Effect of stellate gangliectomy on basal cardiovascular function
and responses to $\beta_1$-adrenoceptor blockade in the rat

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Yoshimoto M, Wehrwein EA, Novotny M, Swain GM, Kreulen DL, Osborn JW. Effect of stellate gangliectomy on basal cardiovascular function and responses to $\beta_1$-adrenoceptor blockade in the rat. Am J Physiol Heart Circ Physiol 295: H2447–H2454, 2008. First published October 17, 2008; doi:10.1152/ajpheart.00958.2008.—Cardiac sympathetic nerve activity is an important short-term controller of cardiac function and arterial pressure. Studies also suggest that long-term increases in cardiac sympathetic nerve activity may contribute to hypertension, coronary artery disease, and cardiac remodeling in heart failure. However, our understanding of the role of cardiac sympathetic nerves in chronic models of cardiovascular disease has been limited by inadequate experimental approaches. The present study was conducted to develop a surgical method to surgically denervate the sympathetic nerves of the heart for long-term cardiovascular studies. We characterized the effect of cardiac sympathetic denervation on basal levels of mean arterial pressure (MAP) and heart rate (HR) and the responses to a chronic administration of atenolol, a $\beta_1$-adrenoceptor antagonist. Rats were instrumented with telemetry transmitters for continuous recording of MAP and HR. After a 4-day baseline period, the rats were subjected to bilateral stellate gangliectomy (SGX; $n = 9$) or sham surgery (Sham; $n = 8$). Seven days following SGX or Sham, the rats were administered atenolol for 5 days, followed by a 7-day recovery period. Following a transient decrease, SGX had no effect on basal MAP but decreased HR compared with baseline and Sham rats. Five days of atenolol treatment decreased MAP similarly in SGX and Sham rats. Atenolol resulted in a marked bradycardia in Sham rats but had a negligible effects on HR in SGX rats. The measurement of the content of cardiac catecholamines in all cardiac chambers at the end of the study verified a successful sympathetic denervation. This study confirms that bilateral SGX is a useful method to study the contribution of cardiac sympathetic nerves to the regulation of cardiac function. Moreover, these results suggest that cardiac sympathetic nerves are relatively unimportant in maintaining the basal level of MAP or the depressor response to atenolol in conscious, unrestrained rats.

cardiac sympathetic nerve activity; heart rate

AT ANY MOMENT IN TIME, cardiac output is the product of the rate and stroke volume of the heart. Under normal physiological conditions, changes in cardiac sympathetic nerve activity (SNA) play an integral role in regulating the rate and force of contractions of the heart, ultimately influencing cardiac output and therefore arterial pressure. In addition to an important role in the regulation of cardiac function and arterial pressure in normal physiological states, recent evidence suggests that cardiac SNA is increased in human essential hypertension (6) and heart failure (5) and may contribute directly to the development of cardiac hypertrophy and coronary vascular disease in patients with these cardiovascular diseases (23).

The importance and relative contribution of cardiac nerves specifically to the pathogenesis of hypertension and cardiac disease in humans are difficult to establish since there are no routine treatments available to block cardiac SNA independent of other physiological effects. However, it has been reported that the surgical removal of the stellate ganglion, the source of sympathetic innervation of the heart, alleviates hypertension following coronary bypass surgery (32). The pharmacological antagonists specific for the $\beta_1$-adrenoceptor, which mediates the actions of the sympathetic neurotransmitter norepinephrine (NE) on cardiac function, are useful antihypertensive agents, but their mechanism of action is not limited to the heart. In addition to blocking cardiac $\beta_1$-receptors to decrease cardiac rate and contractility, $\beta$-blockers also act on renal $\beta_1$-receptors to inhibit the stimulatory actions of neuraly released NE, as well as circulating catecholamines, on renin release from the kidney to decrease the activity of the renin-angiotensin-aldosterone system (4). $\beta$-Blockers have also been reported to act directly on the brain to decrease sympathetic nerve discharge (9, 10, 12, 35), although this is controversial (2). Therefore, the antihypertensive effects of $\beta$-blockers may not be due to their direct effect on cardiac $\beta_1$-adrenoceptors specifically.

