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# Afferent vs. efferent cervical vagal nerve stimulation: effects on blood glucose, insulin, and glucagon concentrations in rats

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# AFFERENT VS EFFERENT CERVICAL VAGAL NERVE STIMULATION: EFFECTS ON BLOOD GLUCOSE, INSULIN, AND GLUCAGON CONCENTRATIONS IN RATS

by

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Thesis Supervisor: Associate Professor Harald M. Stauss

Graduate College
The University of Iowa
Iowa City, Iowa

CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

Erin Elizabeth Meyers

has been approved by the Examining Committee for
the thesis requirement for the Master of Science degree
in Health and Human Physiology at the May 2016 graduation.

Thesis Committee: _____
Vitor Lira, Thesis Chair

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Harald M. Stauss

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Kamal Rahmouni

To my husband Cody, for being with me every step of the way, even when we are thousands of miles apart. To my parents and siblings, for their unwavering support.

## ACKNOWLEDGEMENTS

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## ABSTRACT

Cervical vagal nerve stimulation (VNS) has been studied in the context of several conditions including epilepsy and depression. However, its effects on glucose metabolism, and its potentially beneficial effects in type II diabetes, have not yet been evaluated in humans. Efferent parasympathetic activation reduces hepatic glucose release and increases pancreatic insulin secretion, while afferent parasympathetic activation may increase hepatic glucose release and inhibit insulin secretion potentially through sympathetic activation. Thus, the effect of combined afferent and efferent cervical VNS is difficult to predict. We hypothesized that selective efferent VNS would decrease blood glucose concentration [Glu] and that selective afferent VNS would increase [Glu].

To investigate these potentially contrasting effects of efferent vs. afferent parasympathetic signaling, we recorded [Glu] and serum insulin and glucagon levels before and during 120 min of VNS in anesthetized rats. The nerve was left intact for combined afferent and efferent VNS (n=9) or sectioned proximal or distal from the stimulation electrode for selective efferent (n=8) of afferent (n=7) VNS, respectively.

We found that afferent VNS caused a strong and sustained increase in [Glu] (+108.9±20.9% or +77.6±15.4% after 120 min of combined afferent and efferent VNS or selective afferent VNS) that was not accompanied by an increase in serum insulin concentration. Combined afferent and efferent VNS significantly increased serum glucagon concentration (57.6±23.4% at 120 min of VNS), while selective afferent VNS did not increase glucagon levels. Conversely, selective efferent VNS increased [Glu] only temporarily (+28.8±11.7% at 30 min of VNS). This response coincided with a transient increase in serum glucagon concentration at 30 min of VNS (31.6±8.3%) and a strong

and sustained increase in serum insulin concentration (+71.2±27.0% after 120 min of VNS).

These findings demonstrate that afferent VNS may increase [Glu] by suppressing pancreatic insulin release, while efferent VNS transiently increases [Glu] by stimulating glucagon secretion before reducing levels to or below baseline values by stimulating the release of insulin. Thus, selective efferent VNS may be potentially effective in the treatment of type II diabetes.

## PUBLIC ABSTRACT

Glucose uptake into the cells is impaired in type II diabetes, leading to elevated blood glucose levels. The hormone insulin lowers blood glucose levels by increasing cellular glucose uptake. The hormone glucagon increases blood glucose levels through increased glucose release from the liver. Nerves in the body send signals about glucose homeostasis from the peripheral organs to the brain, where these signals are processed and then returned to peripheral organs to maintain glucose homeostasis. We investigated the potentially differential effects of selectively activating nerve fibers traveling from the peripheral organs to the brain vs. nerve fibers traveling from the brain to the peripheral organs on glucose homeostasis. Therefore, we electrically stimulated either the central or peripheral end of the cut vagus nerve in anesthetized rats and measured glucose, insulin, and glucagon blood concentrations.

Stimulating fibers running from the periphery to the brain strongly increased blood glucose concentration. Interestingly, this was not associated with an increase in insulin that would normally be seen when glucose levels increase, suggesting that insulin secretion is suppressed in this condition. Stimulating fibers traveling from the brain to the periphery transiently increased blood glucose concentration before returning to or below baseline levels. This response was associated with a strong increase in insulin secretion. These findings are important because they may potentially lead to novel treatment strategies for type II diabetes based on electrical stimulation of nerve fibers traveling in the vagus nerve from the brain to the periphery.

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# CHAPTER I INTRODUCTION

## The Vagus Nerve

The vagus nerve, or cranial nerve X, is the longest cranial nerve in the human body and originates in the medulla oblongata (20). It is a mixed nerve, composed of roughly 80% afferent fibers and 20% efferent fibers (17). The afferent sensory fibers project to the nucleus of the tractus solitarius (NTS), with cell bodies residing in the nodose ganglion (19). These fibers relay sensory information from the respiratory tract, heart, esophagus, gastrointestinal tract, liver, and pancreas, as well as afferent taste information (8). The efferent motor fibers originate from the dorsal motor nucleus and nucleus ambiguus. Fibers from the dorsal motor nucleus send information to the esophagus, gastrointestinal tract, lungs, heart, and pancreas; fibers from the nucleus ambiguus supply somatic motor output to the pharynx and larynx and parasympathetic output to the heart (19).

The majority of fibers in the vagus nerve are unmyelinated. According to Foley and DuBois, 10-20% of all afferent fibers and 48-71% of all efferent fibers are myelinated (17). Nerve fibers of the vagus can be classified as A-, B-, or C-fibers (16). A-fibers are further subdivided depending on size and conduction velocity, but all of these are myelinated. The largest A-fibers range from 13-22 μm in size and 80-120 m/s in conduction velocity and carry somatic afferent and efferent information. The smallest A-fibers range from 1-5 μm in size and 3-30 m/s conduction velocity and conduct visceral afferent information (52). B-fibers are also myelinated; they are 1-5 μm in size, conduct at a velocity of 3-15 m/s, and provide efferent parasympathetic preganglionic innervation (52). Finally, C-fibers are unmyelinated, range from 0.4-2 μm in size and 0.5-2 m/s

conduction velocity, and primarily carry afferent visceral information (52). With stimulation of the vagus nerve, the larger A- and B- fibers will be activated at lower stimulation intensities while the smaller C-fibers have a higher activation threshold (20).

As stated previously, the vagus nerve sends efferent parasympathetic activity to a variety of visceral organs and monitors these structures via afferent sensory fibers. The most relevant organs to this study include the heart, liver, and pancreas. Parasympathetic stimulation to the heart decreases heart rate. This effect is caused by B-fibers originating from the nucleus ambiguus and C-fibers originating from the dorsal motor nucleus (36). The C-fibers evoke changes in heart rate that are smaller in magnitude and have a less rapid onset than those evoked by B-fibers (26).

