Effects of Chronic Vagus Nerve Stimulation on Cardiac Function in Obese-Insulin Resistant Rats

ผลของการกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังต่อการทำงานของหัวใจในหนูอ้วนที่มีภาวะดื้อต่อฮอร์โมนอินซูลิน

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ABSTRACT

Long-term high-fat diet (HFD) consumption leads to not only obese-insulin resistance, but also cardiac dysfunction and decreased parasympathetic activity. Recently, several studies indicate that increased parasympathetic activity by vagus nerve stimulation (VNS) exerts cardioprotective benefits. However, the effect of chronic VNS on the heart in obese-insulin resistant condition has never been investigated. We hypothesized that VNS treatment provides the protective effects on the metabolic parameters and cardiac function. Ten rats were fed with high-fat diet for 12 weeks, and then all rats were randomly divided into 2 groups (n=5/group): sham group and VNS group. VNS was applied for 8 weeks. Blood pressure and echocardiography in each animal was examined at 4 and 8 weeks following treatment. Blood samplings were also collected at those time courses for the measurement of glucose and cholesterol levels. The results showed that VNS treatment reduced plasma glucose and plasma total cholesterol levels, decreased blood pressure and increased cardiac function, compared with those in the sham group. These findings suggest that chronic VNS therapy can improve cardiac function, blood pressure and metabolic parameters in obese-insulin resistant rats.

บทคัดย่อ

การรับประทานอาหารที่มีไขมันสูงเป็นระยะเวลานาน ไม่เพียงแต่นำไปสู่การเกิดภาวะอ้วนที่ดื้อต่อฮอร์โมนอินซูลิน แต่ยังส่งผลทำให้เกิดการทำงานของหัวใจที่ผิดปกติและลดการทำงานของระบบประสาทพาราซิมพาเทติก โดยในช่วงเวลาที่ผ่านมาไม่นานมานี้มีหลายๆการศึกษา พบว่าการเพิ่มการทำงานของระบบประสาทพาราซิมพาเทติกโดยการกระตุ้นเส้นประสาทเวกัสนั้นให้ประโยชน์ที่ดีต่อหัวใจ อย่างไรก็ตามผลของการกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังต่อการทำงานของหัวใจในภาวะอ้วนที่ดื้อต่อฮอร์โมนอินซูลินยังไม่มีผู้ทำการศึกษา ผู้วิจัยจึงมีสมมติฐานว่าการกระตุ้นเส้นประสาทเวกัสจะให้ผลที่ดีต่อค่าเมตาบอลิกและการทำงานของหัวใจ โดยหนูจำนวน 10 ตัว จะได้รับอาหารที่มีไขมันสูงเป็นระยะเวลา 12 สัปดาห์ จากนั้นหนูจะถูกแบ่งเป็น 2 กลุ่ม (จำนวน 5 ตัว/กลุ่ม) คือกลุ่มที่ไม่ได้รับการ

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กระตุ้นและกลุ่มที่ได้รับการกระตุ้นเส้นประสาทเวกัส โดยในกลุ่มที่ได้รับการกระตุ้นเส้นประสาทเวกัส จะได้รับการกระตุ้นเป็นเวลา 8 สัปดาห์ ความดันโลหิตและการทำงานของหัวใจจะถูกประเมินในสัปดาห์ที่ 4 และ 8 หลังการกระตุ้นเส้นประสาทเวกัส การเก็บเลือดจะเก็บในช่วงเวลาดังกล่าวข้างต้นเพื่อตรวจวัดระดับกลูโคส และคลอเรสเตอรอล ผลการศึกษาพบว่าการกระตุ้นเส้นประสาทเวกัสสามารถลดระดับกลูโคส คลอเรสเตอรอล ความดันโลหิต และเพิ่มการทำงานของหัวใจเมื่อเปรียบเทียบกับกลุ่มที่ไม่ได้รับการกระตุ้น จากผลการศึกษาดังกล่าวแสดงให้เห็นว่า การกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังสามารถช่วยปรับปรุงการทำงานของหัวใจ ความดันโลหิต และค่าเมตาบอลิกในหนูที่มีภาวะดื้อต่อฮอร์โมนอินซูลิน

