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Endothelin B Receptors Impair Baroreflex Function and Increase Blood Pressure Variability During High Salt Diet

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Abstract

Baroreflex function is an integral component maintaining consistent blood pressure. Hypertension is often associated with baroreflex dysfunction, and environmental risk factors such as high salt diet exacerbate hypertension in subjects with baroreflex dysfunction. However, the interactions between high salt diet, baroreflex dysfunction, and hypertension are incompletely understood. The endothelin system is another potent mediator of blood pressure control especially in response to a high salt diet. We hypothesized that the endothelin B (ET_B) receptor activation on adrenergic nerves decreases baroreflex sensitivity. We utilized male ET_B receptor deficient (ET_B-def) rats that express functional ET_B receptors only on adrenergic nerves and transgenic (TG) controls to evaluate baroreflex function during normal (0.49% NaCl) and high (4.0% NaCl) salt diets. In conscious rats equipped with telemetry, ET_B-def rats had an increased lability of systolic blood pressure (SBP) compared to TG controls as indicated by higher standard deviation (SD) of SBP under both normal (10.2±0.6 vs. 12.4±0.9 mmHg, respectively, p=0.0001) and high (11.7±0.6 vs. 16.1±1.0 mmHg, p=0.0001) salt diets. In anesthetized preparations, ET_B-def rats displayed reduced heart rate (p genotype=0.0167) and renal sympathetic nerve (p genotype=0.0022) baroreflex sensitivity. We then gave male Sprague-Dawley rats the selective ET_B receptor antagonist, A-192621 (10 mg/kg/day), to block ET_B receptors. Following ET_B receptor antagonism, even though SBP increased (131±7 before vs. 152±8 mmHg after, p<0.0001), the lability (standard deviation) of SBP decreased (9.3±2.0 vs. 7.1±1.1 mmHg, p=0.0155). These data support our hypothesis that ET_B receptors on adrenergic nerves contribute to baroreflex dysfunction.

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Keywords

baroreflex; endothelin; sympathetic nerves; salt; hypertension

1. Introduction:

Healthy baroreflex function is an integral part of maintaining stable blood pressure through reflexive adjustments to heart rate and sympathetic nervous system activity. Aberrations to baroreflex function occur in a number of cardiovascular diseases such as hypertension (Han et al., 2015; Ilescu et al., 2013; Sheng et al., 2020) and heart failure (Becker et al., 2016; Zucker et al., 2012). Although numerous factors can influence baroreflex function, there remains a large gap in our current understanding of how environmental factors such as dietary salt influence the baroreflex. High salt diet has been shown to increase baroreflex sensitivity in healthy adults (Babcock et al., 2018) and animal models (Huang et al., 1994; Simmonds et al., 2014). Conversely, in diseases such as salt-sensitive hypertension, baroreflex dysfunction is thought to play a causative role in the progression of the hypertensive response to high salt diet (Bugenhagen et al., 2010; Coruzzi et al., 2005). Activation of the baroreflex may even be a therapeutic option in treatment resistant hypertensive patients by increasing sodium excretion (Lipphardt et al., 2019), which has also been demonstrated in animal models (Hildebrandt et al., 2014; Hildebrandt et al., 2016).

A previous study in rats demonstrated a robust salt-sensitive increase in blood pressure when the baroreflex was disrupted by sinoaortic denervation (Osborn et al., 1998). An interesting observation in these studies was that denervated animals on a high salt diet displayed an increased amplitude of the diurnal blood pressure rhythm (i.e. greater night/day difference) after being placed on high salt diet. A similar finding was also observed in Sprague-Dawley rats given a specific ET_B receptor blocker and high salt diet (Pollock et al., 2001), and we have also reported robust high salt diet-induced increases in diurnal blood pressure amplitude in endothelin B (ET_B) receptor deficient (ET_B-def) rats (Becker et al., 2017a; Speed et al., 2018). These rats lack functional ET_B receptors except where a transgene with functional endothelin B receptors are expressed under dopamine beta-hydroxylase promoter control (Garipey et al., 1998), such as efferent sympathetic neurons and adrenals.

The endothelin system is an integral modulator of blood pressure through its actions on the vasculature, renal excretion of sodium, and autonomic nervous system function (Davenport et al., 2016; Kohan et al., 2011). Although the endothelin system is a likely mediator of baroreflex function through its modulation of these organ systems, very little is known about the contribution of the endothelin system to baroreflex control in normal physiology or disease. Both the endothelin A and B receptors appear to influence sympathetic norepinephrine release to the vasculature (Kita et al., 1998) and heart (Lehmann et al., 2014), especially following cardiac ischemia (Backs et al., 2005; Yamamoto et al., 2005), but the specific role of either receptor in baroreflex function is not well understood.

Given the similar phenotype of the ET_B-def rat to sinoartically denervated rats in relation to salt-sensitive increases in blood pressure and diurnal blood pressure amplitude and the role of endothelin signaling in sympathetic activity, we hypothesized that the ET_B receptor

activation on sympathetic nerves decreases baroreflex sensitivity. Our approach will focus on the ET_B-def rat where we and others have extensively characterized the salt-sensitivity of blood pressure (Becker et al., 2017a; Garipey et al., 2000; Matsumura et al., 2000; Ohkita et al., 2005; Speed et al., 2018) and the potential role of ET_B receptors on sympathetic nerve activity (Becker et al., 2017b; Dai et al., 2004; Lau et al., 2006). Herein, we attempted to further probe the contribution of endothelin B receptor activation specifically on baroreflex function utilizing both a conscious, telemetry-based approach measuring spontaneous baroreflex activation and an anesthetized, baroreflex curve generation approach in normal salt and high salt fed rats.

2. Materials and Methods:

2.1 Animals and Surgical Preparation:

ET_B-def rats and transgenic (TG) controls were bred from an in-house colony. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th ed., National Academy of Sciences, 2011) by the NIH. Animals were housed in 12:12 hr light cycle with lights on at 7 am and lights off at 7 pm. We and others have previously described the ET_B-def and associated TG control rats in depth (Becker et al., 2017a; Becker et al., 2017b; Garipey et al., 2000; Garipey et al., 1998). In brief, the ET_B-def rat is derived from the spotting lethal rat, which has a spontaneous mutation in the *EDNRB* gene rendering ET_B receptors nonfunctional. This results in the failure of enteric nerve development and causes a lethal megacolon in homozygous rats. This lethal megacolon was prevented by the insertion of a functional *EDNRB* gene under human dopamine- β -hydroxylase (D β H) promoter control (Garipey et al., 1998). Thus, ET_B-def rats have functional ET_B receptor expression in D β H positive tissues, but lack functional receptors elsewhere. Our breeding scheme consists of mating rats homozygous for transgene (D β H-EDNRB) expression and heterozygous for the spotting lethal (sl) mutation. ET_B-def offspring are homozygous for both the transgene and spotting lethal mutation (D β H-EDNRB^{+/+};sl^{+/+}). The littermate TG controls, possess the transgene but are spotting lethal negative (D β H-EDNRB^{+/+};sl^{-/-}) and therefore retain functional endogenous *EDNRB* expression. Offspring heterozygous for the spotting lethal mutation (D β H-EDNRB^{+/+};sl^{+/-}) are euthanized or used as breeders. Tail snips are sent to Transnetyx (Transnetyx Inc, Cordova, TN) for genotyping and confirmation of presence of transgene.

Male ET_B-def and TG rats of starting weights from 250–350g were instrumented with telemetry transmitters (HD-S10, Data Sciences International, Minneapolis, MN) implanted in the abdominal aorta as previously described (Becker et al., 2017a). Animals were allowed to recover for one week following surgery before baseline blood pressure recordings were completed. Following approximately one week of baseline recordings during which time animals were fed a 0.49% NaCl, normal salt diet (TD.96208; Envigo, Indianapolis, IN), rats were switched to a high salt (4.0% NaCl) diet (TD.92034) for two weeks. Figures 1–3 and Tables 1–2 are a further analysis of previously published telemetry data (Becker et al., 2017a). All other data and analyses are derived from newly instrumented animals specific to this study.

2.2 Telemetry Analysis:

Telemetry recordings were taken in 10-minute sections every 20 minutes for 24 hrs per day throughout the experiment using Ponemah v6.0 (Data Sciences International). Data were analyzed offline in 10 second bins using Ponemah v6.5, and blood pressure and heart rate were rounded to the nearest whole integer in Microsoft Excel. Using pivot tables in Microsoft Excel, the number of instances for each given mmHg of blood pressure or bpm of heart rate were determined for 3 consecutive days of baseline, days 8–11 following high salt diet, and days 3–5 of A-192621 treatment. Because of differences between animals in the number of total bins due to telemetry dropouts or noise, the count of each mmHg of blood pressure or bpm of heart rate was divided by a count of the total number of bins to calculate the proportion of each mmHg or bpm to total number of bins. The standard deviation (SD) of blood pressure and HR was calculated from pivot tables in Microsoft Excel. To evaluate the impact of diurnal factors in SD and range of blood pressure and HR, data were separated into active (zeitgeber time 12–24; 7 pm to 7am) and inactive (zeitgeber time 0–12; 7 am to 7pm). Spontaneous baroreflex measurements were analyzed as previously described utilizing Hemolab software using the sequence method (Harald Stauss Scientific, Iowa City, IA) (Becker et al., 2017a; Bertinieri et al., 1985; Stauss et al., 2006). Spontaneous baroreflex measurements were completed during the same time frame as lability parameters, 3 consecutive days of baseline, days 8–11 following high salt diet, and days 3–5 of A-192621 treatment.