In the liver, efferent activation of the vagus nerve promotes the formation of glycogen by activating glycogen synthetase (48). Parasympathetic innervation of the liver also reduces hepatic glucose release (41). These two mechanisms can function to reduce glucose concentrations in the blood. The liver is also innervated by afferent fibers of the vagus nerve as shown by retrograde tracing techniques using horseradish peroxidase (32). Niijima et al. found that these afferent hepatic vagal fibers were sensitive to the glucose concentration in the portal vein (35). When glucose concentration was high, the afferent discharge rate of the fibers decreased. The increased firing rate when glucose levels are low may initiate food intake by signaling to the central nervous system (25).

In addition to vagal effects on glucose metabolism in the liver, preganglionic efferent fibers of the vagus nerve also synapse on postganglionic nerves innervating the islets of Langerhans (10). Stimulation of the subcardiac thoracic vagus nerve has been shown to increase the release of both insulin and glucagon from the pancreas in animal

models (2, 21). However, this response is dependent on glucose concentration; with efferent stimulation, high glucose levels caused greater insulin release while low glucose levels caused greater glucagon release (21). The islet cells are also innervated by afferent vagal neurons as identified by retrograde tracing techniques (34). These afferent neurons, which project to the NTS via the nodose ganglion, are sensitive to insulin and may convey changes in insulin levels to the central nervous system (23).

## Clinical Vagal Nerve Stimulation

Early animal studies have shown that cervical vagal nerve stimulation (VNS) alters brain activity (20). In particular, Zabara found that cervical VNS suppresses seizures and tremors in canines, and the inhibition of the seizure activity exceeded the length of the stimulation period (53). Based on these studies, the first human vagal nerve stimulator was implanted for the treatment of seizures in 1988 (39). Out of these first 11 patients, five reported a reduction in seizures, with two patients being completely seizure free. Less than ten years later, cervical VNS was FDA approved for the treatment of therapy-refractory epilepsy (37).

Patients receiving VNS treatment for epilepsy also demonstrated improved moods, independent of seizure activity (15), leading researchers to investigate the effects of VNS on depression. Rush et al. conducted the first experiment specifically studying the effects of VNS in patients with severe, treatment resistant depression (44). After only 10 weeks of VNS, 40% of the patients had improved moods. In July 2005, the FDA approved the use of VNS for treatment-resistant depression (37).

In addition to its anticonvulsant and antidepressant effects, VNS has also been shown to elicit weight loss. Pardo et al. and Burneo et al. demonstrated that VNS is

associated with a substantial weight loss in patients treated with chronic VNS for treatment resistant depression and epilepsy, respectively (12, 38). Importantly, the reduction in weight is directly proportional to the patient's initial body mass index. Studies conducted in experimental animal models of obesity have also confirmed the weight reducing effects of cervical VNS (6, 11, 51).

These physiological responses of VNS depend on the stimulation parameters being employed. In patients, low stimulation parameters are used when VNS is first activated to ensure that patients can tolerate it. The parameters are then gradually increased until a maximum tolerable level is achieved (20). Typical stimulation parameters range from 20-30 Hz frequency, 0.25-0.5 ms pulse width, and 1.0-1.5 mA (up to a maximum of 3.5 mA) current (29). As stated previously, A-, B-, and C-fibers have differing activation thresholds. Low stimulation of the A- and B-fibers causes EEG synchronization, whereas stronger stimulation, which also recruits C-fibers, causes desynchronization of the EEG (20). This shows that the central effects of VNS depend on the stimulation parameters.

## Blood Glucose Regulation by the Liver and Pancreas

In healthy individuals, blood glucose concentration is tightly regulated by neuronal, hormonal, and nutritional signals, and two main organs involved in glucose regulation are the liver and pancreas. When blood glucose concentration is high, such as after a meal, glucose will be taken up in the liver and stored as glycogen. This process is called glycogenesis and is controlled by the enzyme glycogen synthase (43). When blood glucose concentration is low, glycogen will be broken down into glucose through a process known as glycogenolysis, which is controlled by the enzyme glycogen

phosphorylase (43). Depending upon the blood glucose concentration, the activity of these enzymes can be upregulated or downregulated by a variety of factors. For example, as stated previously and shown in Fig. 1, the parasympathetic nervous system promotes the formation of glycogen by activating glycogen synthase, while the sympathetic nervous system promotes the breakdown of glycogen by activating glycogen phosphorylase (47).

In addition to autonomic regulation, hormonal signals also regulate these processes. Insulin increases glucose uptake by the liver and stimulates glycogen synthase. Glucagon and catecholamines, however, increase blood glucose levels by activating glycogen phosphorylase and initiating glycogenolysis (43). Glucocorticoids and growth hormone can also increase blood glucose concentration by decreasing glucose uptake from cells and stimulating gluconeogenesis, a process that synthesizes glucose from amino acids, glycerol, pyruvate, and lactate (43).

The islets of Langerhans in the pancreas also play an important role in glucose metabolism, as they secrete insulin and glucagon. Blood glucose concentration is the main regulator of insulin and glucagon; however, other regulators can be involved. As stated earlier and shown in Fig. 1, the parasympathetic nervous system increases the secretion of insulin and glucagon, while the sympathetic nervous system inhibits the release of insulin and stimulates glucagon release (1-3, 21, 22). In addition to autonomic regulation, nutritional signals such as increased plasma amino acid levels can also stimulate insulin release (7). Incretins, a group of hormones secreted from intestinal cells in response to food ingestion, can also increase insulin release and inhibit glucagon

release (4). Two well-known incretins are glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) (7).

While glucose concentration is tightly regulated in healthy individuals, disturbance of glucose maintenance can lead to the development of type II diabetes. Type II diabetes is caused by having chronically elevated levels of blood glucose due to reduced glucose uptake from the bloodstream into cells. This reduced glucose uptake is due to the development of insulin resistance, which is closely associated with obesity (46). Initially, the pancreatic β-cells will increase the production of insulin to compensate for the cells' reduced sensitivity. However, over time, β-cells will begin to fail, and insulin production will decline. Glucotoxicity, lipotoxicity, inflammation, and apoptosis may all contribute to β-cell dysfunction and loss of mass (18). Lausier et al. demonstrated that the vagus nerve contributes to β-cell proliferation (30). Therefore, vagal nerve stimulation may prevent the decline in pancreatic β-cell function associated with type II diabetes.