Key Words: Chronic vagus nerve stimulation, Obese-insulin resistant

คำสำคัญ: การกระตุ้นเส้นประสาทเวกัสแบบเรื้อรัง ภาวะอ้วนที่ดื้อต่อฮอร์โมนอินซูลิน

Introduction

Currently, the incidence of cardiovascular disease (CVD) is increasing and still is the major cause of death worldwide (Mathers & Loncar, 2006). Moreover, obesity rates have also increased for all population groups in the industrialized countries (Sharma, 2008). Increased fast food consumption and predominance of sedentary life style have led to this high incidence. Obesity is an important factor that can lead to the metabolic syndrome (MetS), which includes insulin resistance, dyslipidemia, hyperglycemia and hypertension (Reaven, 1997). Several conditions in obesity can increase the morbidity and mortality in relation to the development of MetS (Hall et al., 2010). The MetS has been shown to increase a number of pro-inflammatory cytokines, including the abnormal production and secretion of adipokines (Chaudhary, Buddineni, Nistala, & Whaley-Connell, 2011). The increased pro-inflammatory cytokines leads to increased sympathetic activity and increasing the risk of CVD (Ford, Li, Zhao, Pearson, & Mokdad, 2008).

Although pharmacological agents still play a vital role in both MetS and CVD treatments, a growing number of studies have demonstrated the beneficial effects of increasing parasympathetic activity. Parasympathetic fiber can be stimulated by vagus nerve stimulation (VNS) leads to the secretion of acetylcholine (ACh). Thus, VNS has been regarded as a promising novel therapeutic intervention. Implanted VNS has been used as a treatment for epilepsy, seizure and depression for many years (Groves, Bowman, & Brown, 2005). Several studies have suggested that VNS has beneficial effects greatly through the complex interaction, specifically modulated through afferent fibers of vagus nerve (Cadeddu et al., 2010). For the cardiovascular system, the efferent fibers are responsible for controlling heart rate and blood pressure (Groves & Brown, 2005). Now, a growing number of evidence indicates that VNS exerts cardioprotective benefits in several pathological conditions of the heart (De Ferrari et al., 2011). Several studies have shown that VNS can improve left ventricular function in a model of acute myocardial infarction (MI) (Kong et al., 2012). MI reduces the vagal activity which can cause the increased mortality in heart failure (HF) (Walton et al., 2013). In a rat subjected to global ischemia/ reperfusion (I/R) with an intact vagal innervation,

VNS-treated left ventricle (LV) showed significantly high performance throughout the 120 minutes of reperfusion period (Katare et al., 2009). Moreover, VNS-treated heart exhibited a significant improvement in LV developed pressure. Furthermore, in rats subjected to regional ischemia for 30 minutes, VNS during myocardial ischemia exerts negative chronotropic effects (Ando et al., 2005). In another rat model, VNS also significantly decreased heart rate (HR) and mean arterial pressure (MAP) after left anterior descending coronary artery (LAD) ligation for 30 minutes in both adults and aged rats (Wu & Lu, 2011). Since VNS can induce negative chronotropic effects and attenuate LV dysfunction, it may improve progression of myocardial I/R injury by decreasing myocardial oxygen consumption. Interestingly, the study on spontaneously hypertensive rat (SHR) by Heaton et al. suggested that VNS stimulated cardiac parasympathetic function via NO-cGMP signaling pathway (Gil, Bugajski, Kurnik, Zaraska, & Thor, 2009).

CVD is a major health problem that can be caused by MetS, and VNS has been shown to exert cardioprotection. Therefore, the effect of VNS on MetS is an interesting topic. Chronic VNS has been shown to cause weight loss, as a result of its effect on eating behavior and appetite (Gil, Bugajski, & Thor, 2011). Moreover, long-term VNS treatment has been shown to reduce food intake, body weight and mass cells through an increase in vagal afferent satiety signals in obese rats (Ogbonnaya & Kaliaperumal, 2013). Chronic VNS has also been shown to increase serum ghrelin and nesfatin-1 in combination with a reduction of serum leptin, resulting in a decrease in body weight, food intake and epididymal fat pad in obese animals model (Simpson, Shaw, & McNamara, 2011).