2.3 Acute Baroreflex Measurement:

A separate group of 26 male TG and ET_B-def rats were fed normal salt (0.49% NaCl) or high salt (4.0% NaCl) diet for 7–11 days and subsequently prepared for acute, anesthetized baroreflex experiments. Rats were anesthetized with an IP injection of 100 mg/kg body weight Inactin[®] (thiobutabarbital sodium salt hydrate; Sigma-Aldrich, St. Louis, MO), a tracheostomy was performed to allow for free breathing, and catheters were placed in the right femoral artery and vein for measurement of blood pressure and administration of fluids respectively. Sterile saline was administered at a rate of 2 mL per hr via the femoral vein to maintain electrolyte stability throughout the course of the surgical preparation and experiment. Electrodes were placed in the skin of the right arm and left leg, and the differential signal amplified by 5k and low-pass filtered at 30 Hz with a Brownlee Precision Model 200 amplifier (NeuroPhase, SantaClara, CA) for generation of a single lead electrocardiogram. The left kidney was exposed and deflected via a flank incision, and a left renal sympathetic nerve bundle carefully dissected from surrounding tissues by fine forceps. The nerve was placed on a pair of stainless-steel recording electrodes and covered in warmed mineral oil for measurement of bipotential extracellular electrophysiological activity, and a reference electrode was placed nearby in surrounding tissue. The electrodes were connected to an HZP high impedance probe (Natus Neurology, Oakville, Ontario), and the signal amplified with a Grass P511 AC preamplifier (Natus Neurology), amplified at 100k, low pass filtered at 100 Hz, and high pass filtered at 1 kHz. Renal sympathetic nerve activity (RSNA) was visualized on an LG OS-3020D oscilloscope, and the auditory signal output for monitoring. All signals were recorded using Powerlab 4/30 and Labchart v8 (AD Instruments, Colorado Springs, CO) at 1k samples/sec. Blood pressure was lowered by a bolus IV infusion of 25 µg sodium nitroprusside (Sigma-Aldrich, St. Louis, MO) and raised

by an IV infusion of 10 μ g phenylephrine (Sigma-Aldrich, St. Louis, MO) as previously described (Becker et al. 2016). Interbeat interval (IBI) was calculated as time between each R wave from the electrocardiogram in ms. Nerve activity was rectified and integrated (timed constant of 10 ms). To minimize animal to animal differences in renal sympathetic nerve activity, resting/baseline discharge was set as 100% and background (following proximal and distal sectioning of nerve) was set as 0%. Three rats (1 TG on normal salt diet, 1 ET_B-def on normal salt diet, and 1 ET_B-def on high salt diet) did not have high quality RSNA recordings (such as signal to noise ratio < 2 or lack of characteristic cardiac synchronous bursting) and are only included in the HR data set. Voltages were calibrated using the Grass P511 AC preamplifier controls for each individual experiment.

2.4 Endothelin B Antagonism:

In a separate set of experiments, male Sprague-Dawley or TG and ET_B-def rats (250–300g) were instrumented with telemetry transmitters as described above and allowed to recover for one week. Baseline telemetry collections were acquired and drinking water was switched to that containing the selective ET_B antagonist, A-192621 (PepTech Corp., Bedford, MA) (von Geldern et al., 1999, Wessale JL et al., 2002), measured and adjusted daily to administer a dose of 10 mg/kg/day in four rats. In a different group of four rats, Alzet minipumps (Model 2ML1, DURECT Corporation, Cupertino, CA) were implanted IP to deliver 10 mg/kg/day of A-192621. Blood pressure was recorded for one week following drinking water or minipump administration of A-192621. Telemetry data were analyzed as in the ET_B-def experiment. The sampling period of the rats implanted with minipumps was shorter than needed for adequate spontaneous baroreflex analysis, thus only the 4 rats receiving drug in drinking water are included in these analyses.

2.5 Statistical Analysis

Data are presented as means \pm standard error of the mean (SEM) or as mean and 95% confidence interval. Comparisons are made using one-way analysis of variance (ANOVA) testing, two-way repeated measures ANOVA, three-way repeated measures ANOVA, unpaired Student's t-test, or paired Student's t-test as appropriate. The particular statistical comparison used is designated within the text or figure legends. P values < 0.05 were considered statistically significant.

3. Results:

3.1 Blood Pressure and Heart Rate Lability

ET_B-def rats demonstrated an increased blood pressure lability compared to TG controls as demonstrated by a flattening and broadening of the 24-hr blood pressure frequency distribution plots during both normal (Figure 1 A) and high (Figure 1 B) salt. This was quantified by an elevated range (Figure 1 C) and SD (Figure 1 D) of SBP in ET_B-def rats compared to TG controls. Both genotypes had an increase in lability both in range and SD of SBP following high salt diet (Figure 1 C–D). However, the change in range of SBP following high salt diet was not significantly different between genotypes (Figure 1E), although the change in SD of SBP was significantly greater in ET_B-def rats compared to TG controls (Figure 1F). Average, 24-hr SBP was higher in ET_B-def compared to TG controls

during both normal salt (ET_B-def 162 ± 2, TG 141 ± 2 mmHg P < 0.0001) and high salt (ET_B-def 177 ± 2, TG 142 ± 1 mmHg P < 0.0001). SBP statistically increased following high salt diet only in ET_B-def (ET_B-def P < 0.0001; TG P = 0.7899), P_{Genotype} < 0.0001, P_{Salt} < 0.0001, P_{Genotype*Salt} = 0.0002, 2-way RM ANOVA.

The range of HR was not statistically different across genotypes and salt diet as shown in the 24-hour HR frequency distribution plots (Figure 2 A–B) and quantified HR range (Figure 2 C). ET_B-def rats had a greater SD of HR during normal salt diet compared to TG controls, and this difference became non-significant during high salt diet (Figure 2 D). Only ET_B-def rats displayed a significant decrease in SD of HR following high salt diet (Figure 2 D), and there were no significant differences between genotypes in the change in HR range or change in HR SD following high salt diet (Figure 2 E–F). Average, 24-hr HRs between genotypes were not significantly different and high salt diet significantly lowered HR only in ET_B-def rats (Normal Salt: ET_B-def 350 ± 4, TG 341 ± 3; High Salt: ET_B-def 333 ± 5, TG 335 ± 4, P = 0.0019 vs Normal Salt) bpm; P_{Genotype} = 0.5511, P_{Salt} = 0.0013, P_{Genotype*Salt} = 0.0962 2-way RM ANOVA.

The SD of SBP demonstrated a strong diurnal rhythm, being overall higher during rats' active period; however, the range of SBP was not significantly affected by time of day (Table 1). Similarly, the range of HR was not affected by time of day and SD of HR demonstrated a significant influence from time of day, with higher SD during the active period (Table 1). Cosinor analysis further revealed that neither salt diet nor genotype had a significant effect on acrophase of any parameter, but that high salt diet caused a significant increase in SD of SBP amplitude only in ET_B-def rats (Table 2).

3.2 Baroreflex Responses

We had previously reported no significant differences in spontaneous baroreflex sensitivity (i.e. the gain of the spontaneous baroreflex sequence) between ET_B-def and TG controls (Becker et al., 2017a). One limitation of the sequence method of spontaneous baroreflex sensitivity; however, is the inclusion of only true baroreflex sequences in the analysis (i.e. only those with sequential increases or decreases in both SBP (ramps) and interbeat interval between heart beats are included in analysis of gain, Figure 3 A, top panel). We further probed the spontaneous baroreflex sequences of ET_B-def and TG control rats on normal and high salt diets and evaluated the relative proportion of true baroreflex sequences to non-baroreflex sequences defined as sequential increases or decreases in SBP (ramps) without reciprocal changes in heart rate (Figure 3 A, bottom panel). We found that under high salt diet, the proportion of true baroreflex sequences to non-baroreflex sequences was significantly lower in ET_B-def rats compared to TG controls (Figure 3 B). Also, TG rats had an increase in baroreflex to non-baroreflex sequences following high salt diet, an effect that was not significant in ET_B-def rats.

We then further evaluated baroreflex function utilizing the modified Oxford method of baroreflex testing in anesthetized rats fed a normal salt diet or a high salt diet for 7–11 days by IV infusions of sodium nitroprusside and phenylephrine and plotting RSNA or IBI across mean arterial blood pressure (MAP). Representative traces are shown in Figure 4 and composite baroreflex curves and gain (first derivative of the 4-parameter baroreflex curve) in

Figure 5 A–C. Overall, we did not observe any significant effects of genotype or salt diet on parameters of the RSNA curves as it relates to the bottom plateau, top plateau, or MAP BP50 (the MAP at halfway through curve). On a normal salt diet, there was a significant blunting of the max gain of RSNA response to changes in MAP in ET_B-def rats compared to TG controls and this was not further affected by high salt diet (Figure 5 A,C; Table 3). Similarly, the bottom plateau and MAP50 of the HR response to changes in pressure were not significantly affected by genotype or salt diet; however, the top plateau and max gain were significantly lower in ET_B-def rats compared to TG controls indicating a reduced ability to lower heart rate in response to increased MAP (Figure 5 B,D; Table 3).

Because ET_B-def rats did not display a robust, typical sigmoidal HR curve during the baroreflex function testing (Figure 5B), we also calculated the slope of baroreflex sensitivity during the pressor response from IV infusion of phenylephrine and fit this portion with a linear regression model to isolate the sensitivity of HR and RSNA responses to increased MAP. Although not a complete picture of baroreflex function, this linear regression analysis removes the assumptions present in fitting the responses to a 4-parameter sigmoid curve. Similar to the sigmoid curve analysis, ET_B-def rats had a lower RSNA gain compared to TG controls, and there was an overall trend of salt toward blunting the RSNA gain (Figure 5E). Heart rate responses to increases in MAP were significantly lower in ET_B-def compared to TG controls and salt diet had a significant effect of lowering HR gain. Furthermore, ET_B-def animals on normal salt diet had a significantly lower HR gain compared to normal salt TG controls, and high salt diet significantly lowered HR gain in TG animals compared to normal salt diet but did not significantly lower the already blunted gain of ET_B-def (Figure 5F).

3.3 ET_B Receptor Antagonism

Because ET_B-def and TG control animals express ET_B receptors via a transgene in DBH-positive tissues, the altered baroreflex activity could be due to the presence of ET_B receptors in efferent sympathetic nerves. Therefore, we administered a selective ET_B receptor antagonist, A-192621, in order to globally block ET_B receptor activity. Following 4–6 days of ET_B receptor antagonism, SBP increased in all animals (Figure 6 B), and this was accompanied by a decrease in variability measured by SD of SBP (Figure 6 C) along with an increase in the range of SBP (Figure 6 D). The route of administration of A-192621 (PO vs IP) did not have a significant effect on either SBP or SD of SBP as measured by two-way RM ANOVA (SBP: P Route*A-192621 = 0.1823; SD of SBP: P Route*A-192621 = 0.8430) thus these data were combined. The changes in blood pressure were accompanied by a decrease in heart rate; however, measures of heart rate variability were not significantly altered (Figure 7). There was no significant effect of ET_B antagonism on the overall gain of the spontaneous baroreflex sensitivity (Figure 8 A), but there was a significant increase in the ratio of true baroreflex sequences to non-baroreflex sequences (Figure 8 B).

Antagonism of ET_B receptors in ET_B-def and TG controls resulted in a rise in blood pressure only in TG controls while ET_B-def remained unaffected (Table 4). The range of SBP increased in both TG and ET_B-def rats following A-192621 (Figure 9A–C); however, the lability of SBP as measured by SD of SBP was not significantly affected by A-192621 (Figure 9D). Spontaneous baroreflex sensitivity was not significantly different genotypes or

following A-192621 (Figure 9E), nor where the ratio of true sequences:non-sequences although there was a trend towards reduction of true:non-sequences in TG controls following A-192621 (Figure 9F).

