Autonomic Neuroscience: Basic and Clinical 183 (2014) 30–35

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Role of cardiac sympathetic nerves in blood pressure regulation

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ARTICLE INFO

Article history:
Received 5 October 2013
Received in revised form 19 February 2014
Accepted 22 February 2014

Keywords:
Ganglionectomy
Stellate ganglion
Catecholamines
Heart

ABSTRACT

Stellate ganglionectomy (SGx) was used to assess the contribution of cardiac sympathetic nerves to neurogenic hypertension in deoxycorticosterone (DOCA)–salt treated rats. Experiments were conducted in two substrains of Sprague–Dawley (SD) rats since previous studies reported bradycardia in Charles River-SD (CR-SD) rats and tachycardia in SASCO-SD (SA-SD) rats with DOCA treatment suggesting different underlying neural mechanisms. Uninephrectomized male rats underwent SGx or SHAM surgery and were instrumented for telemetric monitoring of mean arterial pressure (MAP) and heart rate (HR). After recovery, 0.9% saline solution and DOCA (50 mg) were administered. Baseline MAP (Days 0–5 average) after SGx in CR-SD rats (96 ± 2 mm Hg; $n = 7$) was not significantly different ($p = 0.08$) than CR-SD SHAM rats (103 ± 3 mm Hg; $n = 9$); however, there was a significantly lower HR during the baseline period (377 ± 7 vs. 432 ± 7 bpm, $p < 0.05$) in SGx rats. In SA-SD rats baseline MAP was not different between SGx and SHAM rats and HR was lower in SGx rats (428 ± 8 vs. 371 ± 5 bpm, $p < 0.05$). After DOCA treatment in both substrains, MAP and HR were elevated similarly in SHAM and SGx groups showing minimal impact in both groups of SGx on hypertension development. However, overall MAP in SA-SD SHAM rats reached a significantly higher level (155 ± 10 mm Hg vs 135 ± 5 mm Hg, $p < 0.05$) than that observed in CR-SD SHAM rats demonstrating that the magnitude of hypertensive response to DOCA–salt treatment varies between substrains. In conclusion, removal of cardiac sympathetic nerves did not alter the development or maintenance of DOCA–salt hypertension in SD rats.

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1. Introduction

It is now well accepted that human essential hypertension is associated with increased sympathetic nervous system activity (SNA). Most notably, the studies of Esler and colleagues have demonstrated that both cardiac and renal norepinephrine (NE) spillovers are elevated in human hypertensives (Ferrier et al., 1993; Esler et al., 2001). Also, in support of a role of the cardiac sympathetic system in hypertension, local anesthetic blockade of cardiac projecting neurons in the stellate ganglia decreases blood pressure in humans with hypertension following cardiopulmonary bypass surgery (Fee et al., 1979). Although these measurements do not establish a causal link between increased SNA to the heart and kidneys and development of hypertension, the recent demonstration that radiofrequency ablation of renal nerves in humans with drug resistant hypertension decreases arterial pressure for a long as 2 years following the procedure, provides strong evidence that organ specific sympathetic denervation may be an important therapeutic approach to the treatment of hypertension. This therapeutic strategy was based on a large number of reports in which renal denervation was found to delay and/or prevent some (Katholi et al., 1980; Katholi et al., 1983; O'Hagan et al., 1990; Jacob et al., 2005), but not all (Dzielak and Norman, 1985; Kandlikar and Fink, 2011a), animal models of neurogenic hypertension.

In contrast to numerous studies on the role of renal nerves in regulation of arterial pressure under normal or pathophysiological conditions, the contribution of SNA to the heart has received relatively little attention in animal models (Bell and McLachlan, 1979). This is due, in part, to the challenges of recording cardiac SNA in conscious animals and the fairly accepted view that neural control of arterial pressure in the long-term primarily involves regulation of kidney function. However, it has been reported that systemic pharmacological blockade of beta-adrenergic receptors or surgical destruction of cardiac sympathetic nerves prevents the development of a model of neurogenic hypertension, the deoxycorticosterone acetate (DOCA)–salt model in the rat (Bell and McLachlan, 1979). In that study arterial pressure was measured intermittently in restrained rats using the indirect tail cuff method and the effectiveness of the denervation was confirmed for the atria but not the ventricles (Bell and McLachlan, 1979). Thus,

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http://dx.doi.org/10.1016/j.autneu.2014.02.005
1566-0702/Published by Elsevier B.V.

interpretation of these findings is complicated by the fact that this method of arterial pressure measurement may result in acute stress-induced increases in arterial pressure that may be mediated by increased cardiac SNA.

In order to investigate the contribution of cardiac sympathetic nerves in the regulation of arterial pressure, we recently developed a method to chronically denervate the sympathetic innervation of the rat heart by bilateral surgical resection of the stellate ganglia (Yoshimoto et al., 2008). Our procedure significantly reduces tissue NE in all four chambers of the heart and chronically decreases heart rate, but not baseline arterial pressure, as measured continuously in conscious unrestrained rats using radiotelemetry (Yoshimoto et al., 2008).

The present study was conducted to determine if cardiac sympathetic nerves play a role in the development and/or maintenance of DOCA–salt hypertension in the rat. Experiments were conducted in two substrains of Sprague–Dawley (SD) rats; one derived from the original strain and transferred to Charles River Laboratories (CR), in which heart rate has been reported to decrease during DOCA–salt treatment (Jacob et al., 2005) and the other from SASCO (SA), in which a marked tachycardia has been reported in DOCA–salt rats (O'Donaughy et al., 2006). We hypothesized that the proposed neurocardiogenic nature of DOCA–salt hypertension would be revealed and SGx would attenuate the rise in MAP in this model of hypertension similar to observations in renal and splanchnic denervation (Kandlikar and Fink, 2011b).