The rat is the most commonly used species for basic research into the mechanisms of hypertension. However, investigations into the contribution of cardiac SNA to the pathogenesis of experimental models of hypertension in this species are almost nonexistent. This is in stark contrast to studies on the role of renal nerves that have been extensively investigated in almost all rat models of hypertension (4, 36). The reason for this is twofold. First, the surgical denervation of the renal nerves is technically much easier than denervating the heart in the rat. Second, it has been theorized that the renal nerves are more important in the long-term control of arterial pressure and hypertension than sympathetic nerves to other vascular beds such as the heart (13). As a result, the contribution of changes in cardiac SNA to the development of hypertension is relatively unknown, in part, because methods for selective cardiac denervation have not been developed and extensively validated in the rat.

To our knowledge there is only one published report in which cardiac denervation was conducted in the rat and changes in cardiovascular function were measured (1). In that
study, stellate ganglionectomy (SGX) was performed retropleurally using a dorsal approach without removing the ribs, an approach that allows only the partial removal of the ganglia. Indeed, a histochemical confirmation of the denervation, which was conducted on the atria but not on the ventricles, revealed that the denervation was not complete in every animal (1). Although it was reported that SGX had no effect on the basal level of arterial pressure, measurements were made intermittently in restrained rats using the tail-cuff method, which complicates the interpretation of these findings. Also, since the tail-cuff method does not allow for the accurate measurement of heart rate (HR), the effect of cardiac denervation on this variable was not determined. There is one other report in which the effect of SGX on cardiac NE content was measured, but the effects on cardiovascular function were not determined (26).

The ability to detect small but physiologically significant changes in arterial pressure and HR in conscious rats has been greatly advanced by the development and application of radio-telemetry. This technology allows for continuous 24 h/day monitoring of arterial pressure and HR in freely moving unrestrained rats in their home environment. Using this approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in unrestrained rats in their home environment. Using this approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18). Subsequently, we established that, approach, we reported that bilateral renal denervation results in small but highly reproducible decreases in arterial pressure in normotensive rats (17, 18).

The present study was conducted with two objectives in mind. The first was to develop and validate a method for surgical denervation of the rat heart for long-term cardiovascular studies. The second objective was to compare the cardiovascular responses to the chronic administration of atenolol in control and denervated rats to determine the contribution of cardiac sympathetic nerves to the hypotensive response to this β-blocker. Rats were instrumented for continuous telemetric monitoring of arterial pressure and HR throughout the protocol. Our results suggest that a bilateral SGX results in a complete sympathetic denervation of all cardiac chambers and that the hypotensive response to atenolol is not mediated by the blockade of cardiac β1-adrenoceptors.

METHODS

Animals

Male Sprague-Dawley rats (250–275 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed in small groups in a temperature- and light-controlled room until the time of study. During this period, the 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

Experimental groups and anesthesia. The time line for surgical procedures and the experimental protocol are shown in Fig. 1. To assess the effect of cardiac sympathetic denervation on the basal levels of arterial pressure and HR, rats underwent two separate surgical procedures. The first surgery was for the implantation of a radiotelemetry transmitter (telemeter) for the continuous measurement of arterial pressure and HR before and after cardiac denervation or sham surgery. Following a recovery period (7 days) and a period of basal measurement of arterial pressure and HR (5 days), the rats were randomly assigned to undergo either bilateral SGX (n = 9) or sham SGX (Sham, n = 8) surgery. For both procedures, rats were anesthetized (pentobarbital sodium; 50 mg/kg) and administered atropine sulfate (0.4 mg/kg) with a single intraperitoneal injection. A prophylactic antibiotic (gentamicin sulfate; 10 mg/kg) was given intramuscularly before each surgery.

