Published in final edited form as:

Auton Neurosci. 2014 July ; 0: 30–35. doi:10.1016/j.autneu.2014.02.005.

ROLE OF CARDIAC SYMPATHETIC NERVES IN BLOOD PRESSURE REGULATION

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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 (day 0-5 average) after SGx in CR-SD rats (96 ± 2 mmHg; $n=7$) was not significantly different ($p=0.08$) than CR-SD SHAM rats (103 ± 3 mmHg; $n=9$); however, there was a significantly lower HR during the baseline period (377 ± 7 vs. 432 ± 7 beats/min, $p<0.05$) 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 beats/min, $p<0.05$). 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 mmHg vs 135 ± 5 mmHg, $p<0.05$) 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.

Keywords

ganglionectomy; stellate ganglion; catecholamines; heart

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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 (Esler et al., 2001; Ferrier et al., 1993). 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 as long as 2 yrs 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 (Jacob et al., 2005; Katholi et al., 1983; Katholi et al., 1980; O'Hagan et al., 1990), but not all (Dzielak et al., 1985; Kandlikar et al., 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 et al., 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 deoxycortisosterone acetate (DOCA)-salt model in the rat (Bell et al., 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 et al., 1979). Thus, 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'Donoghue et al., 2006). We hypothesized that the proposed neuro-cardiogenic 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 et al., 2011b).

Material and Methods

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.

Surgical Procedures

Figure 1 shows the timeline for surgical procedures and experimental protocol. 10 days before control measurements began, rats were anaesthetized (pentobarbital sodium; 50 mg/kg ip) and administered atropine sulphate (0.4 mg/kg ip). Prophylactic antibiotic (gentamicin sulphate; 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.

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 24hr 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/second for 10 secs every 4 mins 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 hrs 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 mins 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 hrs 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 mins and was executed between the hours of 10 am and 12 pm. Rats were returned to their cages and monitored until day 31.

Measurement of Cardiac Norepinephrine Content

Upon completion of the experiment, rats were deeply anesthetized (sodium pentobarbital, 65 mg/kg, ip) and hearts were removed to assay for content of NE. The heart was rapidly removed and divided into the following samples which were individually weighed and frozen in liquid nitrogen; right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), and ventricular septum (VS). Heart chambers were homogenized in ice-cold 0.1 M perchloric acid. The homogenate was then centrifuged at 10,000 rpm for 15 mins at 4°C and the supernatant was further processed with a 30kD filter. The eluate was analyzed by capillary electrophoresis with electrochemical detection (CE-EC). CE-EC using boron-doped diamond electrodes for detection was employed. The CE-EC system, electrochemical detection cell, and electrode fabrication is described elsewhere (Muna et al., 2005; Novotny et al., 2007). The separation and detection was performed using following conditions: 76 cm long, 362 µm o.d., 29 µm i.d. capillary, 250 mM boric acid - 1 M potassium hydroxide run buffer of pH 8.8, separation voltage 24 kV, detection potential +0.86 V vs. Ag/AgCl and electrokinetic injection at 18 kV for 8 s.

Data and Statistical Analysis

MAP and HR data were sampled every 4 mins and stored for later analysis. Data were initially analyzed by plotting hourly averages for the entire protocol. Baseline data is reported as average of days 0-5, while day 31 values are used for post-DOCA-salt comparisons. Subsequently, 24 hr averages were plotted and analyzed by 2-way analysis of variance for repeated measures followed by the Holm-Sidak method for all post-hoc

comparisons (SigmaStat version 3.5). Cardiac content of NE between SHAM and SGX rats was determined for each heart chamber using an unpaired t-test with Welch's correction as needed to account for differences in variance between groups.

Results

Baseline physiological measurements

Table 1 summarizes the average baseline physiological measurements over the 5 day control period while rats were drinking saline. There was a trend for reduced MAP following SGX in CR-SD rats ($p=0.08$); however, the MAP was not different compared to SHAM rats in either substrain. HR was significantly reduced by SGX in both groups to a similar extent. Fluid intake was similar in all groups prior to DOCA-salt treatment.