## Present Study

Since the implantation of the first vagal nerve stimulator in 1988, cervical VNS has been studied in the context of several conditions including epilepsy, depression, anxiety, Alzheimer's disease, and migraines (20). However, its effects on glucose metabolism, and possibly type II diabetes, have not yet been evaluated in humans. This is surprising as the role of efferent parasympathetic activity in reducing blood glucose is relatively well known and finding new optimal treatment strategies for type II diabetes is of upmost importance. One would expect that efferent cervical VNS would decrease blood glucose concentration by inhibiting hepatic glucose release and stimulating the

secretion of insulin from the pancreas as explained above and referred to in Fig. 1. However, with clinical cervical VNS, the vagus nerve is left intact, and both afferent and efferent pathways are stimulated, as evidenced by the central nervous system effects in the treatment of epilepsy and other neurocognitive disorders and the peripheral side effects in these patients such as bronchoconstriction. While efferent cervical VNS may reduce blood glucose concentration, afferent cervical VNS may falsely signal low glucose levels in the portal vein and high insulin concentrations in the pancreas to the brain, which may initiate hepatic glucose release and inhibition of insulin secretion, causing an increase in blood glucose concentration as illustrated in Fig. 1. Since both of these pathways may be stimulated with cervical VNS, the net effect of combined afferent and efferent stimulation is unknown.

To investigate the potentially contrasting effects of efferent vs. afferent cervical VNS, and to determine the net effect of combined afferent and efferent stimulation, we studied the effects of combined afferent and efferent cervical VNS, selective afferent VNS, and selective efferent VNS on glucose metabolism in an acute study in anesthetized rats. We hypothesized that efferent cervical VNS would decrease blood glucose concentration by inhibiting hepatic glucose release and stimulating pancreatic insulin secretion, while afferent cervical VNS would increase blood glucose concentration by increasing hepatic glucose and pancreatic glucagon release and inhibiting pancreatic insulin secretion. As an experimental approach, we recorded blood glucose concentration and serum insulin and glucagon levels before and during 120 min of continuous cervical VNS with the vagus nerve left intact (for combined afferent and efferent VNS) or

sectioned proximal or distal from the stimulation electrode for selective efferent or afferent VNS, respectively.

# CHAPTER II METHODS

## Animals

Experiments were performed in male normotensive Sprague-Dawley rats at an age of 120±4 days (395±9 g body wt). Rats were housed in clear plastic cages, and temperature and light periods (12-h light-dark cycle; light on between 6:00 AM and 6:00 PM) were controlled. A standard rat chow and tap water were provided ad libitum. Experiments were approved by the Institutional Animal Care and Use Review Committee of the University of Iowa.

## Instrumentation

Rats were first anesthetized using 5% isoflurane in room air. The fur was shaved from the neck and inner leg regions, and rats were then placed on a warming pad to maintain body core temperature during instrumentation and experimental protocols. Anesthesia was then switched to 2.25-3% isoflurane in room air for instrumentation of the animal. First, an incision was made in the right inguinal area, and a telemetric glucose sensor (HD-XG, DSI, St. Paul, MN) was inserted into the femoral artery and forwarded into the abdominal aorta to continuously monitor blood glucose concentration. Then, to record arterial blood pressure and heart rate, a catheter was inserted in the left common carotid artery through a midline neck incision. The right cervical vagus nerve was then isolated from the right common carotid artery, and coil-shaped bipolar stimulation electrodes were wrapped around the nerve. Silicon elastomer (Kwik-Sil, World Precision Instrument, Inc., Sarasota, FL) was applied to the nerve and electrodes to electrically insulate the area from surrounding tissue. Finally, a rectal thermometer was inserted to ensure the animal's core temperature was properly maintained.

## Experimental Protocol

After instrumentation, anesthesia was maintained at 1.2% to 1.5% isoflurane in oxygen. Blood pressure and heart rate were recorded by connecting the arterial catheter to a pressure transducer (P23 ID, Gould-Statham, Oxnard, CA) and amplifier (Series 4000, Gould, Inc., Cleveland, OH). The output of the amplifier was connected to an A/D-converter (ADUSB4CH, Harald Stauss Scientific, Iowa City, IA) for recording with the WinAD module of the freely available HemoLab software suit (http://www.haraldstauss.com/HaraldStaussScientific/hemolab) at a sampling rate of 500 Hz. Blood glucose concentration was recorded using the Dataquest A.R.T. software (Version 4.35, DIS, Saint Paul, MN).

Three experimental conditions were utilized in this study: combined afferent and efferent stimulation, selective afferent stimulation, and selective efferent stimulation. The vagus nerve was left intact in nine animals for combined afferent and efferent stimulation. For selective afferent stimulation, the vagus nerve was sectioned distal from the stimulation electrodes in seven animals. Finally, in eight animals, the vagus nerve was sectioned proximal from the stimulation electrodes for selective efferent stimulation.

The experimental protocol is shown in Fig. 2. After establishing stable baseline conditions, continuous VNS was initiated with a frequency of 5 Hz, voltage of 3 V, and pulse width of 1 ms. These parameters were chosen, since they represent the lowest stimulation intensity that resulted in an immediate and consistent bradycardic response in prior pilot experiments. After two hours of stimulation, the stimulator was turned off, and the animals were euthanized. To measure plasma insulin, glucagon, and glucose

concentrations, blood samples were obtained from the arterial line at baseline before VNS and at 30 min and 120 min after the start of VNS.

## Biochemical Analyses

Blood glucose concentrations were determined using a TRUEtrack glucose meter (Nipro Diagnostics, Fort Lauderdale, FL). The implanted telemetric blood glucose sensor provides an electrical current proportional to glucose concentration. The glucose values obtained from the TRUEtrack glucose meter were used to calibrate this electrical current into glucose concentration values. Serum insulin and glucagon concentrations were quantified using commercially available ELISA kits (Kit #90010 for insulin and Kit #81505 for glucagon, CrystalChem, Downers Gove, IL).

## Data Analyses

Blood glucose data collected using the Dataquest A.R.T. software were combined and synchronized in time with the blood pressure and heart rate data collected using the WinAD software and then analyzed together using the Analyzer module of the HemoLab software. For each animal, values for mean arterial blood pressure, heart rate, and blood glucose concentration were extracted at baseline (before VNS), and at 30 min and 120 min of VNS.

## Statistics

Data provided in the text of the manuscript are presented as arithmetic mean values±SEM. Data presented in figures are shown as box-and-whisker plots with the median, quartiles, and extreme values. Separate statistical analyses were performed for each experimental condition (combined afferent and efferent VNS, selective afferent VNS, and selective efferent VNS). Statistical comparison between the three time points

(baseline before VNS and 30 min and 120 min of VNS) were done by one-way analysis of variance for repeated measures with post hoc Fisher tests. Statistical significance was assumed for $P<0.05$.