4. Discussion:

The major findings of the current study are: 1) ET_B -def rats display increased blood pressure lability and impaired heart rate baroreflex control, which are augmented during high salt diet, 2) pharmacological blockade of the ET_B receptor decreased lability and increased the ratio of true:non-baroreflex sequences even in the face of elevated blood pressure in Sprague-Dawley rats, 3) However, pharmacological blockade of ETB receptors had no significant effect on blood pressure lability or baroreflex control in ETB -def or TG rats. These findings suggest that ET_B receptors influence baroreflex control, particularly during high salt diet..

The role of the endothelin system and specifically the role of ET_B receptors in baroreflex control has not been well defined. Furthermore, there remains a large amount of uncertainty regarding the interplay between endothelin-1 and the ET_A and ET_B receptors in modulating various autonomic functions. Some of the challenge in elucidating the role of endothelin receptors in autonomic function likely lies in the potency of vascular responses to activation of either receptor, as this can obscure or complicate attribution of hemodynamic responses to autonomic activity (Davenport et al., 2016). Results from the current study suggest that activation of ET_B receptors increases efferent sympathetic nerve activity. Prior work has demonstrated an increase in superoxide production in sympathetic ganglia following activation of ET_B receptors (Dai et al., 2004; Lau et al., 2006), and activation of ET_B receptors augments the vasoconstriction of vessels to application of norepinephrine (Kita et al., 1998) and/or angiotensin II (Gossl et al., 2004). We have also previously shown direct effects of activating neuronal ET_B receptors to increase blood pressure using the ET_B -def rats (Becker et al., 2017b). In ET_B -def rats with ganglionic blockade, activation of ET_B receptors with the highly selective agonist, sarafotoxin 6c, caused an alpha1-adrenergic dependent increase blood pressure. Interestingly, higher doses of sarafotoxin 6c resulted in a tachycardia that was blocked by beta-adrenergic receptor blockade. These findings indicated that activation of ET_B receptors on sympathetic neurons caused adrenergic-dependent increases in blood pressure and heart rate. In this current study we demonstrated that the ET_B -def model, which only has functional endothelin B receptors on efferent sympathetic nerves, displays baroreflex dysfunction following high salt diet.

We observed an increased blood pressure lability in our ET_B -def rats indicated by elevated 24-hour SD and range of SBP that was further augmented by placing the rats on a high salt diet. Our observed difference in SD of SBP between ET_B -def and TG control rats during normal salt diet was many orders of magnitude smaller than that seen following complete sinoarotic denervation in studies such as those done by Osborn and Hornfeldt (approximately 20% vs. 200%, respectively) (1998). This highlights that ET_B -def rats still have intact and partially functioning baroreflexes, which is further evidenced by the similar spontaneous baroreflex sensitivity between ET_B -def and TG controls (Becker et al., 2017a). These differences become exaggerated following high salt diet and approach approximately

60% difference between ET_B-def and TG controls suggesting a strong interaction between salt diet and ET_B receptors mediating baroreflex dysfunction. High salt diet has been shown to increase renal levels of endothelin-1 (Becker et al., 2017a; Pollock et al., 2001; Speed et al., 2018) and ET_B-def rats have elevated circulated levels of endothelin-1 compared to TG controls under both normal and high salt diets. In the DOCA-salt model, endothelin-1 levels are similar to controls in celiac ganglia; however, there is a significant increase in ET_B receptor expression (Dai et al., 2004). It is unknown what effect salt diet has on other sympathetic ganglia in terms of endothelin-1 or receptor expression, but it is likely a similar increase in expression may occur in cardiac ganglia. Future work is needed elucidating this potential interaction between ET_B receptor expression in various autonomic ganglia and salt diet.

We also observed an increase in the lability of SBP following high salt diet in the TG controls. This increase in lability in a salt-resistant animal has also recently been shown by Simmons et al (Simmonds et al., 2014) with an isolated increase in blood pressure lability during the animals' active period following high salt diet. Consistent with these findings, we observed an interaction between high salt diet and time of day in both TG and ET_B-def rats. These findings along with the increased blood pressure amplitude in ET_B-def rats (Becker et al., 2017a; Speed et al., 2018) and sinoaortic denervated rats (Osborn et al., 1998) pose a potential interaction between high salt diet and diurnal baroreflex and blood pressure control. The available rodent model data seem to indicate that salt-sensitive blood pressure is associated with increased diurnal blood pressure rhythms, which is in contrast with human data demonstrating a reduction in blood pressure rhythms or "dipping" in salt-sensitive individuals (Higashi et al., 1997; Kimura et al., 2010). The reasons for this difference between humans and rodent models are unknown but worthy of further investigation because dipping status in humans is a strong prognostic indicator of cardiovascular morbidity and mortality (Douma et al., 2018). Furthermore, high salt diet appeared to cause a higher diurnal difference in frequency distribution across blood pressure in both genotypes (Figure 1B). This divergence created the presence of a hump in the 24-hour distribution curve, although there were no statistically significant differences between genotypes. Future work will be instrumental in elucidating these interactions between the endothelin system and diurnal control of blood pressure.

Although to a lesser magnitude than ET_B-def counterparts, the TG rats also had a remarkable inhibition of the heart rate response to modified Oxford baroreflex testing in anesthetized preparations following high salt diet. This could potentially be a feature of the transgene expression in both the ET_B-def and TG animals. As the ET_B receptor expression is no longer under its native control but is driven by the dopamine beta-hydroxylase promoter, it is feasible there is increased expression of ET_B receptors in sympathetic ganglia and central sites important in baroreflex control compared to non-transgenic animals. There is some preliminary evidence that this occurs in the ET_B-def and TG line, specifically in the stellate ganglia (Wehrwein et al., 2007), which would explain the differential responses to high salt diet between cardiac and renal sympathetic beds to baroreflex testing (Figure 5). This further emphasizes that the role of ET_B receptors on efferent sympathetic nerves themselves is responsible for the observed baroreflex dysfunction and not primarily a sensory defect in baroreceptor afferents. However, the current study is limited in that we are

unable to conclusively determine whether the defect in baroreflex function is due to baroreceptor afferents, cardiac efferents, or central neuronal pathways. It is also possible there are localized vascular defects in the ET_B-def rats in the baroreceptors that blunt effective signal transduction. One of the major roles of ET_B receptors in the vascular endothelium is the production of nitric oxide and other vasodilators. As nitric oxide signaling is an important component of baroreceptor function, it is possible that ET_B-def animals lack this signaling pathway. In order to test for the possible contribution of ET_B receptors on vascular tone or baroreflex afferents vs. efferents, we subjected Sprague-Dawley rats to global ET_B receptor blockade. As has been previously demonstrated, global ET_B receptor blockade raised blood pressure; however, even in the face of increased blood pressure we observed a reduction in lability as evidenced by a lowering of the SD of SBP (Figure 6). ET_B receptor blockade also failed to significantly alter spontaneous baroreflex sensitivity but did increase the ratio of true to non-baroreflex sequences (Figure 8). Because these effects are in direct contrast to those observed in the ET_B-def rat, this would indicate that the primary baroreflex defect in ET_B-def rats is the presence of ET_B receptors on sympathetic efferents and not the lack of ET_B receptors on afferent nerves. However, blocking ET_B receptors in ET_B-def and TG rats had little observable effect on either blood pressure lability or ratio of true to non-baroreflex sequences (Figure 9), although there was a trend toward a reduction of the true to non-baroreflex sequences in TG controls. This would indicate that an absence of ET_B receptors on sensory pathways is responsible for the genotype difference as antagonism had virtually no effect on ET_B-def rats. These observed effects were limited to normal salt diet and may be further augmented during high salt diet. Future work will be instrumental in dissecting how high salt diet affects receptor expression and function and how interactions between ET_B receptors on sensory vs efferent pathways contribute to baroreflex dysfunction.

Why is the gain of the spontaneous baroreflex not altered even though the modified Oxford technique demonstrated a large effect of ET_B receptors on baroreflex function? There is evidence that blocking muscarinic receptors with atropine has a strong effect on reducing spontaneous baroreflex gain, but blocking adrenergic receptors with propranolol does not have a significant effect (Stauss et al., 2006). This would imply that the sequence technique is more apt for observing alterations in parasympathetic/vagal modulation of the baroreflex and may be relatively insensitive to sympathetic influences. As our data indicate altered sympathetic tone to the heart due to ET_B receptor activity, we may not be able to observe this in the gain of the spontaneous baroreflex analysis due to these limitations of the system. We therefore extended our evaluation of the spontaneous baroreflex sensitivity by evaluating non-baroreflex sequences, i.e. instances where blood pressure and HR both increase or decrease together rather than reciprocally. There has been some debate regarding the interpretation of non-baroreflex events with the general theory being that they represent a type of feedforward mechanism driven primarily by sympathetic activity to both the vasculature and SA node vs. the feedback mechanism of a classical baroreflex. Investigations that seek to specify the balance between feedforward and feedback mechanisms in an intact animal (closed loop system) are notoriously difficult to fully interpret (Kamiya et al., 2011; Kawada et al., 2016). One potential hypothesis is that under rest and normal conditions, feedforward mechanisms driven by sympathetic activity prevail

and the feedback mechanisms are reactionary, responding to alterations in hemodynamic status (Kamiya et al., 2011; Tan et al., 2011). These feedforward sequences may then represent central outflow of sympathetic tone, which is supported by observations of fewer non-baroreflex sequences following sympathetic or ganglionic blockade but no changes in non-baroreflex sequence number following muscarinic blockade (Legramante et al., 1999; Legramante et al., 2009). This interpretive framework fits well in our observations of increased non-baroreflex sequences in ET_B-def rats during high salt diet and decreased non-baroreflex sequences following ET_B receptor blockade further demonstrating that ET_B receptors on sympathetic nerves contributes to increased sympathetic drive.

Our study has a number of limitations that are worthy of discussion. As already briefly mentioned, the nature of the genetic model used (ET_B-def and TG) rat is both a strength and weakness of the study. The expression of ET_B receptors via a transgene utilizing D β H promoter may result in expression of ET_B receptors where they normally would not be expressed under native conditions. Although this selective expression on adrenergic tissue allows us isolate the effects of ET_B receptor activity, it may not be representative of normal physiology. Furthermore, our results indicate a connection between ET_B receptors and baroreflex dysfunction but do not specifically identify the location of ET_B receptors responsible such as sensory or efferent autonomic pathways. Future studies are needed to clarify the specific interactions involved.