Although chronic VNS treatment exerts cardioprotective effect and reduces body weight and food intake in obese subjects, the effect of chronic VNS on the heart in obese-insulin resistant condition has never been investigated. Therefore, in the present study, we determined the effects of chronic VNS treatment on the cardiac function in high-fat diet induced obese-insulin resistant rats.

Objective of the study

The aim of this study was to investigate the effect of chronic VNS on cardiac function, blood pressure changes and metabolic parameters in obese-insulin resistant rats.

Methodology

Animal Preparation

All experiments were conducted in accordance with an approved protocol from the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee, in compliance with NIH guidelines. The present study was used male Wistar rats (body weight 180-200 g, n=10) from the National animal center, Salaya campus, Mahidol University, Bangkok.

Animal were fed with high-fat diet for 12 weeks, after which rats were randomly divided into 2 treatment groups: sham group and VNS group. Rats were treated for 8 weeks. Echocardiography and blood pressure measurement were performed at baseline and at 12 weeks after high-fat diet consumption, and at 4 and 8 weeks after VNS treatment. Blood samples for the measurement of glucose, insulin and cholesterol levels were also

determined at baseline and at 12 weeks after high-fat diet consumption, and at 4 and 8 weeks after VNS treatment.

Vagus nerve stimulation

In VNS group, rats were anesthetized with xylazine (3 mg/kg) and zoletil (50 mg/kg). After hair shaving and skin cleaning, a bipolar cuff electrode was implanted around the left cervical vagus nerve and connected to a custom implantable pulse generator (Demipulse, Model 103, Cyberonics). A period of one week was allowed for recovery from the surgical implantation of the stimulation system. VNS was continuously delivered at a frequency of 20 Hz, 500-µs pulse width, current of 0.5 - 0.75 mA, with a 14-s ON and 48-s OFF. Prior to data collection, VNS therapy was turned off for 5–10 min and remained off for the entire period of the hemodynamic, echocardiographic and blood pressure (BP) measurements.

In the sham group, similar surgical procedure was performed except that the programmed VNS was turn off.

High fat diet preparation

The high-fat diet (59.28% E fat) was prepared by mixing the following ingredients; standard rat diet (365 g/kg food), casein (250 g/ kg food), lard (310 g/kg food), cholesterol (10 g/kg food), vitamins (60 g/kg food), DL-Methionine (3 g/kg food), yeast powder (1 g/kg food) and sodium chloride (1 g/kg food). The mixture was molded into a spherical shape and then refrigerated until utilization (Pratchayasakul et al., 2011).

Echocardiography protocol

Echocardiography is a non-invasive method for the assessment of the cardiac function. Signals from M-mode echocardiography at the level of papillary muscles were recorded. Parameters obtained from echocardiography are (1) IVSs, d= systolic and diastolic interventricular septum, (2) LVIDs, d = systolic and diastolic left ventricular internal dimension, (3) LVPWs, d = left ventricular posterior wall thickness during systole and diastole. Fractional shortening (FS) was calculated using the following formula (Lekawanvijit et al., 2012):

$$FS\% = [(LVIDd - LVIDs)/LVIDd]*100$$

After the investigation, animals were allowed to fully recover and then returned to the cages.

Measurement of blood pressure (BP)

Rats were preheated under infrared warming pad for at least 5 minutes to dilate the tail vein and let them acclimate to the holder. A volume pressure recording (VPR) and occlusion cuff (O-cuff) were attached to the tail. A blood pressure value was achieved from taking an average of 20 consecutive measurements at a steady state (Lekawanvijit et al., 2012).

