Continuous Vagus Nerve Stimulation Effects on the Gut-Brain Axis in Swine

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ABSTRACT

Objectives. This study was designed to assess vagus nerve stimulation effects on the food intake pattern in swine and determine the electrical stimulus direction. Material and Methods. Fifteen Large White pigs were randomly divided into three groups, groups A–C. All animals underwent implantation of a vagus nerve stimulator at the gastro-esophageal junction. In group A, the stimulation was switched off, whereas stimulation was switched on in groups B and C. Food intake and body weight were registered in groups A and B, but not in group C, which was used to measure direction of stimulation in the vagus and effect on heart rate and blood pressure. Variables measured in group C included the bispectral index, blood pressure, and heart rate. A Student’s t-test and one-way analysis of variance were used to detect differences between groups. All animals were sacrificed to identify effects of implantation and stimulation on the vagus nerve. Results. With respect to food intake, there was no difference between groups A and B; however, body weight did register a continuous increase. During stimulation, in group C arterial pressures decreased significantly, whereas the heart rate and bispectral index increased. Conclusion. The stimulation protocol applied in this study was insufficient to cause changes in the feeding behavior of swine; however, it did increase central nervous system activity.

KEY WORDS: Gut-brain axis, morbid obesity, stimulation, swine, vagus nerve.

Introduction

Vagus nerve stimulation (VNS) is currently used to treat refractory epilepsy (1) and is under study for the management of pain (2), depression (3), and memory enhancement (4). However, some adverse effects of VNS on the gastrointestinal tract have been observed, including chronic diarrhea, nausea, abdominal pain (5,6), and weight reduction (7). Interestingly to many, this weight reduction effect can potentially be used to manage morbid obesity. Because of this fact, we have chosen to study the effects of VNS on food intake and obesity in a swine model. Several experimental studies have demonstrated that VNS reduces food intake and body weight in dogs (8), rats (9,10), and rabbits (11). Despite

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finding no similar study using swine in the literature, we chose to study the effects of VNS on obesity and food intake in this species because the model is considered a good model for the study of gastrointestinal tract physiology (12) and is used for obesity research (13). Accordingly, this study was designed to assess VNS effects on the food intake pattern in swine, determine the electrical stimulus direction, and describe vagus nerve histopathologic features after stimulation.

**Methods**

This study protocol was conducted in compliance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Ethical Committee for Animal Research. Every effort was made to minimize the number of animals used.

Fifteen female Large White pigs (30.43 ± 1.27 kg), allocated under the same environmental conditions, were used. Each pig was housed individually in a pen measuring 2 × 1 m, with a metal bow for food and constantly available water. The pens were arranged in two rows. The animals were fed commercial pig feed with the following nutritional characteristics: crude protein 17%, crude fiber 4.5%, oil 2.7%, and ash 6%, respectively.

Animals were randomly divided into three groups (A, B, and C), each group destined for the study of different parameters and type of electrostimulation. However, all groups were implanted with a vagus nerve stimulator. In order to assess the effect of stimulation on food intake and body weight after implantation of vagus nerve stimulator, group A and group B received implantation of an entire system, but in group A the stimulation was switched off (i.e., a deactivated stimulator) and in group B, the system was activated. Group C also received implantation of an entire system; however, this group was only used to determine the effect of the stimulation on heart rate, invasive arterial and venous pressures, and bispectral index (BIS). BIS monitors are modern neurophysiologic monitoring devices. They continually analyze patients’ electroencephalograms during general anesthesia to assess the level of anesthesia.

In groups A and B, food intake for a period of two hours was recorded daily throughout the second, third, and fourth week after implantation, but not during the first week to avoid any interference that the surgery might have on food intake. This recording of food intake was done manually by weighing the food remaining after the two-hour period (from 9:00 AM to 11:00 AM) and subtracting it from the initial quantity given. The subjects were weighed weekly at the same time period in the morning and after 22 hours of fasting.

All animals underwent implantation under general anesthesia and mechanical ventilation using the same anesthetic protocol, as follows: after 22 hours of fasting the animals were first sedated with a mixture of ketamine (Ketolar® 50 mg, Parke-Davis S.L., Barcelona, Spain), 10 mg/kg, atropine (Atropina Braum® 1 mg, B. Braum Medical S.A., Barcelona, Spain), 0.01 mg/kg, and diazepam (Valium® 10 ampollas, Productos Roche S.A., Madrid, Spain), 0.1 mg. Ten minutes later, all animals were anesthetized with intravenous thiopental sodium (Tiobarbital Braum® 1 g, B. Braum Medical S.A., Barcelona, Spain). Following the introduction of an endotracheal tube, anesthesia was maintained with isofluorane (1–3% as necessary, Aerrane Baxter S.L., Valencia, Spain) during positive pressure ventilation with oxygen. Ketorolac (Toradol® 30 mg, injectable, Productos Roche S.A., Madrid, Spain) 1 mg/kg intravenously was used as an intraoperative analgesic.