Telemeter implantation. Rats were anesthetized and placed dorsally on a heated surgical table. Following a midline abdominal incision, the body of a radiotelemetry transmitter (model TAI1PA-C40, Data Sciences; St. Paul, MN) was placed into the abdominal cavity. An incision was made on the ventral surface of the left leg, and the femoral artery and vein were exposed and bluntly dissected apart. The transmitter catheter was tunneled from the abdominal cavity to the femoral area via a blunt-tipped 14-gauge needle. The femoral artery was tied off distally with 3-0 silk suture, and a small clamp was used to temporarily interrupt the blood flow, at which point a small incision was made in the femoral artery. The catheter tip was introduced through this incision, advanced ~3 cm into the abdominal aorta such that the tip was distal to the origin of the renal arteries, and tied into place. The leg incision was closed, and the body of the transmitter was sutured to the femoral artery. The abdominal musculature was sutured, and the skin layer was closed using 9-mm stainless steel wound clips. Upon recovery from anesthesia, the rats were individually housed for the duration of the study.

SGX or Sham surgery. The surgical denervation of the cardiac sympathetic nerves was accomplished by bilateral SGX. The rats were anesthetized as described in Experimental groups and anesthesia and prepared for intubation as follows. The ventral surface of the neck was shaved, and the rat was placed dorsally on a heated surgical table. The neck was gently extended and held in place by loosely taping the nose to the surface of the surgical table. A fiber optic surgical lamp was directed at the ventral surface of the neck, the tongue was depressed ventrally, and a cotton swap soaked in 2% lidocaine was used to anesthetize the vocal cords for ease of passing the intubation tube. At this point it was possible to visualize the vocal cords and airway because of the illumination of the neck surface. The trachea was intubated with polyethylene-190 tubing, which was then attached to a Harvard rodent ventilator (model 683) via connecting tubing. The ventilator was set at 65 cycles/min and a 2.5-ml stroke volume. Following a midline skin incision, a thoracotomy was performed by cutting the sternum directly on the midline from the manubrium to just above the xiphoid process. The incision was retracted, and the right lung was gently pulled caudally using sterile saline-soaked gauze. A surgical microscope (model M651, Leica) was used to visualize the
right stellate ganglion 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, and the gauze was removed. A 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 the sectioning and removing of the ganglia. The chest was closed by suturing the bone, muscle, and skin in three separate layers. A negative intrathoracic pressure was reestablished by inserting a 23-gauge needle into the thoracic cavity and suctioning air with a 3-ml syringe. This was done on both the right and left sides at the ninth intercostal space. The intubation tube was removed, and the rats remained on the heated surgical table until they were mobile and then returned to their home cage. All SGX rats exhibited bilateral ptosis the day after surgery, an initial indicator of a successful ganglionectomy.

Experimental Protocol

The protocol was designed to address two separate but related questions. First, what is the effect of SGX on the basal level of arterial pressure and HR? Second, to what extent is the depressor response to $\beta_1$-adrenoceptor blockade dependent on cardiac sympathetic nerves? The time line for the experimental protocol is shown in Fig. 1.

Measurement of Cardiac Neurotransmitter and Neurotransmitter Precursor Content

Upon the completion of the experiment, the rats were deeply anesthetized and the hearts were removed to assay for the content of catecholamines [NE, dopamine (DA), and epinephrine (EP)], 3-(3,4-dihydroxyphenyl)-l-alanine (L-DOPA), and serotonin. The heart was rapidly removed and divided into the following samples that were weighed and frozen in liquid nitrogen: right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), and ventricular septum (VS). The heart chambers were homogenized in ice-cold 0.1 M perchloric acid. The homogenate was then centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant was further processed by solid-phase extraction using Oasis MCX cartridges (30 mg/1 ml; Waters, Milford, MA) as previously described (3). 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 are described elsewhere (24, 25). The separation and detection were performed using the following conditions: 76 cm long, 362 $\mu$m OD, 29 $\mu$m ID capillary, a 250 mM boric acid-1 M potassium hydroxide run buffer of pH 8.8, a separation voltage of 24 kV, a detection potential of +0.86 V versus Ag/AgCl, and an electrokinetic injection at 18 kV for 8 s.