Determination of plasma insulin level

Plasma insulin level was detected by sandwich ELISA kit (Millipore, MI, and USA). A microplate reader was used for measurement the intensity of enzyme activity at 450 nm. The intensity of absorbance is directly proportion to the amount of captured insulin in each sample. The insulin level was interpreted by calculation from the standard curve that was generated from the known-concentration standards (Pratchayasakul et al., 2011).

Determination of plasma glucose level

After 5-hour fasting, blood samples were collected from tail vein into NaF-coated tube. Blood was centrifuged at 6000 rpm for 10 minutes and plasma was collected. Plasma glucose was determined by

colorimetric assay from a commercially available kit (Biotech, Bangkok, Thailand). The color intensity of each sample underwent enzyme reaction was measured, at 505 nm by spectrophotometry (BioTek, Winooski, VT, USA), using Trinder indicator reaction. A standard curve was generated and the glucose level of each sample was interpreted by interpolation its absorbance to the standard curve (Pipatpiboon, Pratchayasakul, Chattipakorn, & Chattipakorn, 2012).

Determination of plasma cholesterol level

Plasma cholesterol level was determined by enzymatic colorimetric essay using a commercial kit (Biotech, Bangkok, Thailand). The color intensity of each sample was measured, at 505 nm by spectrophotometer (BioTek, Winooski, VT, USA). A standard curve of cholesterol was generated and the concentration level of each sample was interpreted by interpolation its absorbance to the standard curve (Pipatpiboon et al., 2012).

Statistical analysis

Data were expressed as mean ± SEM. One-way ANOVA followed by Fisher's least significant difference post-hoc were used to test the different among the group. P<0.05 was considered statistical significant.

Results

Echocardiographic parameter

In sham groups, the results indicated left ventricular (LV) systolic dysfunction at 12 weeks after high-fat diet feeding and at both 4 and 8 weeks after post VNS treatment. Interestingly, at 8 weeks after VNS treatment, there was a significant improvement of %fractional shortening in the VNS treated group when compared with the sham group.

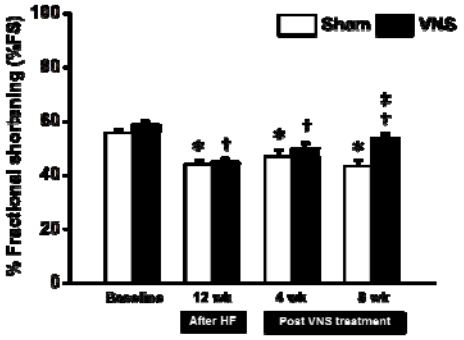

Figure 1 %fractional shortening of obese-insulin resistant rats treated with Sham and VNS (n=5 in each group). *p<0.05 vs sham baseline, †p<0.05 vs VNS baseline, ‡ p<0.05 vs sham group. HF= High-fat diet.

Blood pressure parameter

In sham groups, there was a trend of increased mean arterial pressure after high-fat diet feeding for 12 weeks. Interestingly, at 8 weeks after VNS treatment, there was a significant improvement of mean arterial pressure in the VNS treated group when compared with the sham group. VNS could reduce mean arterial pressure at weeks 4 and 8 after VNS treatment when compared with the sham group.

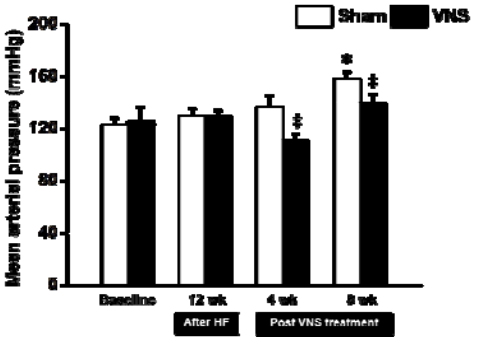

Figure 2 Mean arterial pressure of obese-insulin resistant rats treated with Sham and VNS (n=5 in each group). *p<0.05 vs sham baseline and ‡ p<0.05 vs sham group. HF= High-fat diet.

