Transection of the vagus nerve in rats and the role it plays in altering activity of the baroreceptor reflex

Abstract The baroreceptor reflex is an intricate mechanism that causes blood pressure to be maintained if deviation from blood pressure set point occurs. There are many baroreceptors but the ones of focus in this study are the arterial baroreceptors, which are more prominent in the carotid sinus and aortic arch. Using Sprague Dawley rats, we study the baroreflex by utilizing the femoral vessels and administration of both a vasoconstrictor, phenylephrine (PE) and a vasodilator, sodium nitroprusside (SNP) through the femoral vein. The vagus nerve plays a role in the baroreflex by its association with the aortic arch mechanosensory neurons, providing parasympathetic stimulation to the heart when the reflex is activated. We tested the hypothesis that transection of both vagus nerves alters the baroreflex in its overall function to correct for blood pressure. Evidence points toward the conclusion that the blood pressure was able to be maintained in response to PE or SNP regardless of whether the vagus nerves were intact.

Introduction

The baroreceptor reflex is a compensatory mechanism that exists in vertebrate physiology in response to perturbations in blood pressure that deviate from the set point to maintain the blood pressure itself. This reflex is able to occur quite rapidly, in fractions of a second. In the case of acute hypertension, the reflex mechanism occurs by increasing sympathetic inhibition and parasympathetic excitation to the heart, thus decreasing the heart rate. On the other hand, for acute hypotension, increases in blood pressure will trigger an opposite effect, where an increase in sympathetic excitation and parasympathetic inhibition occurs causing contraction of blood vessels and an increase in heart rate (Heusser, Tank, Luft, & Jordan, 2005). Arterial baroreceptors, which are mechanosensory neurons, facilitate this physiological response by sensing changes in blood pressure, and are located within arterial blood vessels where the most sensitive receptors are localized within the aortic arch and carotid sinus (Heesch, 1999).

Vagus nerves are one of the cranial nerves in the central nervous system, where its function is to provide parasympathetic stimulation to the heart. There exist procedures involving the vagus nerve, one being vagal nerve stimulation, an example in which it was used, would be as an adjunctive treatment for treating patients with seizures (Schachter & Saper, 1998). More relevant to the experiment enacted is a vagotomy, which is when the vagus nerve(s) is transected. One study showcased results that a sympathoinhibitory function was associated with the vagus nerve and removing it increased sympathetic output during pressure inputs (Kawada, Li, Zheng, & Sugimachi, 2016). We hypothesize that the vagus nerve plays a prominent role in the function of the baroreflex and when the vagus nerve is inhibited, the physiological response i.e. the rectification of blood pressure back to baseline will be perturbed.

Aims

Our aims involve observing the baroreflex in rats following phenylephrine (PE) and sodium nitroprusside (SNP) drug administration, which are vasoconstrictors (Mets, 2016) and vasodilators (Tinker & Michenfelder, 1976) respectively. This is conducted by analyzing the mean arterial blood pressure (MAP), and heart rate. Most importantly, we seek to ascertain if any differences occur in physiology before and after a vagotomy with the administration of the aforementioned drugs.

1) Observe and discuss the baroreflex with administration of PE and SNP.
2) Determine if transection of the vagus nerve affects the ability of the baroreflex to correct for blood pressure.
    a. Since the vagus nerve provides parasympathetic stimulation to the heart, when the vagus nerve(s) is inhibited, changes in heart rate and blood pressure in response to the baroreflex may be different.

Methods

Male Sprague Dawley rats (n = 39), ranging from 165 to 282 grams and averaging 225 grams were purchased from Taconic Biosciences and utilized to perform non-survival surgery upon. Rats were housed in pairs, under light-dark cycle (12:12h) conditions and study involving the rats were approved by IACUC. Out of the 39 rats, 17 survived for the duration of the complete

experiment, which were used for data collection, whereas the prematurely deceased rats were not. Rats were initially anesthetized using a concentration of 1.6g/kg urethane. Dosage was applied in two half-doses and toe tests were performed 45 minutes after the second half-dose to observe the responsiveness of the rats before surgery was initialized. If urethane was unsuccessful in anesthetization of the rat, xylazine at 4mg/kg was used as an alternative measure to induce anesthetization. Hereon after, rats were monitored for both respiratory rate and temperature at intervals of 20 minutes.