Responses of Charles River-Sprague Dawley (CR-SD) to DOCA-salt

The cardiovascular and fluid intake responses to DOCA-salt treatment in CR-SD rats are shown in Figure 2. Following DOCA implantation, MAP rose similarly over the next 26 days in both SHAM and SGX groups (Figure 2A). By day 9 (4 days after DOCA), there was a significant elevation in MAP in CR-SD SHAM rats and this remained significant throughout the protocol. MAP increased similarly in CR-SGX rats such that the MAP was significantly different from baseline on day 8 of the protocol. There was no statistical difference in the MAP between groups throughout the DOCA-salt treatment period. MAP on the final day of DOCA-salt in CR-SD SHAM and CR-SD SGX rats was 135 ± 5 mmHg and 140 ± 5 mmHg, respectively. Data for absolute values and delta MAP in response to DOCA-salt are shown in Table 2. The final steady-state increase in MAP from baseline to the last day of DOCA-salt was not different in SHAM versus SGX rats ($p=0.08$).

SGX resulted in a significantly lower baseline HR in CR SD-SGX compared to CR-SD SHAM rats (Figure 2B). HR gradually declined following implantation of DOCA becoming significantly lower than baseline at day 11 in CR-SD SHAM animals and day 14 in CR-SD SGX rats. With the exception of days 27-29 and 30 of the protocol, HR remained significantly lower in CR-SD SGX rats compared to the SHAM group. Data for changes in HR from baseline in response to DOCA are shown in Table 2. The decrease in HR in response to DOCA-salt was significantly blunted in CR-SD SGX rats ($p<0.05$).

Fluid intake was similar in SHAM and SGX CR-SD rats during baseline and increased in both groups following administration of DOCA (Figure 3C). This increase from baseline became significant on day 8 for both groups and remained elevated until day 29 and day 31 for SHAM and SGX groups, respectively. There was no difference between SHAM and SGX groups in fluid intake except on day 29 where fluid intake in SHAM animals was significantly lower than SGX.

Responses of SASCO-Sprague Dawley (SA-SD) to DOCA-salt

The cardiovascular and fluid intake responses to DOCA-salt treatment in SA-SD rats are shown in Figure 3. For the most part, the pattern of responses, and the effect of SGX was similar to CR-SD rats. There was no difference between groups in MAP during the baseline

period and administration of DOCA increased MAP similarly in SA-SD SHAM and SA-SD SGx rats (Figure 3A). MAP on the final day of DOCA-salt was 155 ± 10 and 152 ± 8 mmHg in SA-SD SHAM and SA-SD SGx rats, respectively. Data for absolute values and the DOCA-induced increase in MAP are shown in Table 2. The magnitude of the increase in MAP from baseline to day 31 was not different in SA-SD SHAM versus SGx rats.

Consistent with CR-SD rats, HR was significantly lower in SA-SD SGx rats compared to the SHAM group during the baseline period (Figure 3B). After DOCA implantation, HR was unchanged until day 27 in SHAM animals. In contrast, HR did not change significantly from baseline during the entire DOCA treatment period in SA-SD SGx rat. Data for absolute values and the response to of HR to DOCA-salt are shown in Table 2.

Fluid intake was similar in both groups during the baseline period and throughout the entire DOCA treatment period (Figure 3C). There was no statistical difference between SHAM and SGx SA-SD rats for fluid intake during the entire protocol (Figure 3C).

Comparison of cardiovascular responses to DOCA-salt in SHAM CR-SD and SA-SD rats

The comparison of CR-SD and SA-SD SHAM rats to DOCA-salt treatment are shown in Figure 4. SA-SD rats responded with a more robust increase in MAP such that by day 16 of the protocol MAP was statistically higher compared to CR-SD rats and remained so for the duration of the protocol (Figure 4A). By the end of the protocol, the increase in MAP from baseline was greater in SA-SD SHAM (49 ± 7 mm Hg) than CR-SD SHAM rats (33 ± 4 mm Hg). Despite this greater hypertensive response in SA-SD rats, the fall in HR observed during DOCA-salt treatment was less than that observed in CR-SD rats (Figure 4B, Table 2). These differences in MAP and HR were not the result of differences in fluid intake during the protocol (Figure 4C).

Cardiac norepinephrine content

NE content was significantly reduced in all the chambers of the SGx hearts compared with the SHAM hearts in both rat substrains (Table 3). For CR-SD, the NE content of SGx hearts compared to Sham was reduced in the RA, LA, RV, LV, and ventricular septum by 82.4, 86.5, 89.2, 89.6, and 90.7%, respectively. For SA-SD, the NE content of SGx hearts was reduced compared to Sham in the RA, LA, RV, LV, and ventricular septum by 94.9, 95.8, 97.4, 96.3, and 97.6%, respectively.