Metabolic parameter

At baseline, metabolic parameters were not different between Sham and VNS group (Table 1). After 12 weeks of high-fat diet consumption, the body weight and plasma total cholesterol were increased in both groups. VNS treatment reduced plasma glucose and plasma cholesterol compared with the sham group. No difference on body weight parameter was found between sham and VNS group.

Discussion and Conclusions

The present study demonstrated that high-fat diet led to obese-insulin resistance, increased plasma total cholesterol, and LV dysfunction. Our study demonstrated that VNS treatment improved insulin resistance, reduced plasma cholesterol and attenuated LV dysfunction in obese-insulin resistant rats. However, VNS did not alter the body weight. In this study, rats developed insulin resistance after 12 weeks of high-fat diet consumption. This finding is consistent with previous studies in which long-term high-fat diet consumption could induce insulin resistance (Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2013). Furthermore, previous studies have shown the beneficial effects of VNS on metabolic parameters (Apaijai et al., 2013; Bikman et al., 2010). Our data showed that VNS improved insulin resistance and metabolic parameters in obese-insulin resistant rats. However, some previous studies have found that VNS did not reduce body weight in obese model (Bodenlos et al., 2014). Consistent with the results of our study, we found that VNS does not alter the body weight.

Previous studies have shown that long-term high-fat diet consumption caused LV dysfunction (Supakul et al., 2014). Consistent with previous studies, we found that %LV fractional shortening was reduced after 12 weeks of high-fat diet consumption. VNS improved the %LV fractional shortening in the present study. Chronic VNS can improve left ventricular (LV) function and reduce adverse cardiac remodeling through several processes (Sabbah et al., 2011).

It was thought for many years that neither the vagus nerve nor ACh had direct effects on ventricular tissues (Rosen & Hoffman, 1978). The previous study demonstrated that VNS significantly decreased heart rate (HR) and MAP after left anterior descending coronary artery (LAD) ligation for 30 minutes in both adults and aged rats (Wu & Lu, 2011). Furthermore, the study on spontaneously hypertensive rat (SHR) suggested that VNS stimulated cardiac parasympathetic function via NO-cGMP signaling pathway (Heaton et al., 2007). Moreover, the cervical VNS increased the release of NO at the cardiac level (Brack, Patel, Mantravardi, Coote, & Ng, 2009), which can improve vascular system. These findings suggested that the increased ACh and NO by VNS could be a mechanism responsible for MAP reduction and leading to the improvement of hemodynamic parameters in obese-insulin resistant condition.

In conclusion, long-term high-fat diet consumption induced obese-insulin resistance, LV dysfunction, and hemodynamic imbalance. VNS treatment attenuated these adverse effects. Under obese-insulin resistance condition, VNS may prevent LV dysfunction and improved the metabolic parameters by increase parasympathetic activity. Moreover, VNS may reduce MAP by increasing ACh and NO levels in obese-insulin resistant rats.

Table 1: Metabolic parameters of obese-insulin resistant rats treated with Sham and VNS (n=5 in each group).

*p<0.05 vs sham baseline, †p<0.05 vs VNS baseline, ‡ p<0.05 vs sham group. HF= High-fat diet.

Metabolic parameters	Baseline		After HF 12 wk		Post VNS 4 wk		Post VNS 8 wk	
	Sham	VNS	Sham	VNS	Sham	VNS	Sham	VNS
Body weight (g)	260 ± 4.08	255 ± 2.88	535 ± 17.08*	567.5 ± 16.52†	528.8 ± 14.49*	512.5 ± 27.80†	543.1 ± 5.14*	517.5 ± 18.87†
Plasma glucose (mg/dl)	137.36 ± 4.91	137.19 ± 11.86	138.46 ± 8.49	133.17 ± 7.03	133.88 ± 3.61	136.41 ± 5.18	127.5 ± 3.52*	115.6 ± 3.5†,‡
Plasma total cholesterol (mg/dl)	57.36 ± 2.49	61.31 ± 4.07	69.4 ± 4.43*	71.1 ± 2.54†	93.5 ± 1.95*	83.5 ± 4.79†,‡	98.1 ± 5.48*	85.6 ± 4.85†,‡

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