Initial incisions were made in the lower region of the abdomen towards the left leg to begin locating the femoral artery and vein. Cannulation with a pressure transducer specific for the artery, to measure arterial blood pressure, was then inserted into the femoral artery. The second incision, a tracheostomy, was performed after along with locating the vagus nerves right next to the trachea and creating a suture around both the left and right nerves for preparation in the latter part of the experiment.

Drug administration was conducted through the femoral vein venous line. PE and SNP were administered independently of each other, once before the vagotomy of both nerves and once after. Administration of either drug would only occur after the blood pressure was stabilized post-initial drug administration. PE and SNP were injected at a concentration of 10 μg/kg and 8 μg/kg respectively. We utilized a data collection program called Daisy Lab, which allowed us to collect blood pressure baseline, peak and recovery values and monitored respiratory rate and temperature at those points. After all procedures were conducted, rats were disposed of in a biohazard container.

We determined significance between baseline, peak, and recovery MAP values using Minitab, which allowed us to conduct paired t-tests.

Results

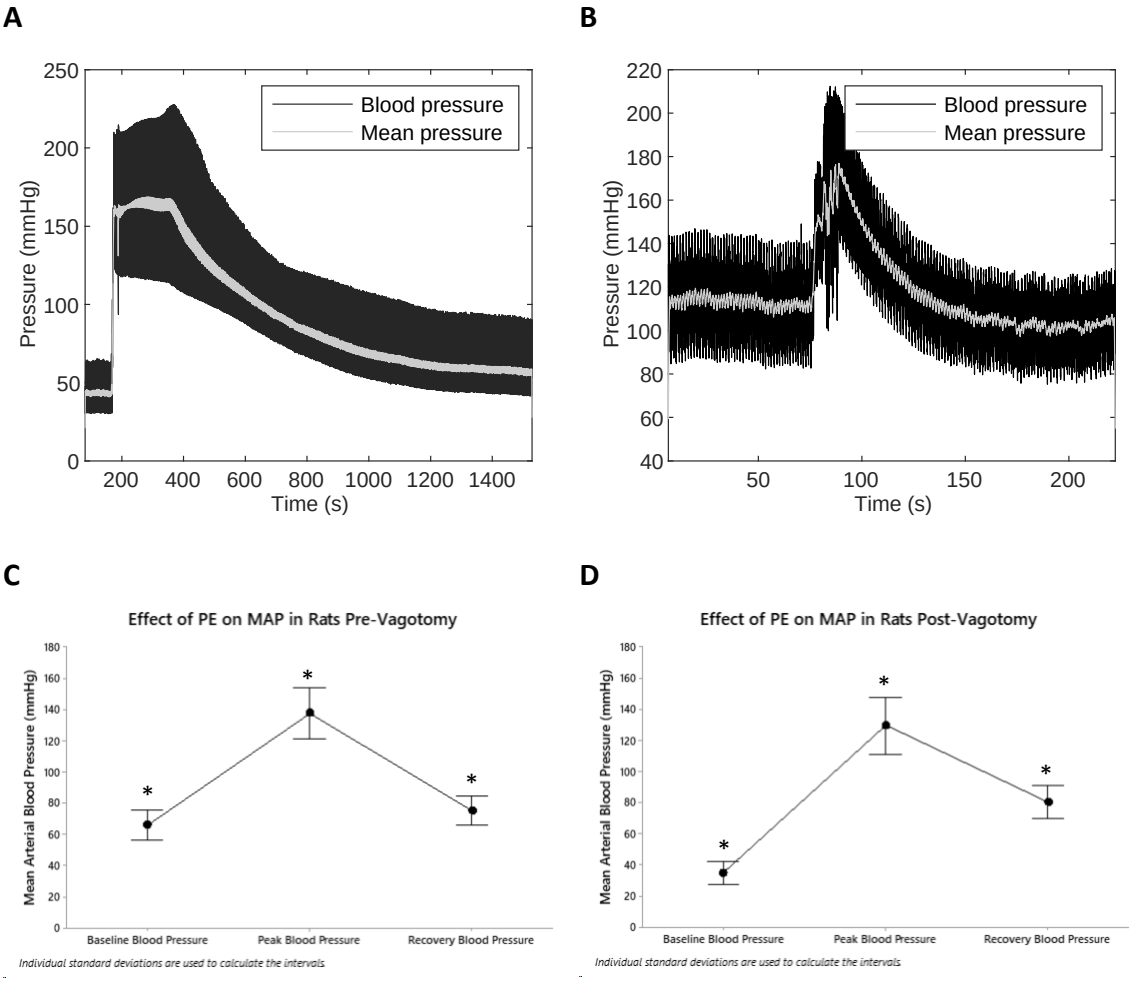

Figure 1. Mean arterial blood pressure: PE administration in rats before and after vagotomy
Raw data showing change in blood pressure due to PE administration before vagotomy (A) and after vagotomy (B). Depiction of MAP baseline, peak, and recovery values (n = 17) with PE administration before vagotomy (C) and afer vagotomy (D). Asterisks (*) indicate significance (*P*-value < .05) in MAP between the other groups on the interval plot. Significance detected between all groups.