Another limitation is issues regarding diurnal variability in our experiments. Telemetry analysis allows us to monitor animals throughout the 24 hrs of the day, but our acute baroreflex measurements were conducted during the animals inactive period. As some of our observed effects, such as the robust increase in SBP phenomenon following high salt diet, occur primarily during the animals active period, this may result in an underestimation of parameters collected during this time. Additionally, the effect of anesthesia on our baroreflex studies cannot be excluded either. Future work utilizing techniques such as reverse lighting in animal housing facilities or indwelling catheters to conduct multiple baroreflex experiments on conscious animals during different periods of the day will be useful in establishing a larger framework of circadian control of baroreflex function.

In conclusion, we have shown that the ET_B receptors are important mediators of baroreflex dysfunction that is augmented by high salt diet and which likely contributes, in part, to the salt-sensitive hypertension of the model. Because baroreflex dysfunction is commonly associated with salt-sensitive hypertension in humans and animal models, further work investigating the ability to modulate baroreflex through the endothelin system in various other models of salt-sensitive hypertension will yield greater understanding of the interplay of this important physiological feedback system.

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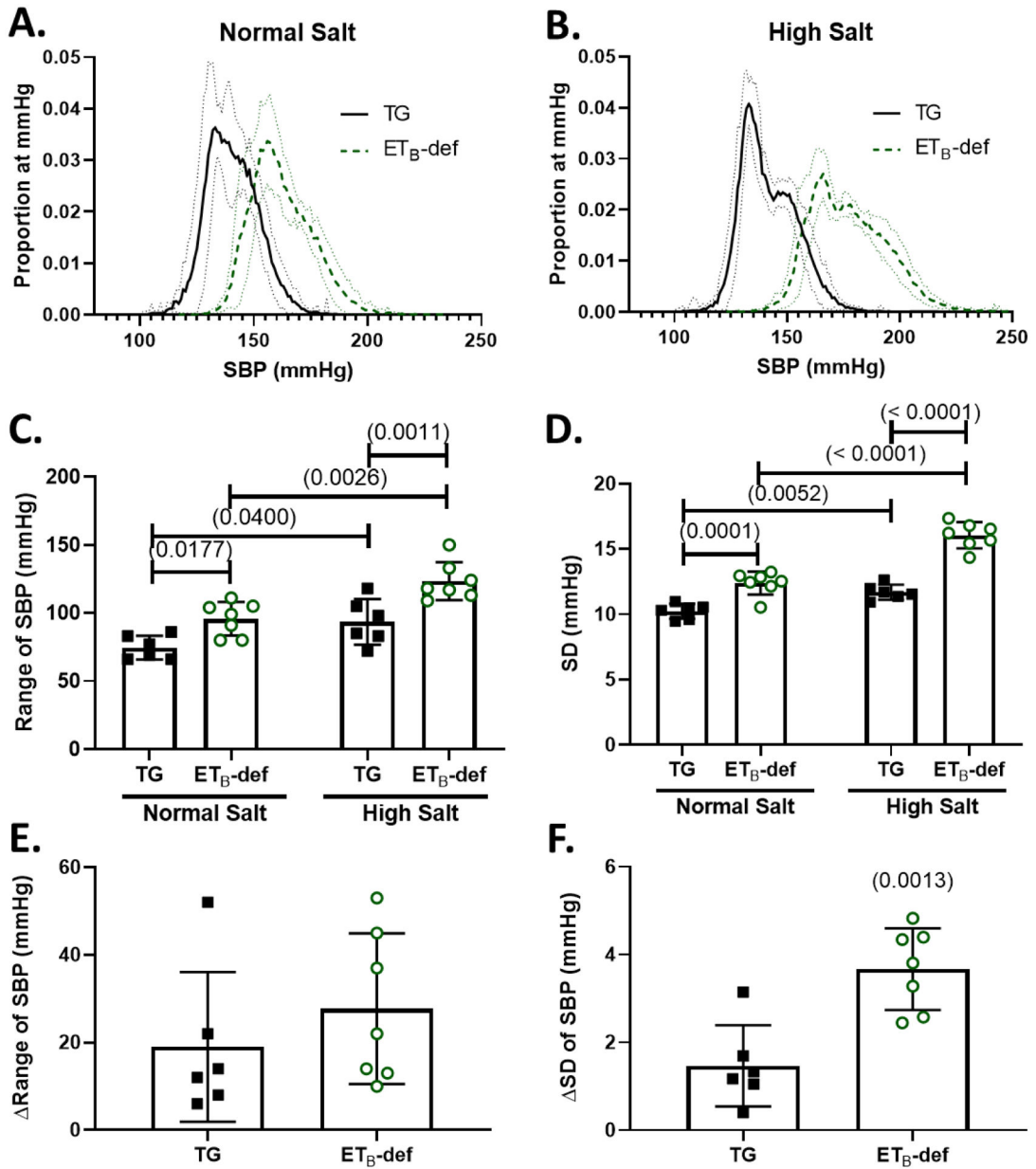


Figure 1: ET_B-def rats have elevated blood pressure lability during normal salt and high salt diet.

A/B: frequency distribution plots of proportion of instances at a given SBP (number of instances of 10-second bins at each mmHg / total number of bins) during normal salt diet (A) and 8–11 days of high salt diet (B) Solid line represents mean ± 95% confidence interval. C: Range of SBP during normal and high salt diet, 2-way repeated measures ANOVA $P_{\text{salt}} = 0.0005$; $P_{\text{genotype}} = 0.0009$; $P_{\text{interaction}} = 0.3808$, with Sidak’s test for multiple comparisons. D: Standard deviation during normal and high salt diet, 2-way repeated measures ANOVA $P_{\text{salt}} < 0.0001$; $P_{\text{genotype}} < 0.0001$; $P_{\text{interaction}} = 0.0013$, with Sidak’s test for multiple comparisons. E: Change in the SBP range between normal salt and high salt diets, unpaired Student’s t-test $p = 0.3808$, F: Change in the standard deviation of

SBP between normal salt and high salt diets, unpaired Student's t-test, SBP = systolic blood pressure, TG = transgenic, ET_B-def = endothelin B deficient, SD = standard deviation.

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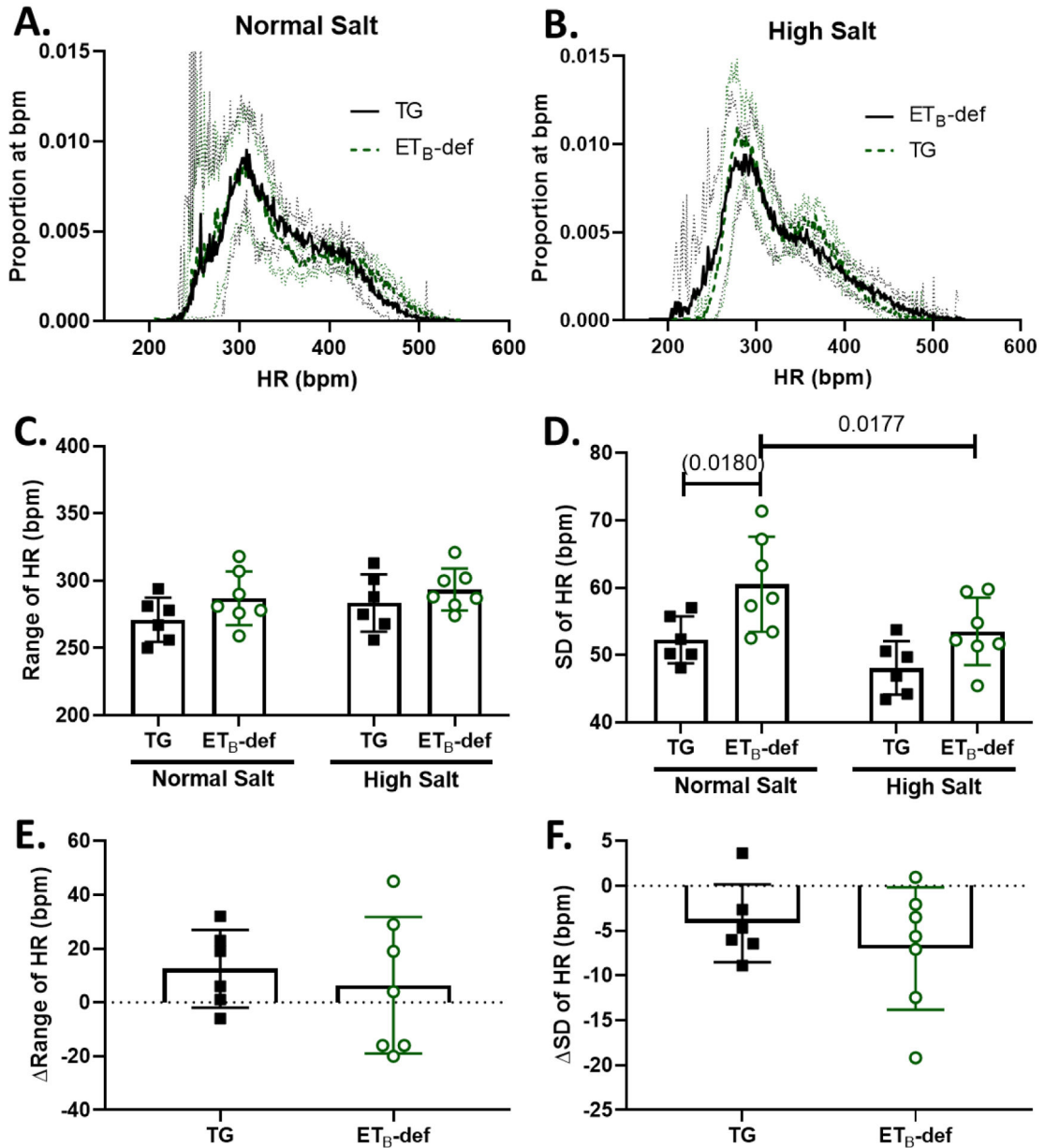


Figure 2: Heart rate lability is similar between TG and ET_B -def rats during normal salt and high salt diet.

A/B: frequency distribution plots of proportion of instances at a given HR (number of instances of 10-second bins at each bpm / total number of bins) during normal salt diet (A) and 8–11 days of high salt diet (B) Solid line represents mean \pm 95% confidence interval. C: Range of HR during normal and high salt diet, 2-way repeated measures ANOVA, $P_{\text{salt}} = 0.1358$; $P_{\text{genotype}} = 0.1510$; $P_{\text{interaction}} = 0.6159$, with Sidak’s test for multiple comparisons (no significant differences). D: Standard deviation of HR during normal and high salt diet, 2-way repeated measures ANOVA, $P_{\text{salt}} = 0.0055$; $P_{\text{genotype}} = 0.0151$; $P_{\text{interaction}} = 0.4046$, with Sidak’s test for multiple comparisons. E: Change in the HR range between normal salt and high salt diets, unpaired Student’s t-test $p = 0.6159$, F: Change in the standard deviation

of HR between normal salt and high salt diets, unpaired Student's t-test $p = 0.4046$. HR = heart rate, TG = transgenic, ET_B-def = endothelin B deficient, SD = standard deviation.