Data and Statistical Analysis

The mean arterial pressure (MAP) and HR data were sampled every 4 min and stored for later analysis. The data were initially analyzed by plotting hourly averages for the entire protocol. Subsequently, 24-h averages were plotted and analyzed by a two-way analysis of variance for repeated measures followed by the Holm-Sidak method for all post hoc comparisons (SigmaStat, version 3.5). The comparison of cardiac content of the neurotransmitters and the neurotransmitter precursors between the Sham and SGX rats were determined for each heart chamber using an unpaired $t$-test with Welch’s correction as needed to account for the differences in variance between the groups.

RESULTS

Hourly Averages of MAP and HR. A comparison of SGX and Sham rats throughout the dark–light cycle over the entire experimental protocol was performed by plotting hourly averages for MAP and HR (Fig. 2). During the 5-day baseline period, the circadian rhythms in both variables were identical in both groups with MAP increasing $\sim$5–10 mmHg and HR increasing $\sim$100 beats/min during the dark cycle. Following the SGX and Sham surgeries, this rhythm was disrupted for 3 to 4 days but returned within 7 days. Note that the HR decreased overall in the SGX rats but that this effect was most notable during the dark cycle. The differences in HR between the two groups was virtually abolished during the 5 days of atenolol administration but returned during the 5-day recovery period. The MAP was not different between the SGX and Sham rats during most of the protocol, including atenolol treatment.

Twenty-Four Hour Averages of MAP, HR, Water Intake, and Body Weight

To simplify the statistical comparison between the SGX and Sham rats, 24 h averages for MAP and HR were plotted and are shown in Fig. 3. The MAP and HR averages were identical in both groups before the SGX or Sham surgeries. Following SGX, the MAP was significantly lower in the SGX compared...
with the Sham rats for 3 days, then there were no statistical differences between the groups for the duration of the protocol. The effect of the SGX and Sham surgeries within the groups was analyzed by comparing days 0–6 to day 1. The MAP was significantly reduced on days 1 and 2 in SGX rats but did not change in Sham rats. Using the same analysis, the HR decreased significantly by day 2 in SGX rats and fell by ~60 beats/min from control levels by the seventh day after surgery. Note, however, that HR also fell slightly in the Sham rats but was statistically higher than in the SGX rats throughout this period.

The effect of atenolol on MAP within both groups was analyzed by comparing days 7–19 to day 6. During the 5 days of atenolol administration, HR was significantly decreased by day 8, reaching a steady-state level of 311 ± 9 beats/min by the final day of the atenolol treatment. In contrast, the atenolol treatment had neglible effects on the HR in SGX rats. Moreover, there were no differences in HR between the groups during atenolol treatment. The MAP was similar in Sham and SGX rats before the initiation of atenolol and remained so during the entire 5 days of β-blockade. Upon the cessation of atenolol treatment, both HR and MAP rebounded toward control levels but HR remained lower in the SGX compared with the Sham rats and MAP was identical in both groups.

The differences in MAP and HR between groups did not appear to be the result of the differences in the rate of recovery from surgery as reflected by food intake, water intake, and body weight over the course of the study (Fig. 4). During most of the protocol, the water intakes averaged 30–40 ml/24 h and were not different between the Sham and SGX rats. The water intake decreased immediately following the Sham or SGX surgeries but returned to control levels within 2 days and remained stable for the duration of the study. Similarly, body weight was not different between groups with both showing a steady rate of growth over the entire study (Fig. 4).

**Cardiac Neurotransmitter Content**

Twenty days after the denervation, the mean cardiac NE content was significantly reduced in all the chambers of the SGX compared with Sham rats (Fig. 5A). When compared with that in the Sham hearts, the NE content of SGX hearts was reduced in the RA, LA, RV, VS, and LV by 92%, 95%, 87%, 83%, and 86%, respectively. The cardiac EP content was also significantly reduced in all chambers of SGX rats compared with Sham rats (Fig. 5B). The denervated RA, LA, RV, VS, and LV were depleted of EP by 80%, 86%, 67%, 48%, and 61%, respectively. The cardiac DA content was significantly reduced in all chambers of SGX rats, except for the LA, which was unchanged, compared with the Sham rats (Fig. 5C). The denervated RA, RV, VS, and LV were depleted of DA by 90%, 61%, 83%, 83%, and 81%, respectively. In contrast, the L-DOPA (Fig. 5D) and serotonin (Fig. 5E) contents were not significantly different between Sham and SGX rats.