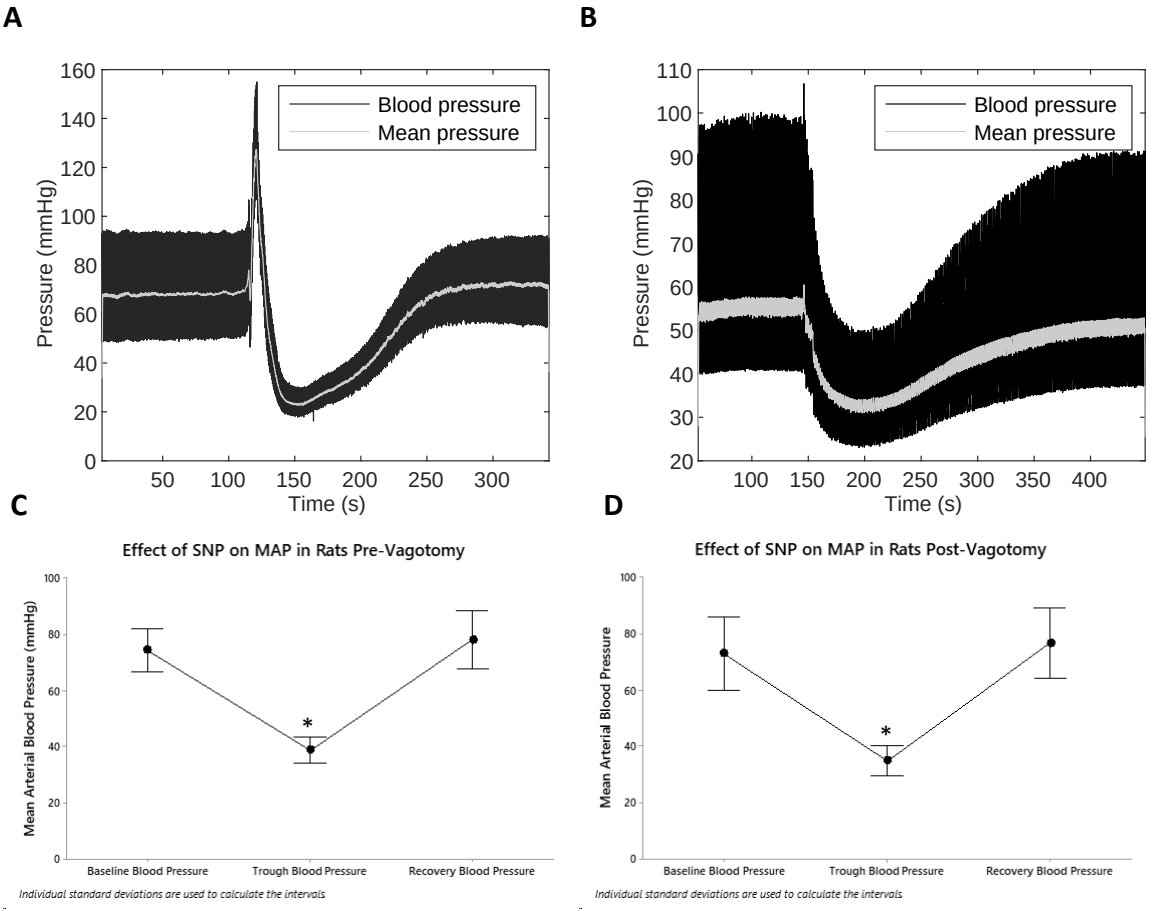

Figure 2. Mean arterial blood pressure: SNP administration in rats before and after vagotomy
Raw data showing change in blood pressure due to SNP administration before (A) and after vagotomy (B). Depiction of MAP baseline, peak, and recovery values (n = 17) with SNP administration before vagotomy (C) and afer vagotomy (D). Asterisks (*) indicate significance (*P*-value < .05) in MAP between the other groups on the interval plot. No significance was detected between baseline and recovery values.