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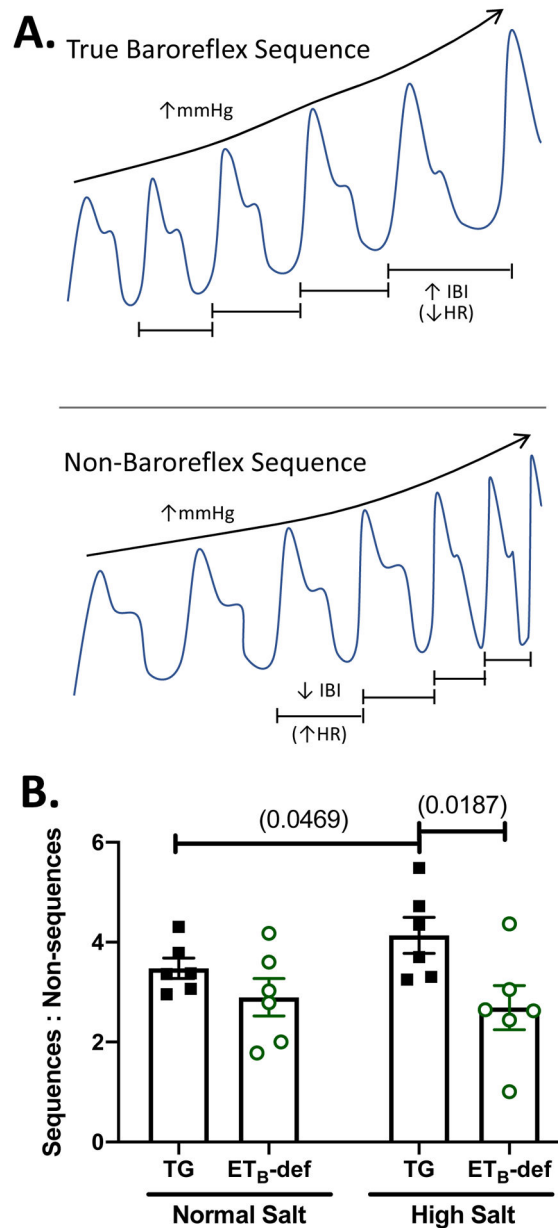
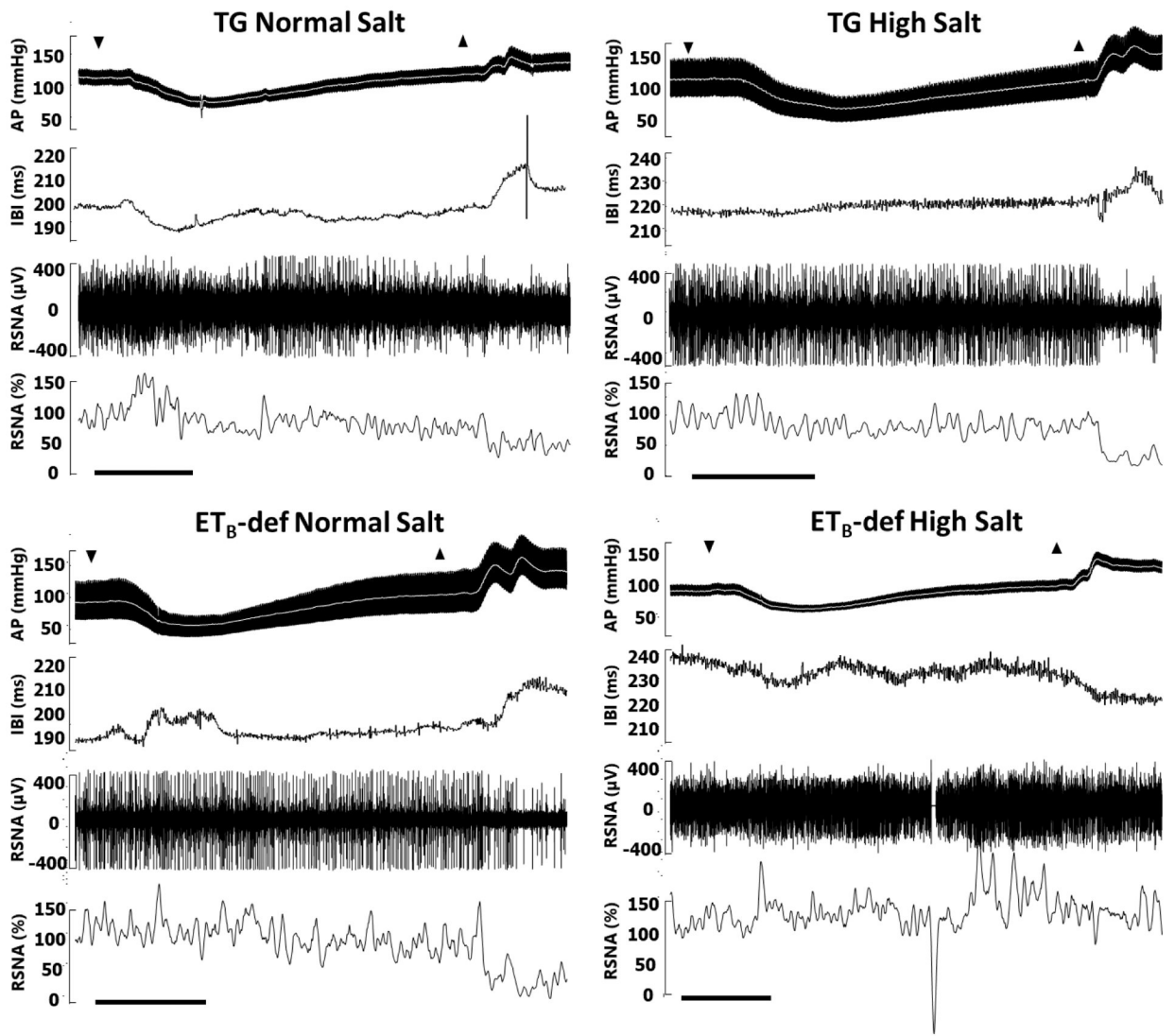


Figure 3: ET_B-def rats have fewer true:non-baroreflex sequences following high salt diet.

A: simplified graphical representation of a true baroreflex sequence (top panel: blood pressure and interbeat interval, or IBI, both increasing) vs. a non-baroreflex/feedforward sequence (bottom panel: blood pressure and IBI change in opposite directions). B: ratio of true baroreflex sequences to non-baroreflex sequences in transgenic TG controls and ET_B-def rats during normal salt (0.49% NaCl) or on days 8–11 of high salt (4.0% NaCl) diet. 2-way repeated measures ANOVA, $P_{\text{salt}} = 0.2233$; $P_{\text{genotype}} = 0.0572$; $P_{\text{interaction}} = 0.0332$ with Sidak's multiple comparison test. IBI = interbeat interval, HR = heart rate, TG = transgenic, ET_B-def = endothelin B deficient.



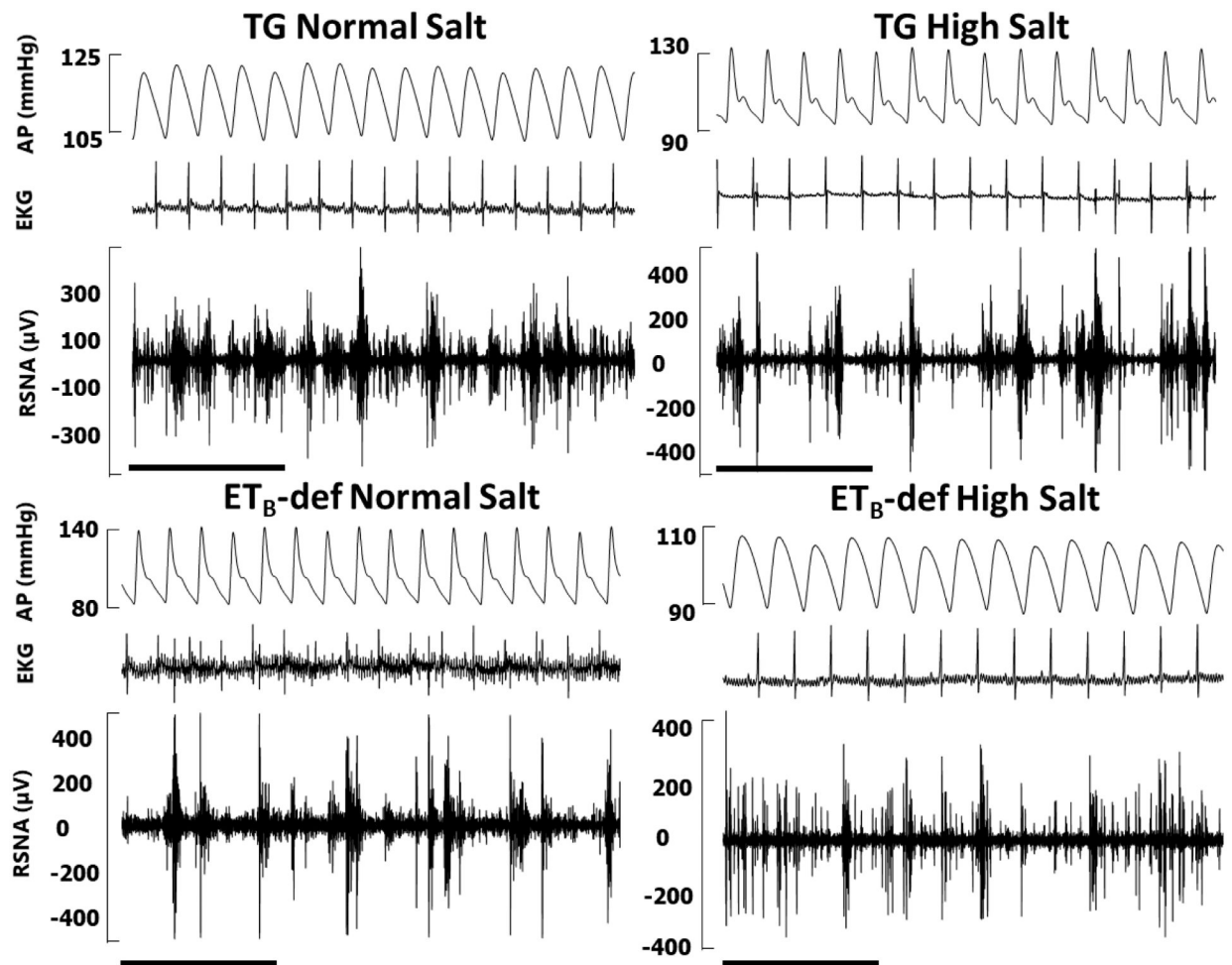


Figure 4: Representative traces of modified Oxford baroreflex experiments.