![Fig. 3. Plot of 24-h averages for MAP and HR in SGX (●) and Sham (○) rats.](image)

![Fig. 4. Daily values for water intake and body weight in SGX (●) and Sham (○) rats.](image)
significantly different in any chambers of the SGX rats compared with the Sham rats.

DISCUSSION

The short-term increases in cardiac SNA enhance the rate and force of cardiac contraction and, as a result, cardiac output and arterial pressure. Under normal conditions, these rapid adjustments in cardiac SNA, often in a synergistic relationship with cardiac parasympathetic activity, act to regulate cardiac function and stabilize arterial pressure.

The contribution of long-term changes in cardiac SNA to the regulation of arterial pressure and cardiac function in health and disease is not as clear. This is partly due to the fact that it is extremely difficult to measure cardiac SNA in experimental animals and that there are currently no pharmacological methods to block the effects of cardiac SNA specifically in animals or humans. This is a clinically relevant issue since many studies suggest that cardiac SNA is increased in humans with essential hypertension (7) and heart failure (5, 23). Although the evidence suggests that an increased cardiac SNA may play a direct role in the development of cardiac hypertrophy observed in hypertensive and heart failure patients (23), it is difficult to separate this factor from other contributors, such as changes in cardiac preload, afterload, and circulating angiotensin II, all of which may contribute to cardiac remodeling (23).

The present study was conducted to develop and validate a surgical method for cardiac sympathetic denervation in the rat as a means to address this issue. Although it is possible to surgically remove cardiac sympathetic nerves in larger species such as the dog (29) and pig (30), we are unaware of any studies in which the effect of cardiac denervation on the development of hypertension has been studied in these species.
Although the rat is the most commonly used species to study hypertension, studies on the effect of cardiac denervation on rat models of hypertension are almost nonexistent, in large part because the method has not been developed and fully validated.

Bell and McLachlan (1) reported that bilateral SGX abolished hypertension in rats treated with mineralocorticoid-deoxycorticosterone acetate in combination with a 0.9% NaCl drinking solution (1). In that study, the ganglia were approached dorsally and excised retropleurally. Because no ribs were removed, it was not possible to excise the entire ganglion on both sides, and tests of the extent of the denervation were inconclusive. The authors reported that denervation was assessed histochemically by an examination of the atria only (ventricles were not examined); however, the details of this analysis, and the data, were not provided. Of the six rats studied, four were completely denervated, one was partially denervated, and one appeared to have intact nerves. The arterial pressure was measured indirectly by tail cuff at a single time point after SGX or Sham surgeries, and the HR was not measured. Since the measurements of cardiovascular function were not extensive and the methods and results for validating the extent of the denervation were not provided, it is difficult to assess the effectiveness of their denervation method in regard to the regulation arterial pressure and HR. In another study in the rat, the effectiveness of SGX via a retropleural approach was validated by the measurement of cardiac NE content, which was reduced by at least 94% in all regions of the heart (26). Unfortunately, these investigators did not measure cardiovascular function in these rats, so the effect of cardiac denervation on the regulation of arterial pressure and HR could not be determined.

The objective of the present study was to use a more aggressive surgical approach to perform SGX to ensure the successful denervation of all cardiac chambers. Rather than using a retropleural approach, we exposed the stellate ganglia via a midline thoracotomy. We have previously used a similar surgical approach for the chronic implantation of aortic flow probes for long-term measurements of cardiac output in conscious, unrestrained rats (8). This approach, combined with the use of a surgical microscope, rather than the standard dissecting scope, made it much easier to visualize the ganglia and therefore excise them, compared with the retropleural approach. The effect of SGX on cardiovascular function was determined using a continuous telemetric monitoring of arterial pressure and HR before and after SGX or Sham surgeries, and during atenolol administration, in conscious, unrestrained rats.

The effectiveness of the denervation was established at the end of the study by the measurement of tissue NE content.