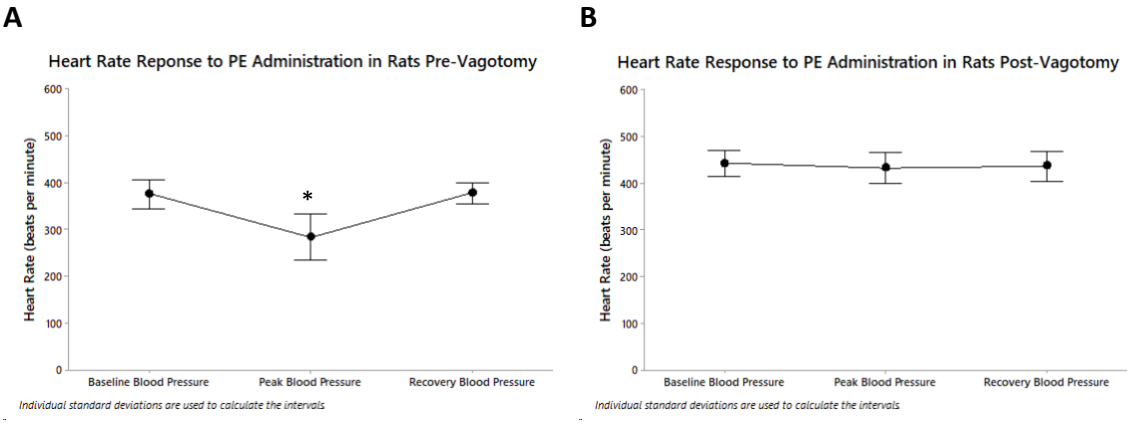

Figure 3. Heart rate: PE Administration in rats before and after vagotomy
Measurement of heart rate in rats administered with phenylephrine collected at baseline, peak, and recovery blood pressure points before vagotomy (A) and after vagotomy (B) was conducted. Asterisks (*) indicate significance (*P*-value < .05) in heart rate between the other means on the graph.

