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Challenging cardiac function post-spinal cord injury with dobutamine

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ABSTRACT

There is general consensus that spinal cord injuries (SCI) above T6 result in altered sympathetic control of the heart, which negatively influences cardiac structure and function. To by-pass disrupted circuitry and investigate cardiac responses under enhanced sympathetic activity we utilized dobutamine (DOB) stress echocardiography. Animals were divided into a T2, 25 g-cm contusive SCI (SCI) or an uninjured control (CON) group. Echocardiography was performed pre-SCI and at 1, 2 and 6 weeks post-SCI. Increasing doses of DOB (5, 10 & 20 μg/min/kg) were infused intravenously pre-SCI and at 1 and 6 weeks post-SCI. Parasternal-short axis images were used to compare group differences in systolic function and track changes in response to SCI and DOB over time. One week post-SCI, stroke volume (SV), end diastolic volume (EDV), cardiac output (CO) and ejection fraction (EF) were all reduced compared to CON and these deficits persisted to 6 weeks. We also found an increase in collagen deposition at 6 weeks post SCI. Pre-SCI, DOB elicited a decrease in EDV and increases in CO, EF and HR but not SV. At 6 weeks following SCI, in addition to increases in CO, EF and HR, DOB also induced increases in SV. This is the first report, to our knowledge, of DOB responses in a contusive SCI model with persistent cardiac impairments. The return of CO to pre-SCI levels and the substantial increase in SV at low DOB dosages shows that impaired descending control of the heart is directly contributing to reduced resting SV after SCI.

1. Introduction

Cardiovascular (CV) dysfunction is the leading cause of morbidity and mortality in the chronic SCI population (Garshick et al., 2005). Following high thoracic and cervical injuries descending sympathetic control of the vasculature and heart is disrupted leading to a dysregulation of blood pressure and heart rate (HR) and ultimately to cardiovascular decline. Cardiac dysfunction is further compromised by a reduction in demand due to immediate immobility and prolonged inactivity. In individuals with chronic tetraplegia, for example, cardiac atrophy (Kessler et al., 1986) is prevalent but can be partially reversed with appropriate training (Nash et al., 1991).

Despite the prevalence of cardiac dysfunction in spinal cord injured individuals (Hopman et al., 1992; Kessler et al., 1986), little is known about cardiac systolic function independent of the disrupted spinal circuitry. West et al. demonstrated that animals with a complete spinal transection at T3 have a blunted ability to develop LV pressure during periods of increased filling (West et al., 2014) and Lujan et al. demonstrated that following a T5 transection there is increased sympathetic support of HR and rates of contraction (Lujan et al., 2012). It is important to note that while the transection model disrupts all descending and ascending circuitry this model does not reflect the majority of clinical injuries which are anatomically incomplete even when deemed functionally complete (according to the National Spinal Cord Injury Statistical Center).

Dobutamine stress echocardiography (DSE) is a clinical technique used to investigate how the heart responds to drug-induced increases in sympathetic activation (Krahwinkel et al., 1997). Since Dobutamine is a sympathomimetic drug that primarily targets β-1 receptors, it stimulates chronotropy and inotropy (Ruffolo, 1987). DSE is classically used as a means to investigate cardiac regional wall motion for detection of

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coronary artery blockage, ischemia, and myocardial viability (Leite et al., 2015; Lualdi and Douglas, 1997; Pellikka et al., 1995; Wu et al., 2004). This clinical technique has recently been applied to the rodent (Plante et al., 2005) and is being implemented in experimental models to evaluate cardiac dysfunction in a variety of diseases/pathologies (Leite et al., 2015; Schneider et al., 2010). Moreover, the rodent responses to DOB infusion strongly resemble those in humans (Plante et al., 2005), most likely because the sympatho-excitatory pathways that innervate the heart are similar across rodents and humans making DSE a highly translatable technique.

The primary objective of this study was to investigate cardiac function and functional reserve following high-thoracic SCI by increasing sympathetic activation with a sympathetic agonist (Dobutamine).

2. Methods

Experiments were conducted on 14 female Sprague-Dawley (SD) rats (age = 15 wks, weight 250–260 g). Group size was determined from power analysis calculations. Based on previous work we would expect a group difference in SV (SD = 27.62–44.46), the power to detect a true significant difference equals 80.4–85% with a sample size of approximately 6–8. All procedures were approved by the University of Louisville Animal Care and Use Committee. Animals were randomly assigned to one of two groups: uninjured control (CON $n = 6$) or T2 25 g-cm SCI (SCI $n = 8$). Cardiac function was assessed prior to SCI and at 1, 2 and 6 weeks post-SCI. Dobutamine stress echocardiography (DSE) was conducted pre-SCI and at post-SCI weeks 1 and 6. Hindlimb function during overground locomotion was assessed weekly using the BBB Open Field Locomotor Scale as described previously (Basso et al., 1995; Magnuson et al., 2009).