# CHAPTER III RESULTS

## Major Findings

Fig. 2 shows typical examples of the blood pressure, heart rate, and blood glucose concentration responses to VNS in the three experimental conditions (combined afferent and efferent VNS and selective afferent or efferent VNS). Afferent VNS (either as combined afferent and efferent VNS or as selective afferent VNS) caused a strong and sustained increase in blood glucose concentration. In contrast, with selective efferent VNS blood glucose concentration increased only temporarily and returned to baseline or even decreased by more than 10 mg/dL below baseline in 5 out of 8 animals after 120 min of VNS.

## Hemodynamic Responses to VNS

Arterial blood pressure and heart rate were recorded to verify the responsiveness of VNS through an immediate bradycardic response (as shown in Fig. 2) and to ensure stable cardiovascular conditions during anesthesia. No significant changes in mean blood pressure were observed in either the combined afferent and efferent VNS or selective afferent VNS conditions at 30 min or 120 min of VNS (Fig. 3, top). However, with selective efferent VNS, a small decrease in blood pressure was observed after 120 min of VNS. After the immediate bradycardic response when the stimulator was turned on, heart rate slowly returned to baseline levels in all three experimental conditions. With selective afferent and selective efferent VNS, heart rate had returned to baseline by 30 min of VNS; however, with combined afferent and efferent VNS, heart rate had only returned to its baseline level by 120 min of VNS (Fig 3, bottom).

## Blood Glucose Regulation during VNS

In both the combined afferent and efferent VNS and selective afferent VNS conditions, blood glucose concentration significantly increased by 82.7±18.9% and 46.8±10.0%, respectively, after 30 min of VNS and remained elevated until VNS was terminated after 120 min at which time blood glucose concentration had increased from baseline by 108.9±20.9% and 77.6±15.4%, respectively (Fig. 4, top). In contrast, selective efferent VNS increased blood glucose concentration only temporarily (+28.8±11.7% at 30 min) followed by a return to baseline levels (Fig. 4, top).

Despite the substantial increase in blood glucose concentration, insulin plasma levels did not increase significantly with combined afferent and efferent VNS or selective afferent VNS, suggesting that afferent VNS suppressed insulin release in these experimental conditions (Fig. 4, middle). In contrast, with selective efferent VNS, insulin levels increased throughout the 120 min of VNS (+57.1±17.4% at 30 min and +71.2±27.0% at 120 min), and this increase in insulin plasma levels reached statistical significance at 120 min.

Plasma glucagon concentration significantly increased by 72.1±14.5% after 30 min of combined efferent and afferent VNS and remained significantly elevated after 120 min of VNS (+57.6±23.4%, Fig. 4, bottom). Selective afferent VNS did not significantly increase plasma glucagon concentration, although a trend towards a temporary increase at 30 min of VNS (+33.6±24.3%) was observed. With selective efferent VNS, plasma glucagon concentration significantly increased temporarily at 30 min into the protocol (+31.6±8.3%, Fig. 4, bottom).

# CHAPTER IV DISCUSSION

The primary focus of this study was to examine the potential contrasting effects of afferent vs. efferent cervical VNS on glucose metabolism. Two important findings were discovered. First, afferent cervical VNS (combined afferent and efferent or selective afferent) caused a strong and sustained increase in blood glucose concentration that was not accompanied by an increase in serum insulin concentration. This suggests that insulin secretion is inhibited with afferent VNS, which could contribute to the hyperglycemic response seen in this condition. Second, selective efferent cervical VNS reduced blood glucose concentration below baseline in 5 out of 8 animals after 120 min of VNS and was associated with increased insulin levels. This finding suggests that selective efferent cervical VNS could be potentially useful in the treatment of diabetes.

## Control of Glucose Metabolism

Combined afferent and efferent VNS caused a strong and sustained increase in blood glucose concentration without stimulating insulin secretion. This response was mirrored with selective afferent VNS, but not selective efferent VNS, suggesting that the hyperglycemic response can be attributed to the afferent stimulation. As elevated glucose concentrations are known to stimulate insulin release from the pancreatic β-cells, the lack of insulin secretion with afferent VNS is surprising and signifies that insulin secretion is inhibited by afferent VNS. This process may be explained by a negative feedback loop. As stated previously, the pancreatic islet cells are innervated by vagal afferent fibers. These afferent fibers, which project to the NTS via the nodose ganglion, are sensitive to insulin and may convey changes in insulin levels to the central nervous system (23). Therefore, by electrically stimulating the vagus nerve, the brain may falsely interpret that

insulin levels are elevated. The NTS sends projections to hypothalamic areas such as the arcuate and paraventricular nuclei (PVN), which are involved in appetite and autonomic nervous system regulation (33, 42). Neurons from the PVN project to the rostral ventrolateral medulla (RVLM) and intermediolateral cell column of the spinal cord (IML) where preganglionic sympathetic neurons originate (5, 40, 45, 49). Activation of this pathway may stimulate the sympathetic splanchnic nerve, which has been shown to inhibit the secretion of insulin from pancreatic islet cells (1, 3, 22). To summarize, insulin secretion may be inhibited by afferent cervical VNS by falsely portraying elevated insulin levels to the CNS and activating a compensatory sympathetic mechanism to the pancreas to lower insulin release. In addition to this possible negative feedback loop, insulin may also be inhibited by afferent parasympathetic information from the liver. Lee and Miller examined this mechanism by electrically stimulating the central end of the sectioned hepatic vagus nerve in rats and found that insulin secretion was suppressed (31).

While the suppression of insulin may contribute to the sustained elevation of blood glucose concentration with afferent VNS, other factors may explain the initial increase in blood glucose levels observed in all three experimental conditions, such as glucagon secretion and hepatic glucose release. With efferent VNS, either selective or in combination with afferent VNS, the initial increase in blood glucose concentration may be attributed to pancreatic glucagon secretion. In both of these conditions, serum glucagon concentrations were significantly elevated. This result is consistent with prior studies that show parasympathetic stimulation to the pancreas increases the release of glucagon (2, 9, 21). However, while combined afferent and efferent VNS significantly increased serum glucagon levels at 30 min and sustained the elevation throughout the

whole 120 min of VNS, selective efferent VNS only transiently increased glucagon concentration. This transient nature can be explained by the large increase in serum insulin levels seen with selective efferent VNS. Studies have shown that insulin inhibits the release of glucagon from pancreatic α-cells (27, 28). Therefore, with selective efferent VNS, blood glucose concentration initially increases due to increased glucagon release but then returns to baseline levels after insulin secretion increases and inhibits glucagon release. With combined afferent and efferent VNS, the suppression of insulin secretion by afferent VNS removes the inhibition of glucagon by insulin, and blood glucose concentration remains elevated throughout the 120 min of VNS.