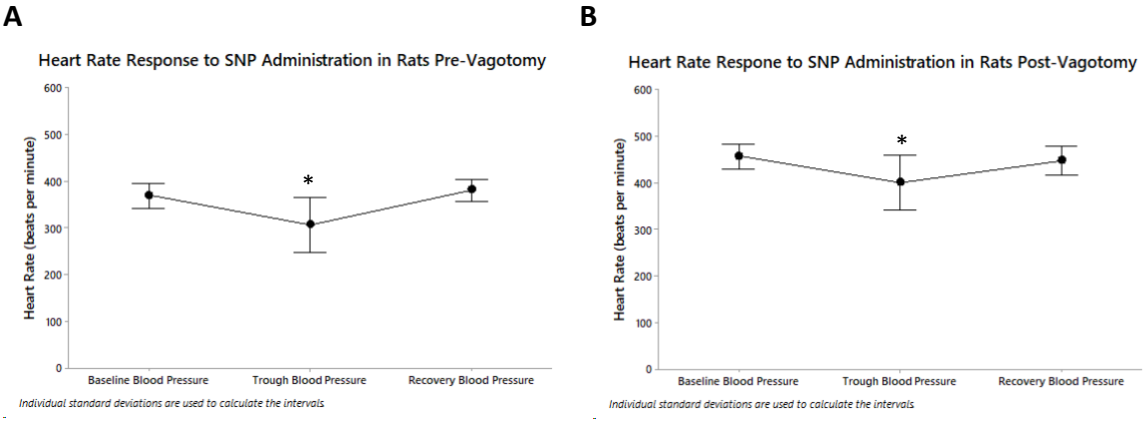

Figure 4. Heart rate: SNP Administration in rats before and after vagotomy
Measurement of heart rate in rats administered with sodium nitroprusside collected at baseline, peak, and recovery blood pressure points before vagotomy (A) and after vagotomy (B) was conducted. Asterisks (*) indicate significance (*P*-value < .05) in heart rate between the other means on the graph.

Table 1	P-Value
PE Baseline vs. Peak	0
PE Baseline vs. Recovery	0.045
PE Peak vs. Recovery	0
SNP Baseline vs. Trough	0
SNP Baseline vs. Recovery	0.305
SNP Trough vs. Recovery	0

Table 1. Mean Arterial Blood Pressure Pre-Vagotomy P-Values

Table 2	P-Value
PE Baseline vs. Peak	0
PE Baseline vs. Recovery	0
PE Peak vs. Recovery	0
SNP Baseline vs. Trough	0
SNP Baseline vs. Recovery	0.185
SNP Trough vs. Recovery	0

Table 2. Mean Arterial Blood Pressure Post-Vagotomy P-Values

Table 3	P-Value
PE Baseline vs. Peak	0.001
PE Baseline vs. Recovery	0.754
PE Peak vs. Recovery	0.001
SNP Baseline vs. Trough	0.024
SNP Baseline vs. Recovery	0.083
SNP Trough vs. Recovery	0.015

Table 3. Heart Rate Pre-Vagotomy P-Values

Table 4	P-Value
PE Baseline vs. Peak	0.350
PE Baseline vs. Recovery	0.232
PE Peak vs. Recovery	0.751
SNP Baseline vs. Trough	0.005
SNP Baseline vs. Recovery	0.090
SNP Trough vs. Recovery	0.007

Table 4. Heart Rate Post-Vagotomy P-Values

Discussion

Observation of the Baroreflex

Figures 1C and 2C are important controls in that they showcase the compensatory mechanism known as the baroreflex occurring in the rat in response to PE and SNP. We expected to see blood pressure increase and decrease for PE and SNP respectively. It is shown that the PE and SNP cause their respective blood pressure changes between baseline and peak/trough after which the baroreflex works to bring the blood back to original levels. Both the difference between MAP values of baseline and peak/trough along with difference in peak/trough and recovery were significant.

Prolonged Effects of SNP and PE on Mean Arterial Blood Pressure and Heart Rate

In our pre-vagotomy protocol, significance is shown between MAP baseline and recovery for PE but not for SNP. This implicates that PE may still have an ongoing effect on blood pressure even after the baroreflex has been activated since recovery MAP is significantly higher than baseline MAP. SNP on the other hand, may have a short half-life due to a lack of difference in MAP between baseline and recovery values.

Effect of Vagotomy on Heart Rate and Blood Pressure with PE Administration

It was interesting to see that transection of the vagus nerve did not change blood pressure, shown in the recovery of blood pressure in Figure 1D, which was significantly decreased from the peak, similar to pre-vagotomy in Figure 1C. Possible reasoning for this may deal with the fact that the vagus nerve is only associated with the aortic arch, leaving another component of the organism, the carotid sinus baroreceptors, to activate and induce the baroreflex (Kieval, Bettett, & Fitts, 2000).

In normal physiology, heart rate will decrease in response to increased blood pressure, which is demonstrated by pharmacological means in figure 3A where heart rate decreases in response to the PE at peak MAP. If the vagus nerve is transected, the expectation would be that heart rate would not be able to drop as low due to a lack of parasympathetic stimulation. This is corroborated by figure 3B in which no statistical significance was achieved between heart rate values at baseline and peak MAP indicating that heart rate more or less stayed constant through administration of the drug. The discrepancy lies in how blood pressure was able to be corrected near to baseline MAP (Fig. 1D) without a substantial decrease in heart rate. As stated before the carotid sinus baroreceptor were still active, facilitating the baroreflex response.