Discussion

The present study was designed to test the hypothesis that removal the input from cardiac projecting stellate neurons by stellate ganglionectomy (SGx) would attenuate or prevent the development of neurogenic hypertension in the rat. We chose the DOCA-salt model because our lab and others have shown that renal denervation attenuates hypertension in this model (Jacob et al., 2005; Katholi et al., 1983; Katholi et al., 1980) supporting the idea that it is at least partially neurogenically driven.

The SGx protocol was conducted first using CR-SD rats since we have used this substrain in several studies investigating the neurogenic mechanisms of this model (Jacob et al., 2005;

Yoshimoto et al., 2008). Contrary to our hypothesis, SGx had no effect on either the developmental or maintenance phases of DOCA-salt hypertension in this substrain. In the present study and our previous investigations (Jacob et al., 2005) we observed a marked bradycardia during DOCA-salt treatment in SHAM rats. In this study heart rate decreased from baseline levels by approximately 70 beats/min in SHAM rats. We reasoned that this bradycardia may indicate a *decrease* in cardiac SNA, rather than an increase, during DOCA-salt treatment in CR-SD rats and therefore SGx would have little to no effect of arterial pressure.

We then repeated the protocol in SA-SD rats since O'Donoghue and colleagues have reported a marked tachycardia, (~100 beats/min) during chronic DOCA-salt treatment in this substrain [15]. We hypothesized that perhaps cardiac SNA is increased in this substrain as compared to CR animals therefore and SGx would attenuate the increase in HR and MAP observed during DOCA-salt treatment. However, in contrast to the studies of O'Donoghue and colleagues, we unexpectedly observed bradycardia in DOCA-salt treated SA-SD rats. We have no explanation for differences between the present study and those of O'Donoghue and colleagues regarding the HR response to DOCA.

It is important to note that, although SGx did not attenuate MAP response to DOCA-salt in either substrain, the steady-state arterial pressure response to DOCA-salt treatment in SHAM rats was greater (~15 mmHg) in SA-SD rats compared to CR-SD rats. This difference in arterial pressure response was not due to differences in the DOCA-induced increase in saline intake which was identical in both groups. This finding is significant in that it strongly suggests that mechanisms underlying the pathogenesis of hypertension in the DOCA-salt model are different in these two substrains of SD rat or at minimum there are differing degrees of sympathetic involvement in hypertension in the substrains.

The failure of SGx to influence hypertension development in this model is unanticipated since the DOCA-salt model is neurogenically driven and denervation of other important sympathetic targets attenuates the hypertensive response (Kandlikar et al., 2011b). It would be expected that, if there is a generalized increase in sympathetic outflow in the DOCA-salt model, denervation of a single organ system would result in a partial reduction in hypertension. Indeed, we have previously reported that renal denervation attenuates, but did not prevent, DOCA-salt hypertension (Jacob et al., 2005). A recent study has demonstrated that denervation of the splanchnic vascular bed also attenuates in DOCA-salt hypertension in the rat (Kandlikar et al., 2011b). In contrast to the importance of sympathetic nerves to the renal and splanchnic vascular beds, the present study suggests that sympathetic support of the heart from NE derived from axonal projections from the stellate ganglion is not a vital component of the DOCA-salt hypertension. This does not however rule out any changes to the intrinsic nervous system of the heart (Armour, 2004) that may provide "cardiogenic" support to hypertension in the absence of sympathetic input from the stellate ganglion. In addition, we do not have any data on possible compensation by changes in stroke volume or increases in SNA to other vascular beds to maintain pressure in the absence of cardiac nerves.

Limitations

The SGx procedure would also presumably partially eliminate some cardiac afferent nerves which are known to influence other systems including the kidney. If there had been an attenuating effect of SGx on BP then it would be difficult to determine if the sympathetic efferent or afferent fibers were primarily involved; however, since there was not an attenuating effect then this issue is a moot point as removal of both efferent and afferent cardiac nerves did not impact MAP control in this study. In addition, there is ample data in human subjects showing that there is increased NE spillover consistent with increase cardiac sympathetic activity in hypertension, yet in this animal model of neurogenic hypertension the role of the cardiac nerves does not have an impact on hypertension. It is possible that since the cardiac sympathetic nerve do not appear to play a role in DOCA-salt hypertension that this model may not be an appropriate one to mimic the cardiogenic hypertension in humans.