AP = arterial pressure, mean arterial pressure denoted by white line; IBI = interbeat interval derived from R-R of electrocardiogram (not pictured); RSNA = renal sympathetic nerve activity in raw voltage and as percent of baseline prior to sodium nitroprusside infusion. RSNA percent is smoothed in rolling 2 second intervals for clarity. A: Down arrows denote beginning of bolus infusion of sodium nitroprusside, up arrows denote beginning of bolus infusion of phenylephrine. Scale bars denote 30 seconds. B: Representative baseline segment from each of the four groups demonstrating signal fidelity, EKG, and pulse wave synchronicity of RSNA. Scale bars denote 1 second.

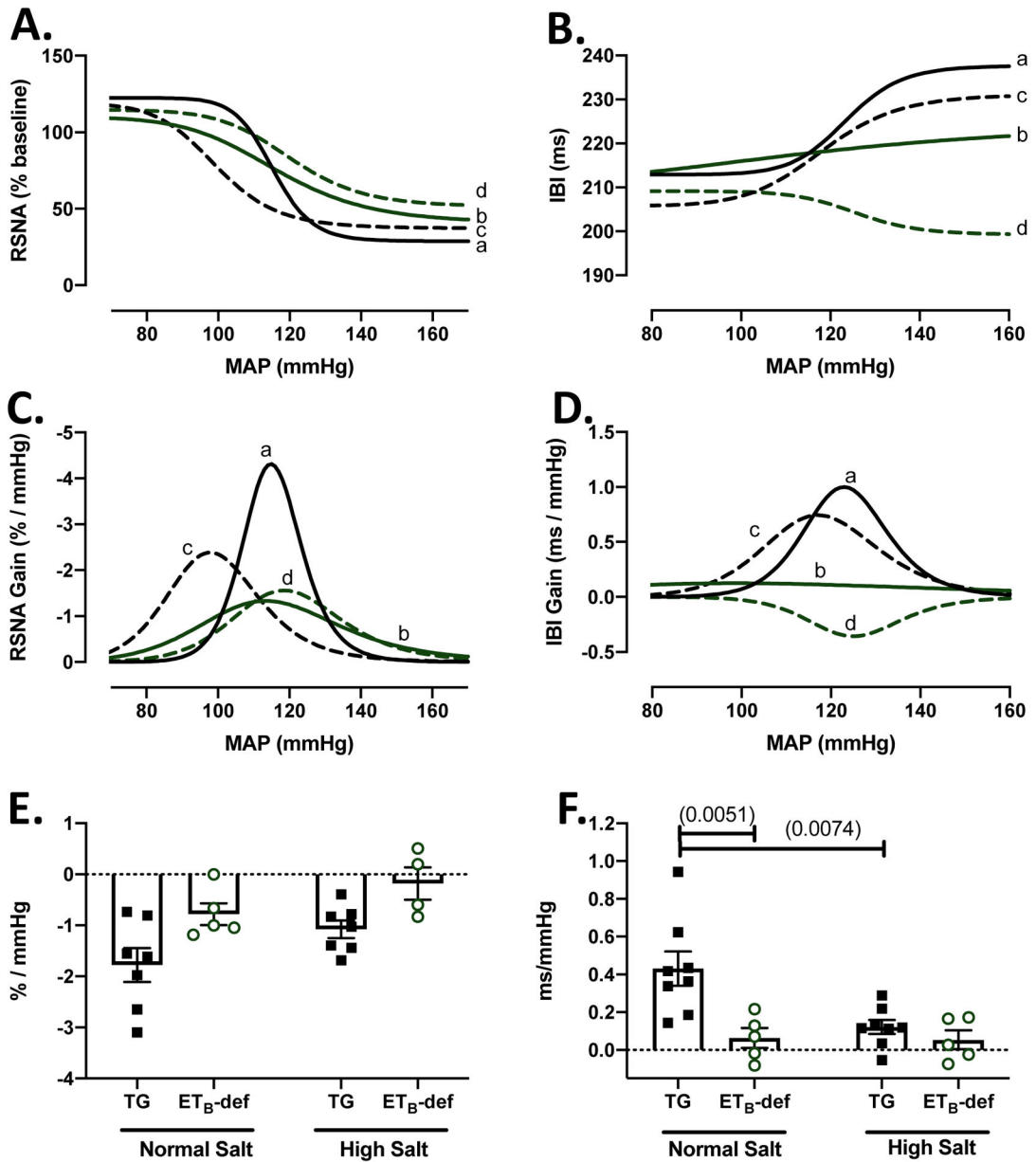


Figure 5: Heart rate baroreflex function is impaired in ET_B-def rats compared to TG controls. A: RSNA composite baroreflex curves. B: heart rate composite baroreflex curves. C: RSNA composite gain curves from first derivative of baroreflex curve. D: heart rate composite gain curves from first derivative of baroreflex curve. (A-D) a = TG NS; b = ET_B-def NS; c = TG HS; d = ET_B-def HS E. Linear regression of RSNA response to increase in pressure during phenylephrine, 2-way ANOVA $P_{\text{salt}} = 0.0309$; $P_{\text{genotype}} = 0.0030$; $P_{\text{interaction}} = 0.8601$, with Sidak's test for multiple comparisons (no significant differences). F. Linear regression of heart rate response to increase in pressure during phenylephrine, 2-way ANOVA $P_{\text{salt}} = 0.0298$; $P_{\text{genotype}} = 0.0044$; $P_{\text{interaction}} = 0.0400$, with Sidak's test for multiple comparisons. p values in (). RSNA = renal sympathetic nerve activity, MAP = mean arterial pressure, IBI = interbeat interval.

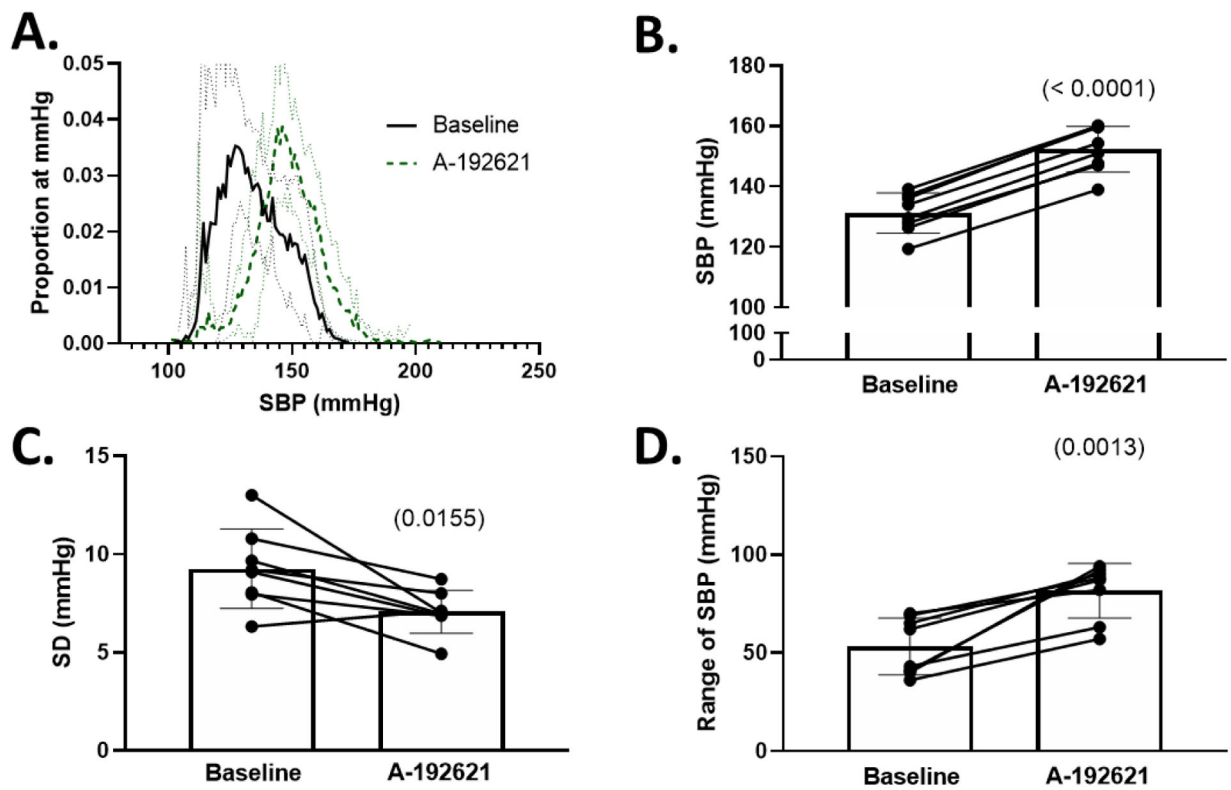


Figure 6: Blood pressure increases and lability decreases in Sprague-Dawley rats after ET_B receptor antagonism.

A. Histograms of proportion of instances at a given SBP (number of instances of 10-second bins at each mmHg / total number of bins) during baseline and following 3–5 days of selective endothelin B antagonism with A-192621. Solid line represents mean \pm 95% confidence interval B. 24-hour average SBP at baseline and following A-192621. C. Standard deviation (SD) of SBP before and after A-192621. D. Range of SBP before and after A-192621. All comparisons made using paired Student's t-test, p values in (). SBP = systolic blood pressure.