Effect of SGX on Regulation of Arterial Pressure and HR under Basal Conditions

When compared to sham-operated rats, SGX resulted in a modest decrease in arterial pressure during the first 2 days following the ganglionectomy procedure, which then rebounded to control levels and remained identical to Sham rats for the duration of the protocol. This observation suggests that cardiac SNA plays little to no role in establishing the basal level of arterial pressure in conscious rats. It is possible that the transient decrease and subsequent recovery of arterial pressure following SGX were due to the compensatory responses of other long-term pressure controlling mechanisms, such as increased SNA to other vascular beds or activation of the renin-angiotensin-aldosterone system. Thus we cannot definitively conclude that cardiac SNA plays no role in maintaining arterial pressure under basal conditions, but it is clear that pressure is well regulated in SGX rats. This is in contrast to our recent studies using telemetric recordings of arterial pressure in rats subjected to the denervation of renal (17, 19) or splanchnic (21) vascular beds that exhibit statistically lower levels of arterial pressure compared with those in sham-operated controls. Taken together, these results suggest that, relative to the renal and splanchnic vascular beds, SNA to the heart plays a minor role in regulating the basal levels of arterial pressure in conscious rats. The role of cardiac SNA under conditions of stress or in models of hypertension or heart failure remains to be established.

As expected, in contrast to the effects on arterial pressure, SGX did chronically alter the regulation of HR. This was evident in the examination of the hourly plots of HR that revealed that the nighttime increase was significantly less in SGX rats compared with the Sham rats, suggesting that a portion of this increase is mediated by cardiac SNA. Although we did not measure or block cardiac parasympathetic activity, we assume that the remaining circadian rhythm in HR in the SGX rats is vagally mediated. The differences in HR between the groups were abolished during the atenolol treatment, suggesting that they were mediated by differences in the sympathetic control of the heart.

Effect of SGX on the Cardiovascular Responses to β-Blockade

Although the administration of β-blockers is commonly used to treat hypertension, the mechanism whereby this class of drugs lowers arterial pressure remains surprisingly unclear (27, 28, 33). We have reported that the chronic administration of atenolol decreases arterial pressure in normotensive rats (17, 18) and that this response is attenuated, but not abolished, by renal denervation (19). This suggests that some, but not all, of the hypotensive response to atenolol in normal rats is mediated by the blockade of neurally mediated renin release.

Since atenolol blocks sympathetically mediated actions on the heart, it is logical to assume that some of the hypotensive response to this drug is due to the blockade of cardiac β1-adrenoceptors. However, the basal level of arterial pressure, as well as the depressor response to atenolol administration, was identical between SGX and Sham rats. In fact, following the initial 2 to 3 days of recovery from SGX and Sham surgeries, the arterial pressure was identical in both groups before, during, and following a chronic atenolol administration. This surprising result strongly suggests that the cardiac sympathetic nerves play little to no role in regulating the baseline arterial pressure or the response to β1-receptor blockade. The ability of atenolol to lower the arterial pressure in cardiac (present study) and renal-denervated (19) rats is consistent with a central nervous system mechanism of action to decrease the overall sympathetic activity.

The extent to which the hypotensive response to β-blockers is secondary to actions within the brain resulting in a decreased SNA is unresolved. Early studies in rabbits reported that renal...
SNA was decreased by the administration of β-blockers (9, 10). The direct measurement of sympathetic nerve discharge to skeletal muscle in hypertensive humans before and during chronic treatment with metoprolol (35) or atenolol (2) suggests that the activity is decreased by β-blockade when SNA is expressed as bursts/minute. However, since muscle sympathetic activity displays a strong cardiac rhythmicity, it is common to normalize SNA to HR by expressing it as bursts/100 beats, and when quantified in this manner, SNA is not changed by β-blocker treatment (2, 35). Thus the interpretation is complicated, leading one group to conclude that the blockers decrease sympathetic nerve discharge (35) and another group to conclude that they do not (2).

