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Chronic spinal cord stimulation modifies intrinsic cardiac synaptic efficacy in the suppression of atrial fibrillation

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ABSTRACT

We sought to determine whether spinal cord stimulation (SCS) therapy, when applied chronically to canines, imparts long-lasting cardio-protective effects on neurogenic atrial tachyarrhythmia induction and, if so, whether its effects can be attributable to i) changes in intrinsic cardiac (IC) neuronal transmembrane properties vs ii) modification of their interneuronal stochastic interactivity that initiates such pathology. Data derived from canines subjected to long-term SCS [(group 1: studied after 3–4 weeks SCS; n = 5) (group 2: studied after 5 weeks SCS; n = 11)] were compared to data derived from 10 control animals (including 4 sham SCS electrode implantations). During terminal studies conducted under anesthesia, chronotropic and inotropic responses to vagal nerve or stellate ganglion stimulation were similar in all 3 groups. Chronic SCS suppressed atrial tachyarrhythmia induction evoked by mediastinal nerve stimulation. When induced, arrhythmia durations were shortened (controls: median of 27 s; SCS 3–4 weeks: median of 16 s; SCS 5 weeks: median of 7 s). Phasic and accommodating right atrial neuronal somata displayed similar passive and active membrane properties *in vitro*, whether derived from sham or either chronic SCS group. Synaptic efficacy was differentially enhanced in accommodating (not phasic) IC neurons by chronic SCS. Taken together these data indicate that chronic SCS therapy modifies IC neuronal stochastic inter-connectivity in atrial fibrillation suppression by altering synaptic function without directly targeting the transmembrane properties of individual IC neuronal somata.

1. Introduction

Clinical evidence indicates that symptoms associated with chronic refractory angina of cardiac origin can be alleviated by delivering high frequency, low intensity electrical stimuli to the dorsal aspect of the thoracic spinal cord–spinal cord stimulation (SCS) therapy (Eliasson et al., 1996; Mannheimer et al., 2002). It is known that the myocardium state is transduced by cardiac sensory neurites that are associated with somata nexus points throughout the cardiac nervous system, including intrathoracic ganglia and central loci (Armour and Kember, 2004; Longhurst et al., 2001; Tjen-A-Looi et al., 1997; Zucker et al., 2012). All of these populations directly and indirectly influence the behavior of intrinsic cardiac neurons, including intrinsic cardiac local circuit neurons (Ardell, 2004; Armour, 2008).

Excessive activation of selective neuronal inputs to the intrinsic cardiac nervous system (ICNS) – for instance by select mediastinal nerve stimulation – consistently initiates atrial tachyarrhythmias in the canine model (Armour et al., 2005; Cardinal et al., 2010). It is also known that SCS, when applied acutely, modifies the behavior of select populations of intrinsic cardiac neurons, in particular its local circuit neuronal population (Armour et al., 2002; Foreman et al., 2000), to obtund atrial tachyarrhythmias of neuronal origin (Cardinal et al., 2006). Recent evidence indicates that the efficacy of acute SCS may reside primarily in its capacity to stabilize ICNS local circuit neurons in the presence of such excessive and heterogeneous neuronal inputs (Gibbons et al., 2012).

While the cardiac nervous system is optimized to respond to every day stressors (heat, exercise, emotion, orthostatic), it can be critically disrupted by cardiac pathology such as myocardial ischemia, myocardial infarction, heart failure and chronic arrhythmias (Ajijola et al., 2013; Dell'Italia, 2011; Kember et al., 2013; Nakahara et al., 2010; Zucker et al., 2012). In contradistinction to global and non-specific effects of pharmacological management of such pathologies (Brunton et al., 2010), chronic neuromodulation based approaches offer the opportunity to target relevant elements of the cardiac nervous system and, as a

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consequence, influence the cardiomyocytes they regulate (Liu et al., 2012; Lopshire and Zipes, 2012; Schwartz, 2012). What remains to be determined is the short *versus* long term effects of such therapy in the context of specific cardiac pathologies.

It is known that long-term application of SCS prevents the development of a tachypacing-induced atrial fibrillation (Bernstein et al., 2012), an effect that has been proposed to depend on electrophysiological remodeling of cardiac myocyte ionic channels (Lopshire et al., 2009). However, based on our prior work (Beaumont et al., 2013; Cardinal et al., 2006; Gibbons et al., 2012), it is likely that the intrinsic cardiac nervous system, especially its local circuit neurons (Gibbons et al., 2012), represent a major target for this reduced arrhythmia potential. It remains to be established whether chronic SCS therapy imparts long-term effects on the intrinsic cardiac nervous system. It also remains to be established whether chronic SCS therapy: 1) targets the function of individual neurons within the intrinsic cardiac nervous system such that their membrane excitability to neuronal inputs becomes modified (Cardinal et al., 2004) vs 2) suppressing the stochastic network hyper-interactivity that occurs among populations of intrinsic cardiac local circuit neurons in the induction of atrial arrhythmias (Beaumont et al., 2013) vs 3) a combination of both processes.

In order to understand these core issues, chronic SCS (3–4 weeks vs 5 weeks) was applied in normal canines to determine the impact of this therapy on the capacity of the intrinsic cardiac nervous system to initiate atrial tachyarrhythmias. By these means, we found that the primary target of chronic SCS therapy in atrial arrhythmia treatment resides in its capacity to regulate intrinsic cardiac neuronal network excitability, rather than solely targeting and remodeling the function of individual intrinsic cardiac neurons. If such a thesis is sustained it implies that suppression of hyper-excited, stochastic interactions within this target organ's nervous system that lead to pathology represents a potential target for anti-arrhythmia therapy.