Conclusion

While there is strong evidence for a generalized increase in sympathetic nerve activity in hypertension, there is also a compelling case to be made for a “sympathetic signature” concept in which distinct patterns of sympathetic discharge to different organ systems occurs under different pathological states. This has recently been demonstrated in the angiotensin II-salt model of hypertension in the rat in which the splanchnic vascular bed is a target of an elevated sympathetic neural discharge (Osborn et al., 2011). From a clinical perspective, the recent reports that renal nerve ablation in humans with drug resistant hypertension decreases arterial pressure also suggests that regionally targeted sympathetic ablation may be an effective therapy for the treatment of hypertension (Esler et al., 2010). Although a similar approach to target cardiac sympathetic nerves has not been developed in humans, the observation that cardiac NE spillover is increased in human hypertensives suggest this may be worthy of exploration. The results of the present study suggests that cardiac sympathetic nerves do not play a role in a rodent model of mineralocorticoid and salt excess induced hypertension, but it remains to be determined whether a similar approach of targeted cardiac denervation would be effective in other models of neurogenically driven high blood pressure or in human patients.

Acknowledgments

None

Sources of Funding: American Heart Association Predoctoral Fellowship (EAW), PO1HL70687 (DLK), and NHLBI R01 HL64176 (JWO)

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Figure 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.

Figure 2. Stellate ganglionectomy (SGx) in Charles River Sprague-Dawley (CR-SD) rats does not attenuate DOCA-salt hypertension

Data was collected by radiotelemetry transmitter in unanaesthetized adult rats. A) Mean arterial pressure (MAP, mmHg) is plotted over 30 days; days 0-5 baseline indicated with vertical dashed line during which 0.9%NaCl/0.2%KCl drinking solution was administered, and days 5-30 DOCA-salt (50 mg/rat subcutaneously plus saline). Charles River Sprague-Dawley (CR-SD) rats were used. Animals underwent either SHAM surgery (Filled squares (■), n=9) or stellate ganglionectomy (SGx, open squares (□), n=7) 3 days before saline drinking water was administered. Values within a substrain of rat for each day were compared to the final day of the baseline period (day 5). Line brackets indicate that all days in the bracketed area are significantly different from baseline. Significance was determined by repeated measures ANOVA and is indicated by # for SHAM and by † for SGx, $p < 0.05$.

B) Heart rate (HR, beats/min) was also monitored in the same animals using methods and statistics as described above. There was a significant difference in HR between SHAM and SGx groups that is maintained from baseline throughout the study (* or †, $p < 0.05$ in SHAM vs. SGx). C) Fluid intake (ml/24 h) was significantly increased following DOCA administration (# or †, $p < 0.05$ vs. baseline) and remained elevated from baseline level throughout the study. Differences between substrains are indicated with *.

Figure 3. Stellate ganglionectomy (SGx) in Sasco-Sprague Dawley (SA-SD) rats does not attenuate DOCA-salt hypertension

Data was collected by radiotelemetry in unanaesthetized adult rats. A) Mean arterial pressure (MAP, mmHg) is plotted over 30 days (days 0-5 baseline with 0.9%NaCl/0.2%KCl drinking solution and days 5-30 DOCA (50 mg/rat subcutaneously (SC)) treatment plus saline). Sasco Sprague-Dawley (SA-SD) rats were used. Animals underwent either SHAM surgery (Filled circles (●, n=7) or stellate ganglionectomy (SGx, open circles (○, n=6)) 3 days before saline drinking water was administered. Values for each day were compared to the final day of the baseline period (day 5). Line brackets indicate that all days in the bracketed area are significantly different from baseline. Significance was determined by repeated measures ANOVA and is indicated by # or †, $p < 0.05$. There was no significant difference between SHAM and SGx groups after administration of DOCA ($p > 0.05$). B)

Heart rate (HR, beats/min) was also monitored in the same animals using methods and statistics as described above. Initially, there was a significant difference in HR between SHAM and SGx groups but that difference is lost by day 18 (*, $p < 0.05$ in SHAM vs. SGx). DOCA-salt treatment did not reduced HR in SGx animals and only reduced HR during the last 5 days of the protocol in SHAM animals (# or †, $p > 0.05$ vs. baseline). C) Fluid intake (ml/24 h) was significantly increased 8-days following DOCA administration (#, $p < 0.05$ vs. baseline) and remained elevated from baseline level throughout the study. Differences between substrains are indicated with *.