An elevated serum glucagon concentration most likely explains the initial increase in blood glucose concentration seen with combined afferent and efferent VNS and selective efferent VNS. However, the increase in blood glucose levels with selective afferent VNS was not associated with an increase in glucagon concentration, which implies that other factors must contribute to the rise in glucose. One such mechanism could be hepatic glucose release, as many studies have shown that sympathetic innervation of the liver stimulates the release of glucose (14, 24, 47, 50). Afferent cervical VNS may activate the sympathetic nervous system as outlined previously (5, 40, 45, 49). However, while sympathetic activation does increase glucose release from the liver, Järhult et al. found that the hepatic glucose contribution was minor compared to the hyperglycemia induced by combined stimulation to both the pancreas and liver. This hyperglycemic response was also associated with a decrease in serum insulin concentration (24). Based on these findings, selective afferent VNS likely increases blood glucose concentration by activating efferent sympathetic pathways to both the liver and

pancreas, stimulating hepatic glucose release and suppressing pancreatic insulin secretion.

## Limitations

One limitation of this study is that it was performed in an anesthetized state using isoflurane. Desborough et al. have shown that 2% isoflurane inhibits insulin secretion from pancreatic β-cells in rats (13). Thus, the inhibition of insulin secretion seen with afferent VNS in our study may partly be due to the isoflurane. However, we kept the isoflurane below 1.5% for each animal, and we still observed an increase in serum insulin concentration with selective efferent VNS. In addition to these factors, we also measured blood glucose concentration in a conscious spontaneously hypertensive rat in a different study investigating the effects of chronic VNS on hypertension-induced cardiovascular end-organ damage. In this animal, the stimulator was programmed to automatically turn on or off every hour for a two hour cycle length. Blood pressure and heart rate were recorded to determine when VNS was on or off (Fig. 5). When VNS was turned on, mean blood pressure and heart rate decreased; when VNS was turned off, mean blood pressure and heart rate increased. Blood glucose concentrations at the end of the one-hour stimulation periods varied between 134 mg/dL and 138 mg/dL, while the glucose levels at the end of the one-hour periods without stimulation varied between 101 mg/dL and 106 mg/dL. These preliminary data indicate that combined afferent and efferent VNS increases blood glucose concentration not only in the anesthetized state, where insulin secretion may be inhibited by the anesthesia, but also in the conscious state.

## Conclusions

This study demonstrates the contrasting effects of afferent vs. efferent cervical VNS. Selective afferent VNS increases blood glucose concentration by potentially stimulating hepatic glucose release and inhibiting pancreatic insulin secretion (Fig. 6). Selective efferent VNS only transiently increases blood glucose concentration before reducing levels to or below baseline values by stimulating the release of insulin and potentially inhibiting hepatic glucose release (Fig. 6). Combined afferent and efferent VNS closely resembles the hyperglycemic response seen with selective afferent VNS. Thus, patients chronically treated with cervical VNS (combined afferent and efferent stimulation) for the treatment of conditions such as epilepsy, depression, and anxiety may be at risk for developing type II diabetes. Future studies are needed to investigate the potential impact of cervical VNS on glucose metabolism in these patients. Also, the elevated insulin levels observed with selective efferent VNS warrant further investigations to determine if selective efferent stimulation of the intact vagus nerve in patients is technically feasible and if such an intervention could be useful in the treatment of insulin resistance, metabolic syndrome, or type II diabetes. Finally, these future studies should examine alternative placements of the stimulation electrodes, such as on the subdiaphragmatic segment of the vagus nerve, to avoid the bradycardic response observed with cervical VNS.

