analysis (19,20). Spontaneous baroreflex sensitivity was assessed by spectral calculation (21).

Arrhythmia score. Irregular heartbeats were visually inspected from heart rate time series (22). Arrhythmic episodes were defined as premature or delayed beats with changes >3 SDs from the mean beat-to-beat interval duration and reported as events/h.

Evaluation of respiratory variability and ventilatory chemoreflex function. Tidal volume (Vt), respiratory frequency, and minute ventilation (Vt × respiratory frequency) were determined by whole-body plethysmography as previously described (13). Respiratory stability was assessed from resting breathing recordings by Poincaré plots and analysis of SD1 and SD2 of the interbreath interval variability (13). Apnea and hypopnea incidence (cessation or ≥50% reduction in Vt over ≥3 consecutive breaths) was counted and reported as apnea and hypopnea index (events/h). Peripheral chemoreceptors were stimulated preferentially by allowing the rats to breathe hypoxic gas (10% oxygen/balance nitrogen).

Western blotting. Neuronal activation in the rostral ventrolateral medulla (RVLM) was assessed by immunoblot of the fos-related antigen 1 (Fra-1) (1:100, Santa Cruz Biotechnology, Dallas, Texas) in RVLM micropunches as previously described (18). Fra-1 expression is induced during