For SCI surgery, animals were anesthetized with a ketamine/xylazine/acepromazine cocktail (50/0.024/0.005 mg/kg i.p.) and a dorsal mid-line incision was made through the skin and musculature overlying the C8 to T4 spinal segments. A laminectomy was performed at T1 and T2 to expose the underlying T2 spinal cord and moderately-severe injuries (25 g–cm) were delivered with a MASCIS Impactor (Rutgers University, NJ). All animals were given Buprenorphine (0.1 mg/kg, SC) twice a day for three days, gentamycin (Gentamicin sulfate 15 mg/kg SC) once a day for seven days, and 5 ml of Lactated Ringers for five days and as needed for hydration. Bladders were expressed manually for five days or until their bladders emptied spontaneously. After recovery, animals were housed socially, two per cage, on the same 12-h light/dark cycle.

2.1. Dobutamine stress echocardiography (DSE) assessments

During DSE assessments, animals were maintained at surgical levels of isofluorane anesthesia (1.5–2%). Core body temperature was maintained at 36–37 °C and ventilation was monitored with inductance plethysmography. The tail vein was cannulated with a 25-gauge butterfly needle for drug administration. Images were captured along the parasternal short-axis (SAX) at the midventricular level with a high-resolution ultrasound imaging system (VisualSonics VEVO 2100) and probe (24 MHz) secured in a stereotactic stand (VisualSonics). Before drug administration, a pre-DOB image was captured for baseline systolic and diastolic measures. DOB was infused at progressively increasing dosages (5, 10 and 20 μg/kg/min; rates of: 2.10, 4.25 and 8.55 ml/h) for 4 min each, using an automated perfusion pump (KD Scientific, Holliston, MA). Four minutes of continuous drug infusion have previously been shown to elicit a maximal response at each dose (Plante et al., 2005). M-mode images were captured at the end of each four-minute administration. Anesthesia was then discontinued and the animals were monitored until fully recovered. Results for 10 cardiac cycles during expiration along the SAX were averaged for between group and dose response comparisons (West et al., 2014).

2.2. Histology

For histological analyses, animals were perfused with phosphate buffered saline via the ascending aorta to preserve cardiac tissue. The heart was then cleaned of excess fat and vessels and was weighed before being placed in 4% PFA. After 24 h in fixative, hearts were cryoprotected in 30% sucrose with sodium azide for two days and then blocked in cryoprotective media. The entire spinal column was also extracted and placed in 4% PFA for three days. The SC was then removed from the column and cryoprotected in 30% sucrose for at least 48 h.

Hearts were sectioned at 10 μm and processed for collagen deposition with conventional Masson's Trichrome stain. Images were captured at 20× magnification from the left ventricle free wall from five different sections at least 70 μm apart using consistent camera settings. Collagen deposition was quantified as a percent of the total area (40 μm^2) of the image. Specifically, images were set to a threshold to identify collagen-positive tissue and the area of collagen-positive tissue was divided by the total area of the image (in pixels). The percent of collagen was averaged across the five images captured per animals to obtain one data point per animal.

Spinal Cords (SC) were sectioned through the epicenter at 30 μm. Sections were allowed to air dry for 30 min before storing at 4 °C overnight. The following day, SC sections were warmed for 20 min and processed for spared white matter using Eriochrome Cyanin (EC) stain as described previously (Smith et al., 2006).

2.3. Statistical analysis

Repeated measures analyses of variance (RM ANOVAs) were preformed to determine significant main effects and significant interactions between the main effects. For all analyses, parametric ANOVA assumptions were tested (normality and Mauchly's sphericity test). The Greenhouse-Geisser correction was used to adjust degrees of freedom and correct the p value when the variance test was significant revealing unequal variance. Following significant main effects, Tukey HSD post hoc t-tests for multiple comparisons were performed on the relevant comparisons of interest to decrease the occurrence of type 1 errors.

Between group comparisons (CON vs. SCI) for echocardiography assessments without Dobutamine were analyzed with RM ANOVA with one factor for time post-SCI (repeated) and one factor for group (independent). Dobutamine stress echocardiography responses were analyzed using RM ANOVA with repeated factors for time post-SCI (i.e. pre-SCI, 1 and 6 weeks post-SCI) and dose.

Anatomical parameters were analyzed with Independent t-tests between means with equal or unequal variance, as appropriate. Statistical analyses were performed with SPSS (v22). Data are displayed as means ± standard deviation (SD). Significance was set at $P \leq 0.05$.

3. Results

Our between group analysis revealed that stroke volume (SV), end diastolic volume (EDV) and cardiac output (CO) were all reduced at one week post-SCI compared to age matched CON (Fig. 1A, C & E; all $P < 0.05$). At two weeks post-SCI ejection fraction (EF) was also reduced compared to CON (Fig. 1D; $P < 0.05$). By six weeks post-SCI, cardiac flow indices (SV, CO & EF) and HR were all diminished compared to CON levels (Fig. 1A–D; all $P < 0.05$). End diastolic volume was reduced compared to CON (Fig. 1E; $P < 0.05$) although end systolic volume was no different. Relative wall thickness (RWT) and posterior wall thickness (PWT) remained unchanged after SCI.

Our within group analysis revealed that with DOB administration pre-SCI there was a dose-dependent increase in HR and CO (Fig. 2B, C; $P < 0.05$ 0 vs. 5, 10 & 20 μg) but not in SV (Fig. 2A; $P = 1.0$). End systolic and diastolic volumes (ESV, EDV) decreased with increasing concentrations of DOB (Fig. 2E, F; $P < 0.01$ 0 vs. 20 μg), which resulted in an

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