APPENDIX-FIGURES

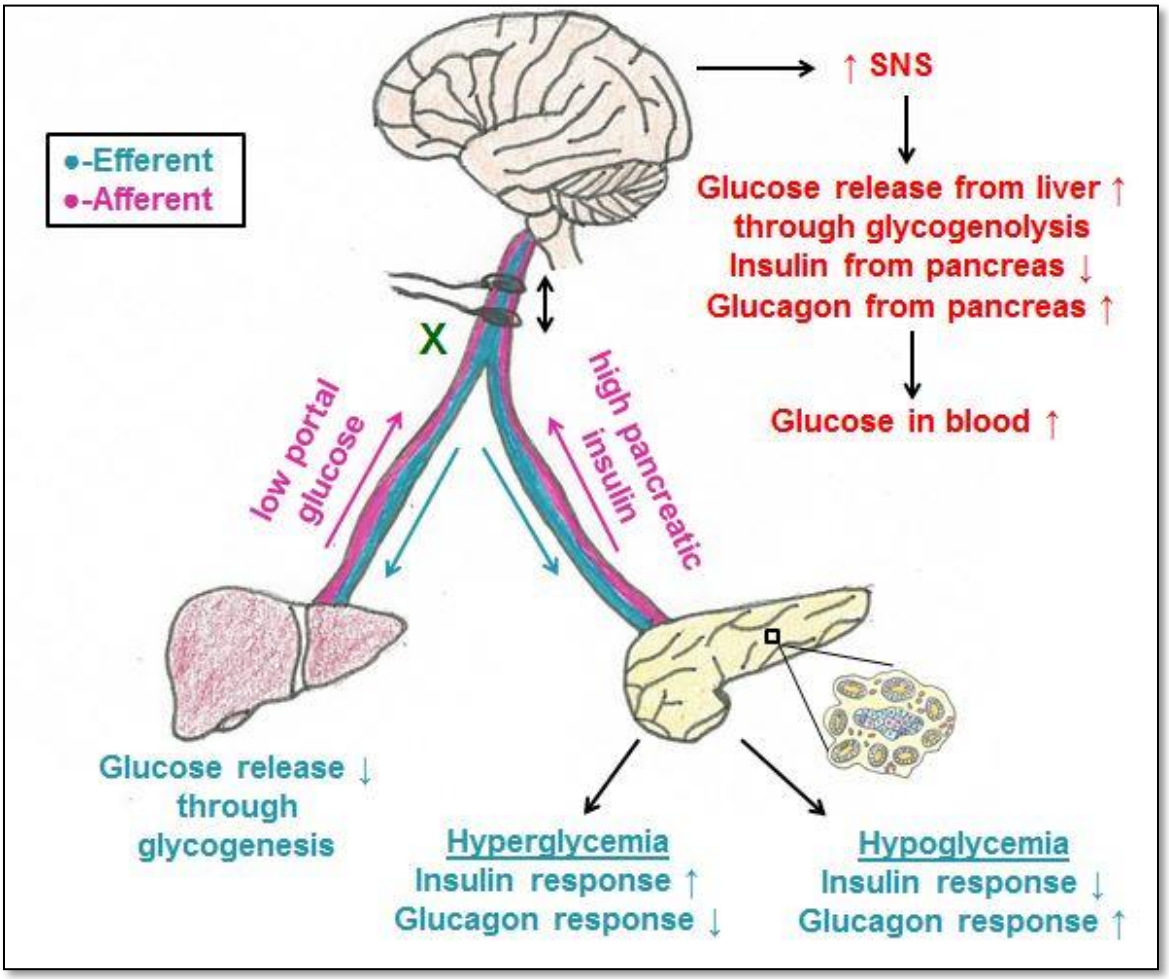


Figure A-1: Autonomic control of glucose regulation. Efferent parasympathetic activation (shown in blue) decreases blood glucose by stimulating glycogenesis in the liver and secretion of insulin from the pancreas. Afferent parasympathetic activation (shown in pink) signals low portal glucose levels and high pancreatic insulin levels to the brain, which could potentially stimulate a compensatory sympathetic response (shown in red) to increase blood glucose concentration. SNS: sympathetic nervous system.

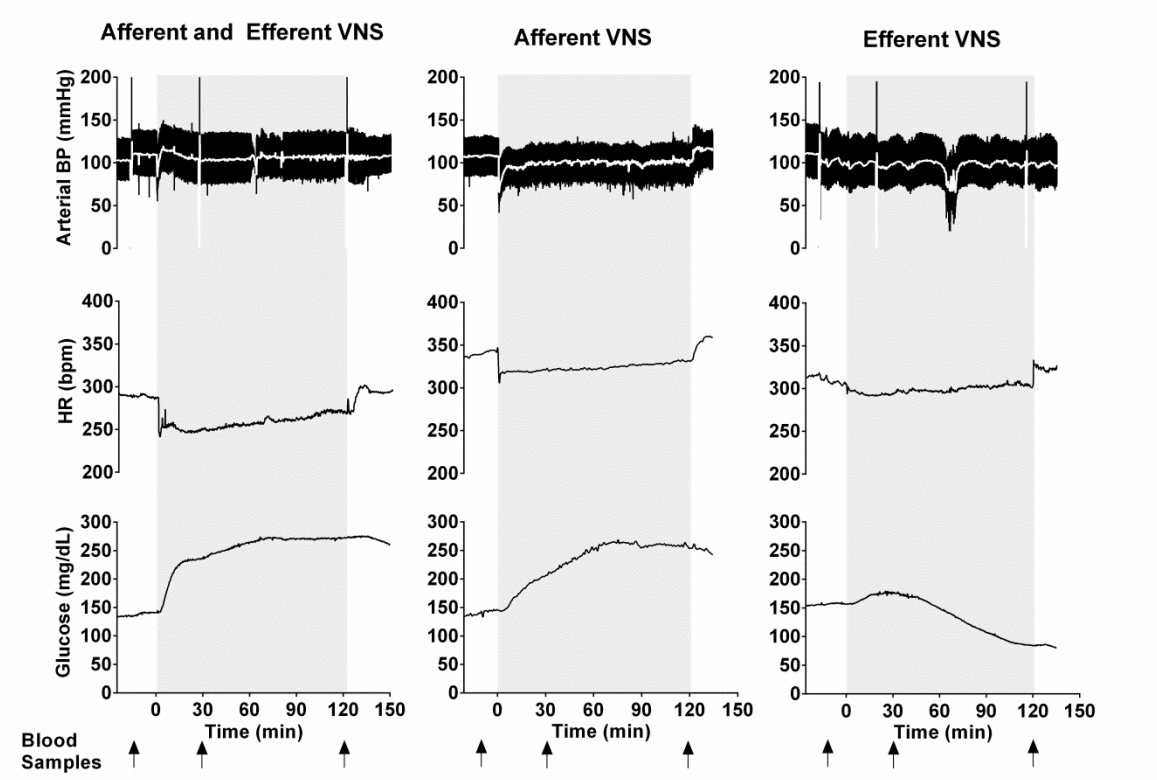


Figure A-2: Typical responses to combined afferent and efferent vagal nerve stimulation (VNS, left) and to selective afferent (middle) or efferent (right) VNS. From top to bottom, recordings of arterial blood pressure (BP), heart rate (HR), and blood glucose concentration are shown. Blood samples for glucose, insulin and glucagon measurements were taken at baseline before initiation of VNS and at 30 min and 120 min of VNS (arrows).