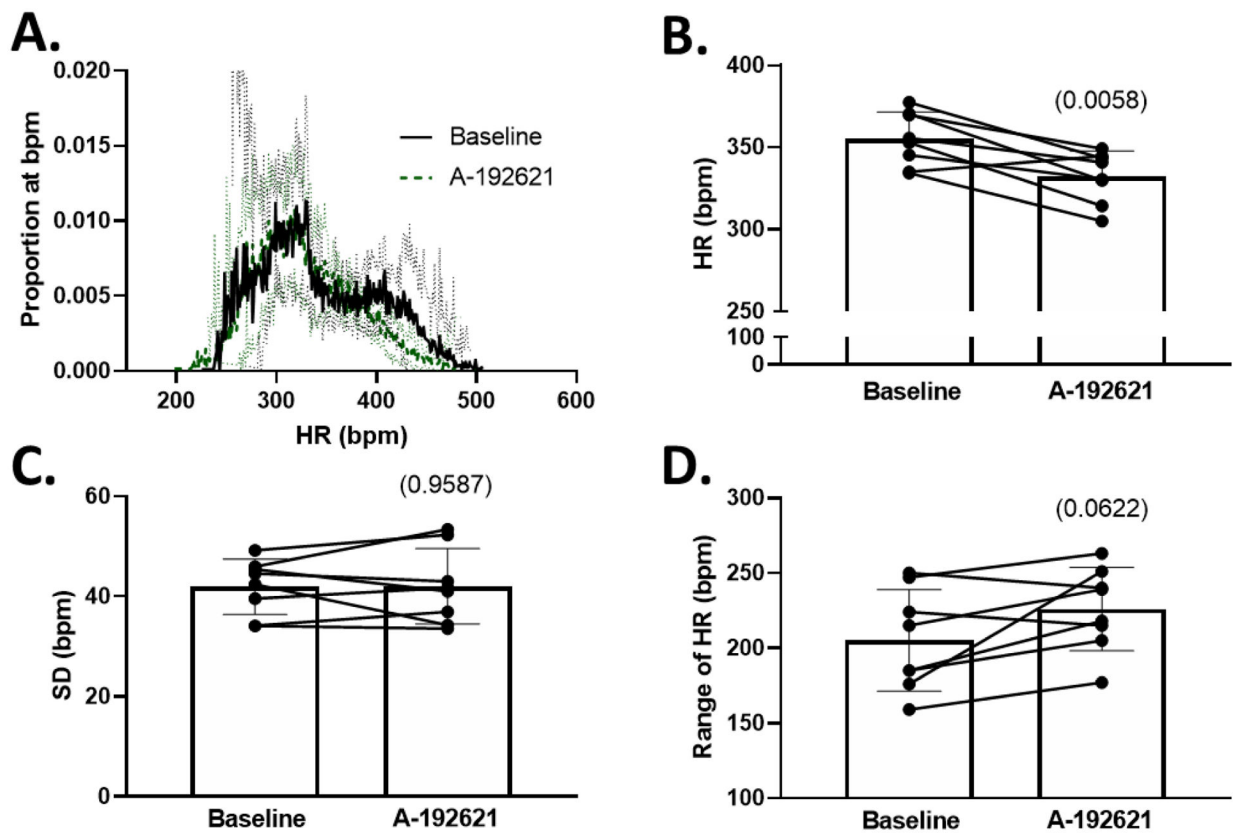


Figure 7: Heart rate decreases but heart rate lability is unchanged in Sprague-Dawley rats after ET_B receptor antagonism.

A. Histograms of proportion of instances at a given HR (number of instances of 10-second bins at each bpm / total number of bins) during baseline and following 3–5 days of endothelin B antagonism with A-192621. B. 24-hour average HR at baseline and following A-192621. C. Standard deviation (SD) of HR before and after A-192621. D. Range of HR before and after A-192621. All comparisons made using paired Student's t-test, p values in (). HR = heart rate.

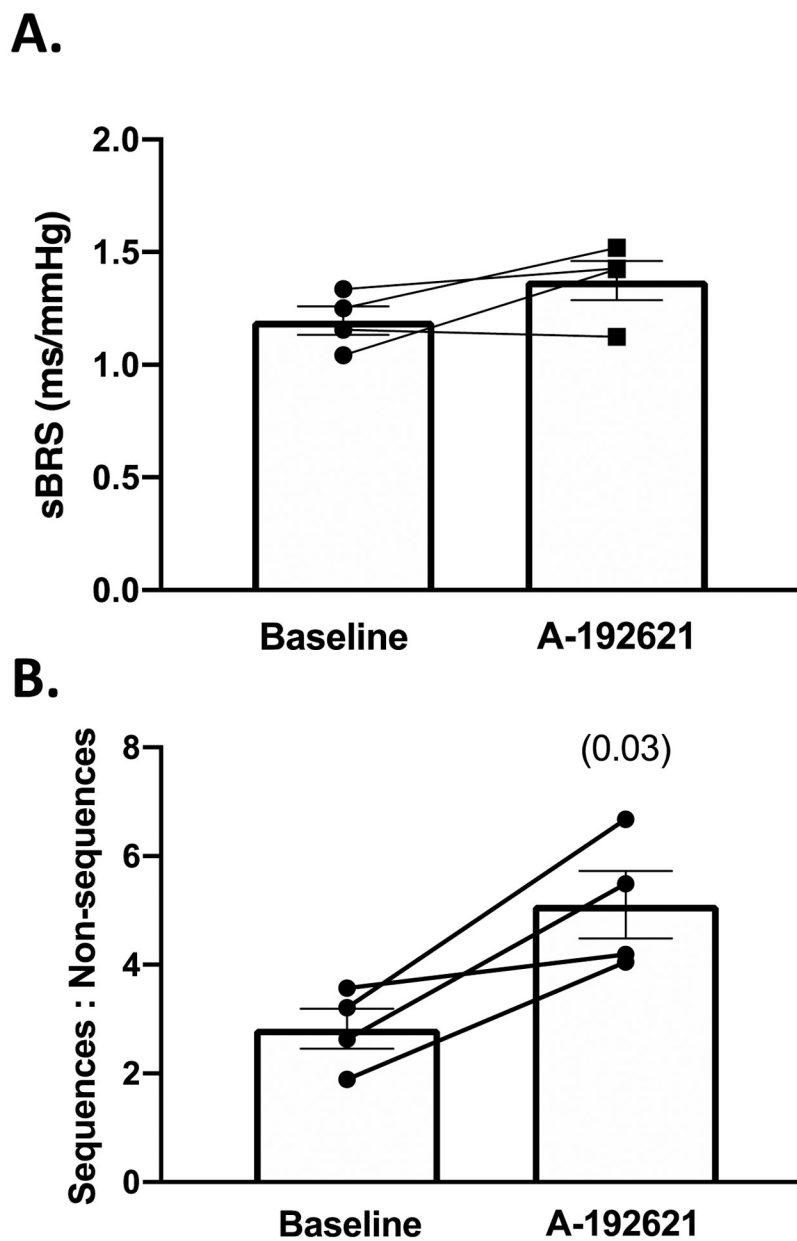


Figure 8: ET_B receptor blockade increases the ratio of true:non-baroreflex sequences in Sprague-Dawley rats.

A: spontaneous baroreflex (sBRS) gain before and after 3–5 days of A-192621. B: ratio of true baroreflex sequences to non-baroreflex sequences during baseline and after 3–5 days of A-192621. All comparisons made using paired Student’s t-test, p values in ().

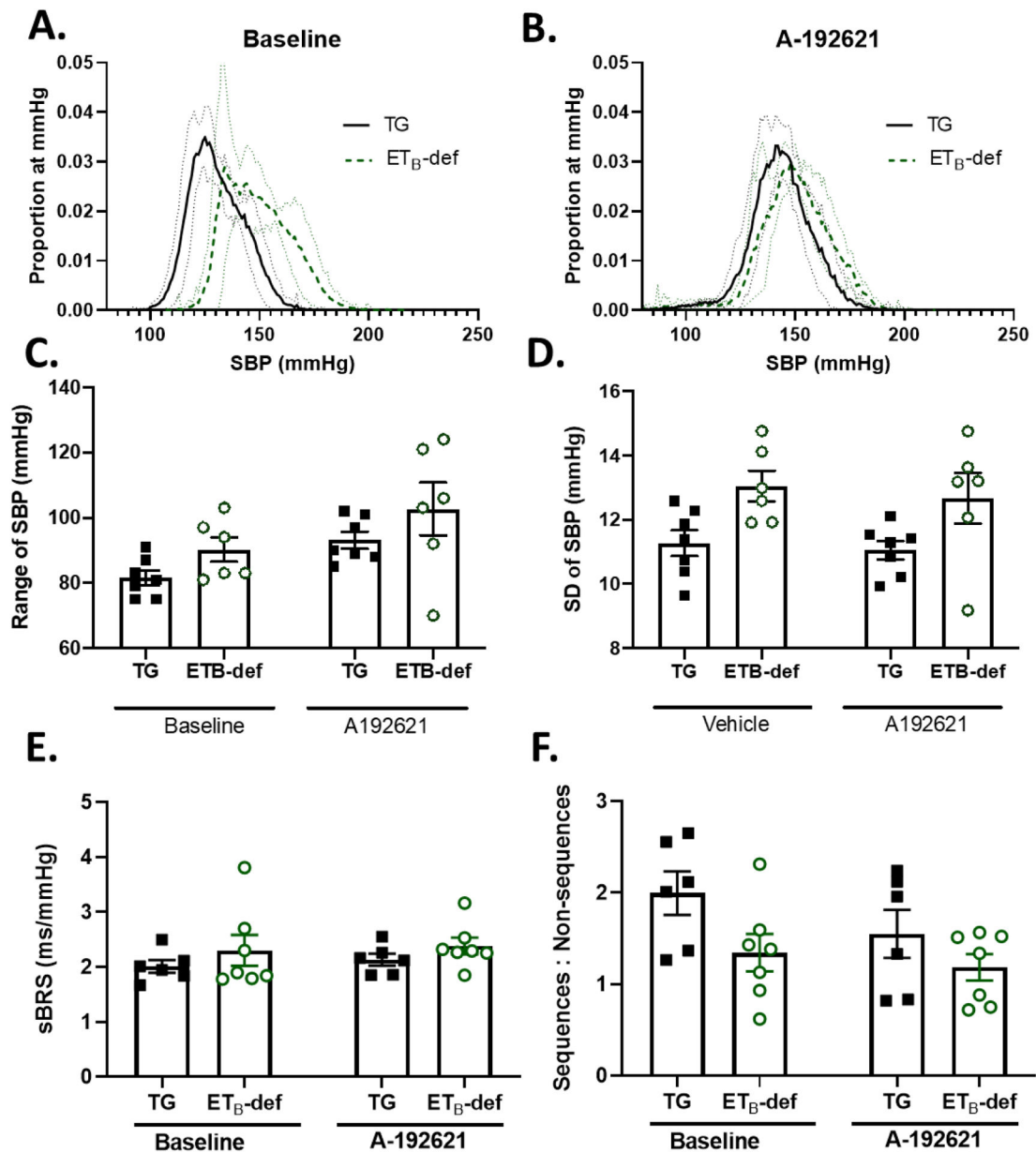


Figure 9: ET_B receptor blockade does not alter blood pressure lability or ratio of true:non-baroreflex sequences in ET_B -def and TG rats.