2. Materials and methods

2.1. Animals

Experiments were approved by the Animal Research Ethics Committee of the Sacré-Coeur Hospital Research Centre and were in accordance with the Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Academy Press, Washington DC, 2010. Three groups of canines were studied: *i)* 5 canines were subjected to continuous spinal cord stimulation for 21–27 days (3–4 weeks); *ii)* 11 canines were subjected to continuous SCS for 33–35 days (5 weeks); and *iii)* 10 control animals were studied that included 4 canines in which a SCS electrode and a neurostimulator were implanted (without being activated) for 5 weeks (sham SCS) and 6 control animals without electrode implantation, all kept in the same housing environment.

2.2. Spinal cord stimulator implantation and SCS implementation

Under isoflurane (2%) anesthesia and sterile conditions, animals were placed in the prone position; a small incision was made on the dorsal surface (T4–T6 level) and the epidural space of the mid-thoracic spinal column penetrated percutaneously with a Touhy needle, using fluoroscopic guidance and loss-of-resistance technique. Then an octopolar electrode (Octrode™ Model 3086, St. Jude Medical, Plano, TX) was introduced *via* this cannula into the epidural space and its tip advanced to the T1 spinal level, slightly to the left of midline. The rostral and caudal poles selected for subsequent use (spacing of 52 mm) were placed at the T1 and T4 levels. These were connected to a neurostimulator (EonC™ Model 3688, St. Jude Medical) generating 50 Hz, 0.2-ms duration pulses. An intensity setting of 90% of motor threshold (contraction of proximal forepaw, shoulder, and thoracic trunk musculature) was determined. After fixing the electrodes in place, the lead was tunneled to a subcutaneous pouch created on the animal flank, both incisions were closed in layers and the animal recovered from surgery. The pulse generator was inactive for the duration of the recovery period (~1 week). Following recovery from implant, SCS was applied for 3–4 (n = 5 dogs) or 5 (n = 11 dogs) weeks.

2.3. Terminal in situ studies

Anesthesia was reapplied with Na thiopental (25 mg/kg i.v.). Animals were intubated and ventilation maintained under positive-pressure. Through a midline neck incision each vagosympathetic trunk was exposed and sectioned cephalad to the site of electrical stimulation electrode application. A bilateral thoracotomy was then performed and the pericardium was incised to expose the heart. Both stellate ganglia were exposed and their central connections severed. Left ventricular pressure (model SPC-350 electronic pressure sensors, Millar, Houston, TX) and a lead II ECG were recorded on a rectilinear pen recorder (Nihon Kohden, Tokyo, Japan). All hemodynamic data were digitized (Cambridge Electronic Design power 1401 acquisition system with Spike 2 software) for subsequent off-line analysis.

After completion of these surgical manipulations, the anesthesia was changed to α-chloralose (25–50 mg/kg i.v. bolus supplemented with 25 mg/kg iv as required). To establish baseline levels for extrinsic efferent control of regional cardiac function, the right and left vagosympathetic trunks (frequency: 3, 5, 10 and 20 Hz) and stellate ganglia (4 Hz) were stimulated individually using bipolar electrodes connected to a battery-driven current source controlled by a programmable stimulator (2 ms pulse duration; 3× threshold intensity). To evaluate the atrial arrhythmogenic substrate, mediastinal nerves were first visualized coursing over the ventral or ventrolateral surface of the superior vena cava within the pericardial reflection. Trains of 5 electrical stimuli (2 ms pulse width; 5 ms inter-pulse interval) were then applied *via* bipolar electrodes (1.5 mm apart) to identified nerves once *per* cardiac cycle during the refractory period of neighboring atrial tissues (*i.e.* ~30 ms after excitation of a reference bipolar atrial electrogram). This procedure was employed to avoid direct atrial muscle capture *via* the bipolar electrodes. The electrodes, mounted on a hand held probe, were connected to a battery-driven constant current stimulus isolator (model A385, World Precision Instruments, Inc., Sarasota, FL). The current delivered *via* this probe was controlled by a programmable stimulator triggered by the reference atrial electrogram. With this technique, atrial arrhythmias can be reproducibility activated over hours and the efficacy of specific neuromodulation therapies evaluated (Armour et al., 2005; Gibbons et al., 2012; Richer et al., 2008).

Three mediastinal nerve sites were identified in each animal. The stimulus intensity applied to identified mediastinal nerves *via* the bipolar electrodes was 1 mA at first in order to determine if stimuli applied to an identified nerve could elicit sinus bradycardia that was rapidly followed by atrial tachyarrhythmia induction, as reported previously (Armour et al., 2005; Beaumont et al., 2013). The stimulus current was stopped immediately upon tachyarrhythmia induction. After a few minutes of recovery, stimulus application was repeated at 2 mA intensity and a third trial was later performed at 5 mA.

2.4. Terminal in vitro studies

In 4 control (sham SCS) and 8 of the SCS 5 weeks animals (at the end of the experimental period), in the presence of general anesthesia the heart was excised. The part of the right atrial free wall which contains the right atrial ganglionated plexus was removed quickly from the heart and placed in a dish containing cold (4 °C) Tyrode's solution (composition in mM: NaCl 120, $NaHCO_3$ 25, NaH_2PO_4 1, KCl 5, $MgCl_2$ 2, $CaCl_2$ 2.5, D-glucose 11; pH 7.4). The myocardium surrounding the fat containing the right atrial ganglionated plexus was trimmed away. The remaining tissue was pinned with the endocardial surface placed on the silicone-rubber covering the bottom of a recording chamber.