2. Materials and methods

2.1. Animals

Two substrains of adult, male SD rats (250–275 g) were purchased Charles River Laboratories (Wilmington, MA). One substrain (strain code 001), which was originated in 1925 by Robert Dawley and moved to Charles River in 1950, is referred to as Charles River SD (CR-SD). The second substrain (strain code 400), which was transferred to SASCO in 1979 and then to Charles River in 1996, is referred to as SASCO-SD (SA-SD). All animals were housed in small groups in a temperature and light controlled room until the time of study. During this period rats had access to standard rat chow and distilled water ad libitum. All procedures were approved by the University of Minnesota Animal Care and Use Committee and were conducted in accordance with the institutional and National Institutes of Health guidelines.

2.2. Surgical procedures

Fig. 1 shows the timeline for surgical procedures and experimental protocol. 10 days before control measurements began, rats were anesthetized (pentobarbital sodium; 50 mg/kg, ip) and administered atropine sulfate (0.4 mg/kg, ip). Prophylactic antibiotic (gentamicin sulfate; 10 mg/kg, im) was given prior to surgery. Following a midline abdominal incision, a right unilateral nephrectomy was performed as previously described (Jacob et al., 2005). Then the catheter of a radiotelemetry transmitter (model TA11PA-C40, Data Sciences, St. Paul, MN) was implanted via the femoral artery into the abdominal aorta as previously described (Yoshimoto et al., 2008). After implantation of the radiotelemetry transmitter, the abdominal musculature was sutured and the skin layer was closed using 9 mm stainless steel wound clips. Finally, surgical denervation of cardiac sympathetic nerves was accomplished by bilateral SGx (Yoshimoto et al., 2008). Briefly, rats were intubated and a midline a thoracotomy was performed. The right stellate ganglion was isolated between the first and second ribs beneath the parietal pleura. All nerve branches running into the ganglion were isolated and cut, the ganglion was excised. Left SGx was performed using the same procedure on the left side. For SHAM rats, the identical surgical procedures were performed with the exception of sectioning and removing the ganglia. All SGx rats exhibited bilateral ptosis the day after surgery, an initial indicator of a successful ganglionectomy. Post-operatively, an injection of ampicillin sodium (22 mg/kg, im) was given and pain management was provided with an injection of buprenorphine hydrochloride (0.05 mg/kg, im). Upon recovery from anesthesia, rats were individually housed for the duration of the study.

2.3. Experimental protocol

As depicted in Fig. 1, rats were allowed 10 days to recover from surgery before beginning the experimental protocol. Water was provided ad libitum during the first 7 days of recovery. Then, water was replaced with a saline solution (0.9%NaCl/0.2%KCl) for the duration of the protocol. On Day 0, daily measurements of MAP, heart rate (HR) and 24 h fluid intake were initiated. The radiotelemetry transmitter signal was monitored by a receiver (Data Sciences, model RPC-1, St. Paul, MN) mounted under the cage and connected to a Data Exchange Matrix. The arterial pressure signal was sampled at 500 samples/s for 10 s every 4 min throughout the protocol using commercially available software (Dataquest A.R.T., Data Sciences, St. Paul, MN). Heart rate was determined from the arterial pressure profile using the same software.

On Day 5 of the protocol, rats were anesthetized with isoflurane for implantation of DOCA subcutaneously. DOCA silicone implants were made at least 72 h prior to surgical implantation. DOCA (50 mg) was added to 2 ml of silicone elastomer® base (Sylgard 184, Dow Corning Corporation, Midland, MI) and mixed for 10 min until homogeneous. Silicone elastomer® curing agent (0.2 ml) was then added to the concoction. The DOCA implants were left curing at room temperature for 24 h and then refrigerated at 4 °C until the day of the surgery. Each 50 mg DOCA silicone implant was cut into 2–3 mm cubes that were then placed subcutaneously between the scapular blades in each rat. The surgical procedure was performed in 15 min

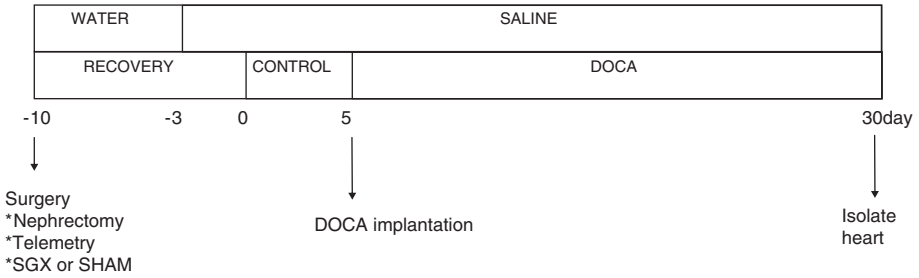

Fig. 1. Experimental timeline. Right uninephrectomy, implantation of telemetry, and SHAM or stellate ganglionectomy (SGx) surgery occurred at Day −10. Animals were allowed to recover for 10 days until the control period measurements were started on Day 0. On Day −3, animals were switched from water to 0.9%NaCl/0.2%KCl drinking solution. Subcutaneous DOCA implantation was done on Day 5. Studies lasted until Day 31 at which time animals were deeply anesthetized and hearts were removed for confirmation of denervation.