A. Histograms of proportion of instances at a given SBP (number of instances of 10-second bins at each mmHg / total number of bins) during baseline and following 3–5 days of selective endothelin B antagonism with A-192621. Solid line represents mean \pm 95% confidence interval. B. 24-hour average SBP at baseline and following A-192621. C. Range of SBP before and after A-192621. 2-way repeated measures ANOVA $P_{A-192621} = 0.0130$; $P_{genotype} = 0.0917$; $P_{interaction} = 0.9112$, with Sidak's test for multiple comparisons. D. Standard deviation (SD) of SBP before and after A-192621. 2-way repeated measures ANOVA $P_{A-192621} = 0.3368$; $P_{genotype} = 0.0224$; $P_{interaction} = 0.8057$, with Sidak's test for multiple comparisons. E. spontaneous baroreflex (sBRS) gain before and after 3–5 days of A-192621. 2-way repeated measures ANOVA $P_{A-192621} = 0.3768$; $P_{genotype} = 0.2905$;

$P_{\text{interaction}} = 0.8513$, with Sidak's test for multiple comparisons. F: ratio of true baroreflex sequences to non-baroreflex sequences during baseline and after A-192621. 2-way repeated measures ANOVA $P_{\text{A-192621}} = 0.0969$; $P_{\text{genotype}} = 0.0661$; $P_{\text{interaction}} = 0.4078$, with Sidak's test for multiple comparisons.

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Table 1 -

Lablity Parameters during Active and Inactive Periods

	TG Controls (n = 6)			ET _B -def (n = 7)			3-way RM ANOVA (p)
	Normal Salt	High Salt	Normal Salt	Normal Salt	High Salt	High Salt	
SBP Range (mmHg)							
Active	72.2 ± 3.1	84.2 ± 6.1	94.6 ± 5.0 ^G	113.0 ± 6.1 ^{GS}			<i>S</i> = 0.0006 <i>S</i> * <i>G</i> = 0.6177 <i>S</i> * <i>TOD</i> = 0.8037
Inactive	70.2 ± 3.8	84.3 ± 4.1	90.9 ± 5.0 ^G	105.0 ± 4.0 ^G			<i>TOD</i> = 0.1404 <i>G</i> * <i>TOD</i> = 0.2711 <i>S</i> * <i>G</i> * <i>TOD</i> = 0.4544
SBP SD (mmHg)							
Active	10.6 ± 0.4	12.0 ± 0.2 ^S	12.7 ± 0.5 ^G	15.2 ± 0.4 ^{GS}			<i>S</i> < 0.0001 <i>S</i> * <i>G</i> = 0.0444 <i>S</i> * <i>TOD</i> = 0.0144
Inactive	9.6 ± 0.2	10.0 ± 0.2 ^T	11.8 ± 0.2 ^G	13.2 ± 0.4 ^{GST}			<i>TOD</i> < 0.0001 <i>G</i> * <i>TOD</i> = 0.9564 <i>S</i> * <i>G</i> * <i>TOD</i> = 0.8546
HR Range (bpm)							
Active	255.8 ± 7.5	278.0 ± 7.6	265.3 ± 8.2	285.1 ± 6.2			<i>S</i> = 0.0004 <i>S</i> * <i>G</i> = 0.9134 <i>S</i> * <i>TOD</i> = 0.3688
Inactive	242.3 ± 4.3	273.7 ± 13.4	262.0 ± 8.1	293.3 ± 6.0			<i>G</i> = 0.0365 <i>TOD</i> = 0.5893 <i>G</i> * <i>TOD</i> = 0.3518 <i>S</i> * <i>G</i> * <i>TOD</i> = 0.9199
HR SD (bpm)							
Active	48.7 ± 1.5	41.7 ± 1.2	57.9 ± 2.7	48.6 ± 1.5 ^S			<i>S</i> = 0.0025 <i>S</i> * <i>G</i> = 0.9481 <i>G</i> = 0.0116 <i>S</i> * <i>TOD</i> = 0.0378
Inactive	42.0 ± 3.5	36.0 ± 1.6	48.3 ± 3.6 ^T	44.2 ± 2.0			<i>TOD</i> = 0.0005 <i>G</i> * <i>TOD</i> = 0.7771 <i>S</i> * <i>G</i> * <i>TOD</i> = 0.1363

SBP: systolic blood pressure, HR: heart rate, Normal salt (0.49% NaCl), High salt (4.0% NaCl), TOD: time of day, G: genotype, S: salt diet, 3-way Repeated Measures ANOVA with Sidak's post-hoc test for multiple comparisons:

G, *p* < 0.05 genotype within salt diet and time of day;

S, *p* < 0.05 salt within genotype and time of day;

T, *p* < 0.05 time of day within salt and genotype. Mean ± SEM.

Group effect significance indicated by italics.

Table 2 -

Cosinor Analysis

	TG Controls (n = 6)		E1p-def (n = 7)		2-Way RM ANOVA (p)	
	Normal Salt	High Salt	Normal Salt	High Salt		
SBP SD Amplitude (mmHg)	1.0 ± 0.1	1.7 ± 0.2	1.2 ± 0.2	2.2 ± 0.2 ^S	<i>S = 0.0032</i>	<i>G = 0.0492</i> <i>S*G = 0.4749</i>
HR SD Amplitude (bpm)	7.5 ± 1.2	7.9 ± 0.5	8.8 ± 0.7	7.9 ± 1.5	<i>S = 0.7123</i>	<i>G = 0.6319</i> <i>S*G = 0.3825</i>
SBP SD Acrophase (ZT)	20.5 ± 0.7	19.8 ± 0.2	20.3 ± 0.7	20.0 ± 0.5	<i>S = 0.4140</i>	<i>G = 0.9608</i> <i>S*G = 0.7426</i>
HR SD Acrophase (ZT)	15.9 ± 0.9	15.9 ± 0.5	16.3 ± 0.2	16.3 ± 0.4	<i>S = 0.9235</i>	<i>G = 0.4757</i> <i>S*G = 0.9685</i>

SBP: systolic blood pressure, HR: heart rate, Normal salt (0.49% NaCl), High salt (4.0% NaCl), G: genotype, S: salt diet, 2-way Repeated Measures ANOVA with Sidak's post-hoc test for multiple comparisons:

^S p < 0.05 vs. normal salt. Mean ± SEM.

Group effect significance indicated by italics.

Table 3 –

4-Parameter Sigmoid Curve Baroreflex Components

Heart Rate Baroreflex Response	TG Controls			ET _B -def			2-way ANOVA (p)		
	Normal Salt (8)	High Salt (5)	Normal Salt (8)	High Salt (5)	High Salt (5)	Normal Salt (8)	High Salt (5)	Normal Salt (8)	High Salt (5)
Bottom Plateau (ms)	213.0 ± 7.5	213.5 ± 11.32	210.3 ± 8.0	191.3 ± 14.5	S = 0.4028	G = 0.2664	S *G = 0.3746		
Top Plateau (ms)	241.7 ± 10.3	239.2 ± 6.6	226.2 ± 13.3	204.5 ± 11.4	S = 0.2799	G = 0.0184	S *G = 0.2145		
MAP BP50 (mmHg)	140.6 ± 7.5	129.8 ± 9.6	146.1 ± 11.8	132.0 ± 13.72	S = 0.7145	G = 0.2514	S *G = 0.3436		
Max Gain (ms / mmHg)	0.99 ± 0.29	0.76 ± 0.21	0.42 ± 0.24	-0.17 ± 0.38	S = 0.1670	G = 0.0167	S *G = 0.5528		
Baseline IBI (ms)	204.5 ± 9.4	235.2 ± 8.4	211.8 ± 9.3	197.2 ± 11.7	S = 0.4273	G = 0.1378	S *G = 0.0327		
Baseline MAP (mmHg)	101.1 ± 2.5	95.1 ± 3.1	96.5 ± 4.2	115.3 ± 3.8	S = 0.0754	G = 0.0348	S *G = 0.0019		
RSNA Baroreflex Response									
Heart Rate Baroreflex Response	TG Controls			ET _{Te} -def			2-way ANOVA (p)		
	Normal Salt (8)	High Salt (5)	Normal Salt (8)	High Salt (5)	High Salt (5)	Normal Salt (8)	High Salt (5)	Normal Salt (8)	High Salt (5)
Bottom Plateau (%)	28.8 ± 9.3	37.22 ± 7.3	40.6 ± 6.9	51.3 ± 12.3	S = 0.3097	G = 0.1758	S *G = 0.9008		
Top Plateau (%)	122.5 ± 13.6	118.8 ± 7.8	109.9 ± 4.0	114.6 ± 8.2	S = 0.9605	G = 0.4363	S *G = 0.7882		
MAP BP50 (mmHg)	115.4 ± 3.8	99.4 ± 8.4	116.3 ± 5.0	120.2 ± 2.7	S = 0.3540	G = 0.1028	S *G = 0.1345		
Max Gain (% / mmHg)	-3.8 ± 0.6	-2.3 ± 0.4	-1.4 ± 0.1 ^G	-1.5 ± 0.1	S = 0.1558	G = 0.0022	S *G = 0.0831		

MAP: mean arterial pressure, IBI: inter beat interval, Normal salt (0.49% NaCl), High salt (4.0% NaCl), TG: transgenic controls; ET_B-def: Endothelin B deficient; G: genotype, S: salt diet. 2-way ANOVA with Tukey's post-hoc test for multiple comparisons:

G, p < 0.05 genotype;

S, p < 0.05 salt; Mean ± SEM.

Group effect significance indicated by italics.

Table 4 –

24-hr Hemodynamic Parameters of TG and ET_B-def before and after A-192621

	TG Controls (n = 7)		ET _B -def (n = 6)		2-Way RM ANOVA (p)	
	Baseline	A-192621	Baseline	A-192621		
SBP (mmHg)	130.5 ± 1.7	148.5 ± 2.3 ^{*****}	150.0 ± 2.5 ^{####}	150.1 ± 2.7	<i>Tx = 0.0002</i>	<i>G = 0.0030 Tx*G = 0.0002</i>
MAP (mmHg)	107.6 ± 1.5	122.8 ± 2.0 ^{*****}	125.3 ± 2.6 ^{####}	125.9 ± 2.9	<i>Tx = 0.0006</i>	<i>G = 0.0044 Tx*G = 0.0003</i>
DBP (mmHg)	88.5 ± 1.4	102.1 ± 1.9 ^{*****}	103.6 ± 2.7 ^{####}	103.0 ± 3.3	<i>Tx = 0.0010</i>	<i>G = 0.0206 Tx*G = 0.0005</i>
HR (bpm)	362.9 ± 4.5	325.9 ± 4.8 ^{*****}	365.9 ± 6.8	359.9 ± 12.2 ^{##}	<i>Tx = 0.0001</i>	<i>G = 0.0827 Tx*G = 0.0015</i>

SBP: systolic blood pressure, MAP: mean arterial pressure, DBP: diastolic blood pressure, HR: heart rate, G: genotype, Tx: treatment with A-192621. 2-way Repeated Measures ANOVA with Sidak's post-hoc test for multiple comparisons:

^{*****} p < 0.0001 vs. baseline;

^{##} p < 0.01 vs. TG,

^{###} p < 0.001 vs. TG

^{####} p < 0.0001 vs. TG Mean ± SEM.

Group effect significance indicated by italics.