Figure 4. Charles River-Sprague Dawley (CR-SD) and Sasco Sprague-Dawley (SA-SD) rats have distinct hemodynamic responses to DOCA-salt treatment

Panels focus on differences between rat substrains that have undergone SHAM surgery. For statistics on change from baseline see Figures 2 and 3 and Table 2. A) Mean arterial pressure (MAP, mmHg) in SA-SD rats (Filled Circles ●, n=7) was significantly higher than CR-SD (Filled Squares ■, n=9) from day 16 onward (*, $p < 0.05$ SA-SD vs. CR-SD SHAM operated rats). B) Heart rate (bpm) tended to be lower in CR-SD rats; however, only reached significance (*, $p < 0.05$) on days 22, 25, and 26. C) Fluid intake (ml/24 h) was not different between rat substrains except on day 28 where CR-SD was significantly lower than SA-SD (*, $p < 0.05$).

Table 1
Average baseline physiological measurements during baseline period for SHAM and stellate ganglionectomized (SGx) rats of both CR-SD and SA-SD

Values reported represent a mean value $\pm$ SEM for all animals in over the entire baseline period (0-5 days).

	N	Body Weight (g)	MAP (mmHg)	HR (bpm)
Sham CR-SD	9	367 $\pm$ 4	103 $\pm$ 3	433 $\pm$ 7
SGx CR-SD	7	330 $\pm$ 5	96 $\pm$ 2	377 $\pm$ 7*
Sham SA-SD	7	336 $\pm$ 5	106 $\pm$ 3	428 $\pm$ 7
SGx SA-SD	8	323 $\pm$ 4	104 $\pm$ 3	371 $\pm$ 13*

* Statistical significance determined by ANOVA between all groups (SHAM vs. SGx, $p < 0.05$).

Table 2
Changes in mean arterial pressure (MAP) and heart rate (HR) in response to DOCA –salt treatment differ based on rat substrain and surgical removal of stellate ganglionectomy (SGx)

Values reported represent a mean value $\pm$ SEM for all animals in over the entire baseline period (“baseline”, 0-5 days) or values on Day 30 (“Post-DOCA”). Delta (Δ) MAP and HR were calculated by taking the MAP or HR average on day 30 minus baseline mean value on day 0-5.

	Mean Arterial Pressure (mmHg)		Heart Rate (bpm)	
	Baseline (Average day 0-5)	Post-DOCA (day 30)	Baseline (Average day 0-5)	Post-DOCA (day 30)
Sham CR-SD	103 $\pm$ 3	135 $\pm$ 5	33 $\pm$ 4	433 $\pm$ 7
SGx CR-SD	96 $\pm$ 2	140 $\pm$ 5	44 $\pm$ 5	363 $\pm$ 7
Sham SA-SD	106 $\pm$ 3	155 $\pm$ 10	49 $\pm$ 7	342 $\pm$ 12
SGx SA-SD	104 $\pm$ 3	152 $\pm$ 8	48 $\pm$ 7	377 $\pm$ 7
				428 $\pm$ 7
				386 $\pm$ 13
				359 $\pm$ 12
				-69 $\pm$ 6
				-35 $\pm$ 14 *
				-35 $\pm$ 14
				-12 $\pm$ 10 *

* Statistical significance (SHAM vs. SGx, $p < 0.05$).

Table 3

Norepinephrine content in heart chambers

Data were collected using capillary electrophoresis as described in the methods section. Values (ug/g tissue) reported represent a mean value $\pm$ SEM for all animals. % decrease from Sham is also reported. Right: Charles-River Sprague Dawley (CR-SD) and Left: Sasco Sprague Dawley (SA-SD).

		CR-SD Sham		CR-SD SGx		SEM		% Decrease		SA-SD Sham		SA-SD SG		SEM		% Decrease	
		SEM		SEM		SEM		SEM		SEM		SEM		SEM		SEM	
Norepinephrine	LA	1228.99	138.47	166.14	98.66	1323.89	124.3	86.5*		415.1	50.4	0.42	0.05	95.8*			
	LV	415.29	36.83	43.17	26.19	2332.73	438.7	89.6*		941.52	213.9	0.94	0.21	96.3*			
	RA	1672.36	131.51	294.25	180.47	332.99	44.8	82.4*		332.99	44.8	0.03	0.02	94.9*			
	RV	773.99	64.61	83.66	37.91			89.2*						97.4*			
	Septum	363.33	35.94	33.79	16.44			90.7*						97.6*			

* Statistical significance (SHAM vs. SGx, $p < 0.05$).