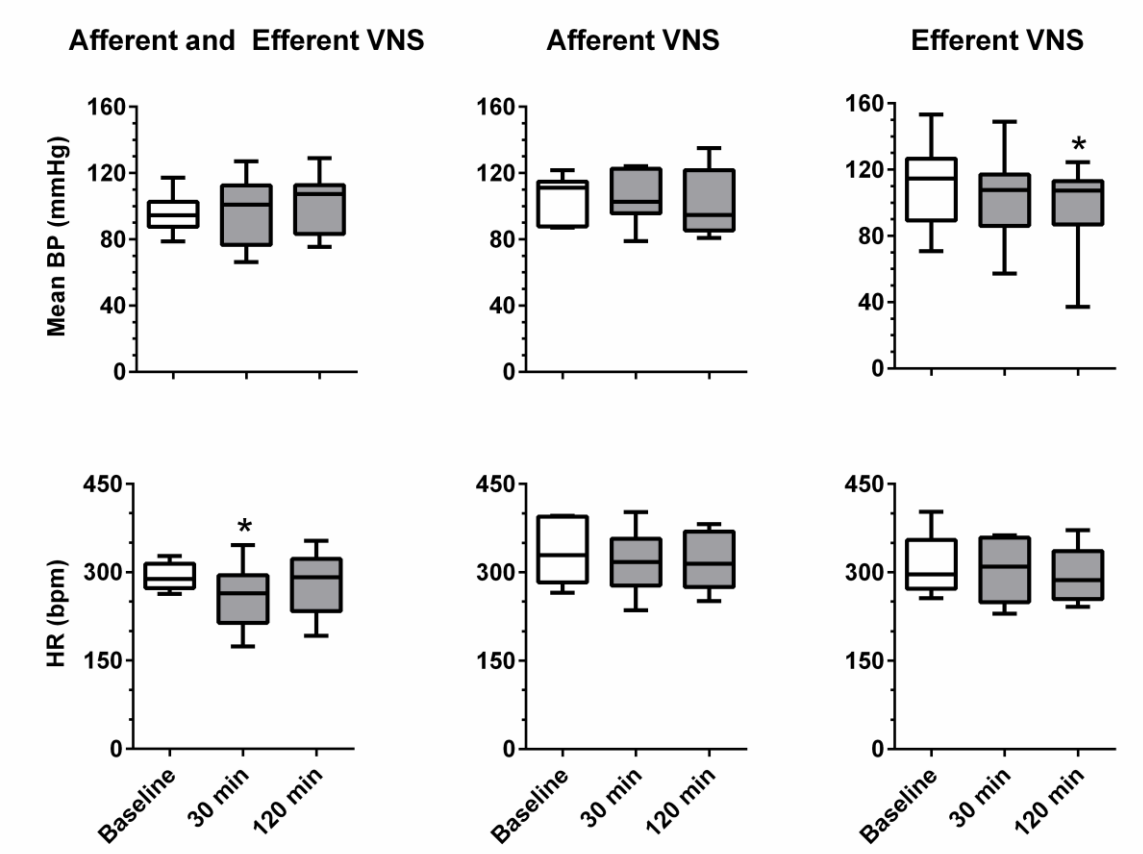


Figure A-3: Mean arterial blood pressure (BP) and heart rate (HR) before vagal nerve stimulation (VNS, Baseline) and at 30 min and 120 min after initiation of combined afferent and efferent VNS (left, n=9) and selective afferent (middle, n=7) or efferent (right, n=8) VNS. *: $p<0.05$ vs. Baseline.

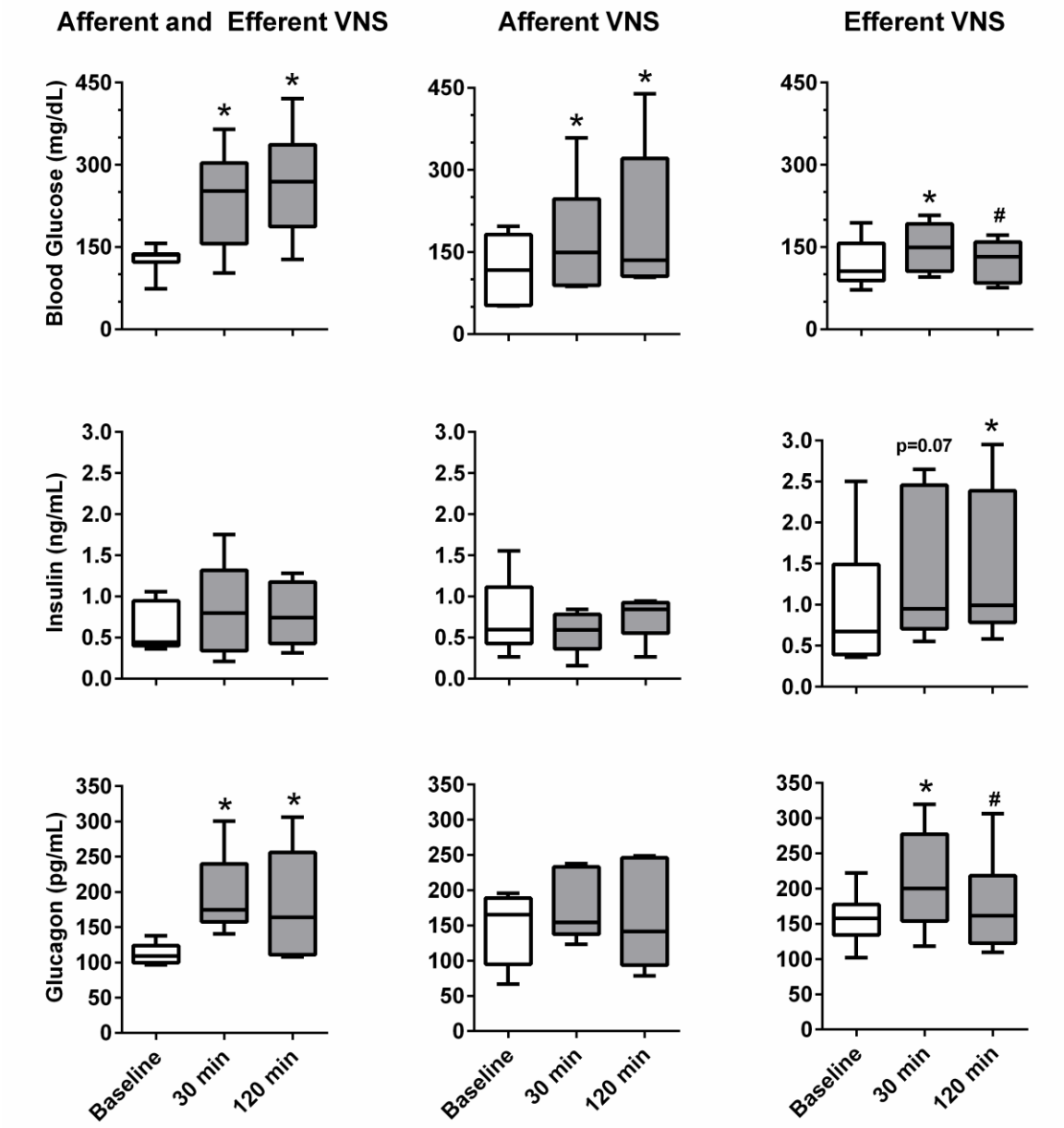


Figure A-4: Blood glucose concentration (top) and plasma insulin (middle) and glucagon (bottom) concentrations before vagal nerve stimulation (VNS, Baseline) and at 30 min and 120 min after initiation of combined afferent and efferent VNS (left, n=9 for glucose, n=5 for insulin and glucagon) and selective afferent (middle, n=7 for glucose, n= 5 for insulin and glucagon) or efferent (right, n=8 for glucose, n=6 for insulin and glucagon) VNS. *: $p<0.05$ vs. Baseline. #: $p\leq0.05$ vs. 30 min.