Effect of Vagotomy on Heart Rate and Blood Pressure with SNP Administration

SNP administration just like PE, showed no changes in significance between pre-vagotomy and post-vagotomy MAP recovery values (Fig. 2). The difference lies in the normal functioning of the baroreflex in terms of heart rate, which was able to increase after the drug was administered, post-vagotomy. To appreciate this difference, specific function of the vagus nerve shall be looked at. Vagus nerve provides parasympathetic stimulation to the heart when the arterial baroreceptors increase firing rate due to increased blood pressure. Since SNP is a vasodilator, the lower firing frequency will cause an increase in sympathetic stimulation of the neural arcs, and inhibition of parasympathetic stimulation. This implicates that regardless

of the presence of the vagus nerve, in times of increased blood pressure, the baroreflex will execute as normal. To take all things into account, if the vagus nerve does play an uncanny role in sympathetic stimulation, other mechanisms exist that would increase blood pressure such as the renin-angiotensin aldosterone system (Atlas, 2007).

Conclusion

The baroreflex response is an intricate mechanism, as seen by the various changes associated with vagus nerve transection. The evidence points towards the vagus nerve only being involved in the decrease of heart rate and even when the vagus nerve is inhibited, there exist other mechanisms in the organism that will facilitate this decrease, and continually maintain blood pressure in both acute hypertension and hypotension. It can be said that mammalian physiology is beautiful in the multide of backup measures that are in play when one component of the system fails, which can be concluded from the results of our study.

Future Directions and Limitations

One limitation is the use of anesthesia, which depresses the response of the organism, in this case Sprague Dawley rats, to drug administration. Urethane specifically, should not be used in the future to study cardiovascular responses in rats due to its inhibition of drugs involved with the heart. (Armstrong, Lefevre-Borg, Scatton, & Cavero, 1982).

Another improvement to be considered is utilizing more representative models of the human cardiovascular system. The disadvantages of using rodents lie in the small size of the heart and their extremely high basal heart rate. A potential more appropriate substitute for this study may be to use canine models, which are similar at the organ and cellular level, specifically beneficial in the similar rate of action potential firing and duration which are important due to the baroreflex directly affecting the excitation of the heart (Milani-Nejad & Janssen, 2014).

References

Armstrong, J., Lefevre-Borg, F., Scatton, B., & Cavero, I. (1982). Urethane inhibits cardiovascular responses mediated by the stimulation of alpha-2 adrenoceptors in the rat. *Journal of Pharmacology and Experimental Therapeutics, 223*(2), 524-535.

Atlas, S. A. (2007). The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. *J Manag Care Pharm, 13*(8 Suppl B), 9-20. doi:10.18553/jmcp.2007.13.s8-b.9

Heesch, C. M. (1999). Reflexes that control cardiovascular function. *Advances in Physiology Education, 277*(6), S234.

Heusser, K., Tank, J., Luft, F. C., & Jordan, J. (2005). Baroreflex failure. *Hypertension, 45*(5), 834-839.

Kawada, T., Li, M., Zheng, C., & Sugimachi, M. (2016). Acute Effects of Vagotomy on Baroreflex Equilibrium Diagram in Rats with Chronic Heart Failure. *Clinical Medicine Insights. Cardiology, 10*, 139-147.

Kieval, R. S., Bettett, T. D., & Fitts, S. M. (2000). Baroreflex modulation with carotid sinus nerve stimulation for the treatment of heart failure. In: Google Patents.

Mets, B. (2016). Should norepinephrine, rather than phenylephrine, be considered the primary vasopressor in anesthetic practice? *Anesthesia & Analgesia, 122*(5), 1707-1714.

Milani-Nejad, N., & Janssen, P. M. L. (2014). Small and large animal models in cardiac contraction research: advantages and disadvantages. *Pharmacology & therapeutics, 141*(3), 235-249.

Schachter, S. C., & Saper, C. B. (1998). Vagus nerve stimulation. *Epilepsia, 39*(7), 677-686.

Tinker, J. H., & Michenfelder, J. D. (1976). Sodium nitroprusside: pharmacology, toxicology and therapeutics. *Anesthesiology, 45*(3), 340-354.