A recent study in rats provided direct evidence of a role for brain β1-receptors in the regulation of SNA in heart failure. A chronic intracerebroventricular administration of metoprolol attenuated the progression of left ventricular remodeling in a rat model of myocardial infarction-induced heart failure (12). Brain microinjection studies suggested that metoprolol acts in the caudal nucleus tractus solitarius, a key site involved in the baroreflex regulation of cardiac SNA (12). Since metoprolol is highly lipophilic, it is possible that during a peripheral administration, it crosses the blood-brain barrier and is able to bind to β1-receptors in the central nervous system? This study supports the hypothesis that cardiac remodeling in heart failure is due to an increased cardiac SNA and that β-blockers can act within brain, as well as cardiac β1 receptors, to reverse this effect.

Effect of SGX on Cardiac Neurotransmitters and Catecholamine Precursor L-DOPA

The catecholamines NE, EP, and DA each have known cardiostimulatory effects, and therefore the depletion of these transmitters would be expected to reduce HR. NE has repeatedly been shown to be depleted following sympathetic denervation procedures (14, 16, 26). Our method of surgical SGX was successful at sympathetically denervating the heart as reflected by a substantial reduction in NE in all heart chambers. In addition, EP and DA were also markedly decreased in all heart chambers of SGX compared with Sham rats, indicating that their primary source in the heart is sympathetic nerve terminals. These findings are consistent with chemical sympathectomy (34) and correspond to a reduction in HR in SGX animals. Although a key intermediate in the synthesis of catecholamines in sympathetic nerves, L-DOPA is not reduced by SGX in any heart chamber. Similar to a report using chemical sympathectomy denervation (20), we show that the amount of L-DOPA is independent of sympathetic innervation even though it is a precursor to catecholamines in the heart that are in fact depleted by the SGX procedure.

The depletion of EP, albeit significant, occurred to a lesser extent than that of NE and DA. This is most likely because phenylethanolamine-N-methyl transferase, the intraneuronal EP synthesis enzyme present in stellate ganglion neurons (22), is also found in large-diameter cardiac intrinsic neurons (31), atrial and ventricular cardiomyocytes (22), and intrinsic cardiac adrenergic cells (16). Thus EP synthesis in the heart occurs both intra- and extraneuronally, and it is possible that there is an upregulation of extraneuronal EP production that partially compensates for the loss of EP in sympathetic nerves. Interestingly, in transplanted human hearts, which are also denervated, phenylethanolamine-N-methyl transferase expression is increased (11), suggesting that an upregulation of extraneuronal EP is a counterregulatory mechanism to NE depletion in the sympathetically denervated or transplanted heart. In contrast, we observed that cardiac EP content was substantially depleted in all cardiac chambers from SGX rats.

In contrast to the tyrosine-derived monoamine transmitters (i.e., catecholamines), serotonin was unchanged following SGX, indicating that it was synthesized independently of a sympathetic innervation to the heart. Serotonin is a potent cardiostimulatory neurotransmitter that is synthesized in small intensely fluorescent cells in the heart by tryptophan hydroxylase (31). These small intensely fluorescent cells are believed to represent a class of serotonergic neurons in the intrinsic nervous system of the heart (15).

Perspectives

Increased SNA to the heart may contribute to cardiac pathologies in a number of cardiovascular disease states, including heart failure and hypertension. The development of a method to selectively denervate the sympathetic nerves to the rat heart provides an effective way to study the impact of these nerves on the regulation of cardiac function and arterial pressure under normal and pathophysiological states. In the present study, the sympathetic denervation of the rat heart had no long-term effect on either the basal level of arterial pressure or the depressor response to atenolol administration. This suggests that, relative to other vascular beds such as the kidney and gastrointestinal tract, the changes in sympathetic activity to the heart play a minor role in the long-term stabilization of arterial pressure in conscious, unstressed rats. In addition, the ability of β-blockers to decrease arterial pressure in cardiodegenerated rats suggests that β1-receptors in the heart are not the target of this class of antihypertensive drugs in regard to the blood pressure effects in normotensive rats. The role of cardiac β1-receptors mediating the depressor actions of β-blockers in animal models of hypertension remain to be determined.

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