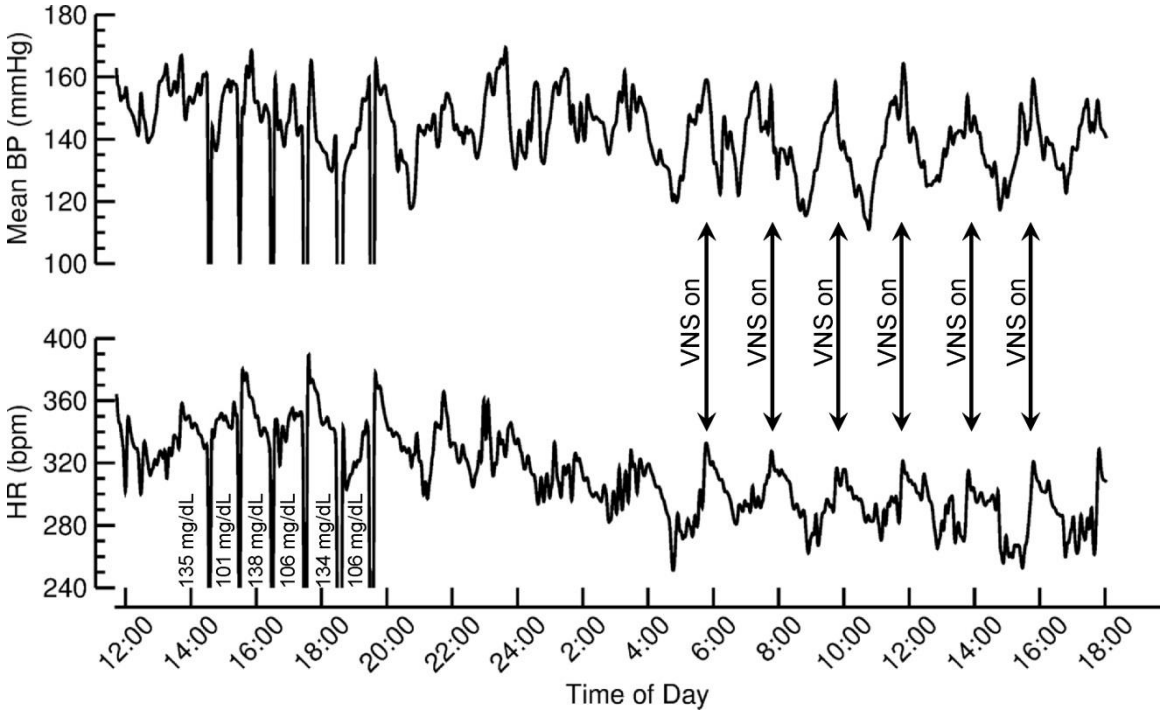


Figure A-5: Original recording of mean blood pressure (BP) and heart rate (HR) in a conscious spontaneously hypertensive rat during periodic vagal nerve stimulation (VNS) with cycles of one hour of VNS (charge balanced impulses of 6 V and 1 ms pulse width at 5 Hz stimulation frequency) followed by one hour without VNS. The stimulator was on during all "even hours" (e.g., 6:00 AM to 7:00 AM) and off during all "odd hours" (e.g., 7:00 AM to 8:00 AM). Between 2:00 PM and 8:00 PM hourly blood glucose concentrations were obtained using the TRUEtrack glucose meter (Nipro Diagnostics, Fort Lauderdale, FL). At the end of the "even hours" (after an hour of VNS) blood glucose readings were 135 mg/dL, 138 mg/dL, and 134 mg/dL, while at the end of the "odd hours" (after on hour without VNS) the blood glucose readings were 101 mg/dL, 106 mg/dL, and 106 mg/dL.

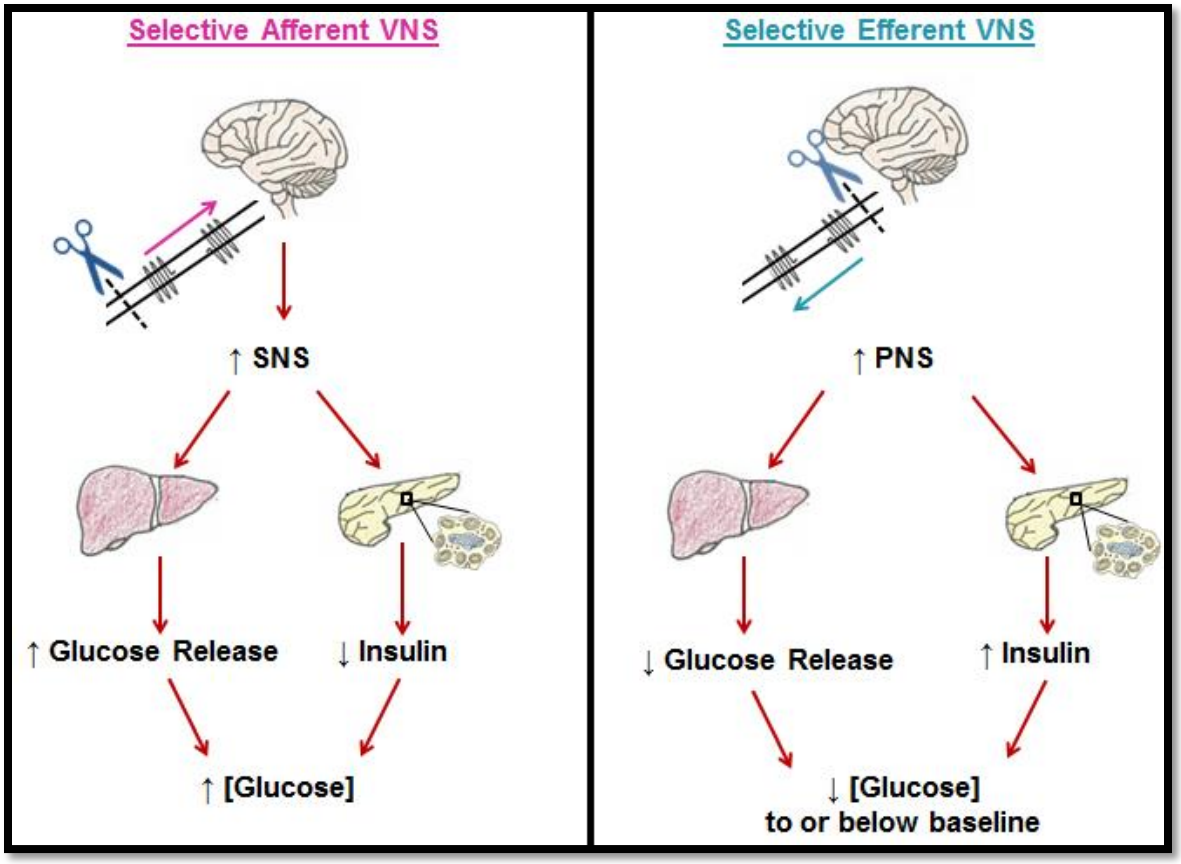


Figure A-6: Conclusions. Selective afferent vagal nerve stimulation (VNS) increased blood glucose concentration potentially by inhibiting pancreatic insulin secretion and increasing hepatic glucose release. Selective efferent VNS decreased glucose levels to or below baseline values by stimulating insulin secretion and potentially inhibiting hepatic glucose release. SNS: sympathetic nervous system. PNS: parasympathetic nervous system.

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