Group C animals, those used to monitor cardiovascular variables and BIS, were monitored with a lead-II electrocardiogram, pulse oxymetry, end-tidal concentration of isofluorane (EtIso), and respiratory rate. Invasive arterial and venous pressures were obtained by a catheter placed in the abdominal aorta and vena cava, respectively. The catheters were connected to the monitoring system via a transducer. Heart rate was measured using the electrocardiogram.

To record the BIS, gel-coated disposable silver–silver chloride electrodes were applied (Zipprep
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Aspect Medical Systems Inc., Natick, MA, USA) to the scalp of all animals. Two electrodes (one for each eye) were placed 1 cm caudal to the lateral canthus of each eye, a central or reference electrode was placed on the midline of the frontal bone, equidistant from each of the previously applied electrodes, and a ground electrode was placed 2 cm to the left or right edge of the central electrode. Before each recording, impedance was checked and maintained below 10,000 Ω at 128 Hz. The electrodes were connected to an electroencephalogram (EEG) monitor (A-1050TM, Aspect Medical Systems Inc.).

These parameters were recorded under deep general anesthesia for 30 min before implantation, 30 min while the stimulator was on, and 30 min when the stimulator was off. Although variables were continuously monitored during our experiments, the EtIso, heart rate, BIS, and arterial and venous pressures were recorded only at 5-min intervals.

The operative technique used for electrode implantation (groups A and B) was performed under sterile conditions as follows: under deep general anesthesia and by laparoscopic approach the ventral vagal trunk was dissected at the gastroesophageal junction. An electrode cuff was installed by wrapping it around the ventral vagal trunk. To prevent migration of the cuff the lesser omentum was closed by suturing. The electrode was connected to a neuropulse generator, manufactured by the electronics and electromechanical engineering department of the University of Extremadura (Spain), and was positioned in a subcutaneous pocket within the fascia of the abdominal wall. The generator delivered a square, monopolar, constant-voltage current of 0.5 V, 0.5 Hz, with a 10-msec pulse width, all through the postoperative period in group B, as had been used previously and reported in our studies with rabbits (11). This system transmitted impulses to the target nerve through a pair of leads connected to a plastic cylinder surrounding the whole vagus nerve. A fixed 10-mm interelectrode distance was maintained in all cases. Upon completion of the study all generators were thoroughly checked and tested by the Electronics and Electromechanics Engineering Department to guarantee their correct functioning during the study.

Once the operation was completed, animals from groups A and B were allowed to recover from anesthesia and surgery for a period of one week. After this one-week rest period, recording of food intake began. Group C animals were immediately sacrificed after surgery.

Portions of the ventral vagal trunk in contact with the electrodes were harvested from groups A and B, four weeks after operation for histologic analysis by two independent pathologists who had no knowledge of the nature of the experiment. Sections of harvested material were stained with hematoxylin and eosin. The severity of nerve lesions found, if any, were graded subjectively, in a blinded fashion, from 0 to 4, according to the extent of fibrosis, vascularization, necrosis, fiber degeneration, and inflammation (0: no lesion; 1: mild; 2: moderate; 3: severe; and 4: very severe). Two samples from healthy, non-study animals were submitted along with the rest of the samples, as controls.

All data derived from these experiments are expressed as mean ± standard error of the mean (SEM). To determine differences in food intake and body weight, the Student’s *t*-test was used. For anesthesia parameters, a one-way analysis of variance followed by a Tukey post hoc test in case of differences being detected was performed. A *p*-value of < 0.05 was considered significant in all cases.

Results

Food Intake and Body Weight

There were no significant change found in food intake in either group A or group B (Fig. 1), but there was a continuous increase in mean body weight registered for both groups (Table 1). There were no significant differences in body weight between groups (group A: 904.65 ± 49.06 vs. group B: 917.02 ± 45.91).

Parameters Under Anesthesia

Arterial blood pressures decreased during the stimulation (P2) and poststimulation periods (P3), compared to baseline (P1) (Table 2). The end-tidal
anesthetic agent (EtIso) increased during stimulation, as did heart rate and BIS (Table 2).

**Histopathologic Changes**

Lesions were concentrated in connective rather than nervous tissue in both groups. A thick fibrous capsule was observed at the implantation site in all animals belonging to groups A and B. Fibrosis, inflammation, vascularization, and nervous fiber degeneration was greater in group B, when compared to group A; however, necrosis was greater in group A. Comparing all aspects of lesions generated, inflammation appeared to be the most severe lesion found in group A and more fibrosis was found in group B (Table 3). Both groups had comparable fiber degeneration (group A: 0.50 ± 0.22 vs. group B: 0.40 ± 0.24).

Although also appearing in the endoneurium of the nerve, inflammation and fibrosis were more acute within the perineurium of the nerve.

**Discussion**

It has been previously stated that stimulation of the vagus nerve reduces food intake and body weight in dogs (8), rats (9,10), and rabbits (11), although its effect in swine had not, to our knowledge, been described previously. We believe

### TABLE 1. Body Weight (kg) (Mean ± SEM) Changes During Postoperative Period. Body Weight Increases During the Postoperative Study in Both Groups

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation</td>
<td>29.33 ± 2.04</td>
<td>31.53 ± 1.57</td>
</tr>
<tr>
<td>1 week</td>
<td>33.31 ± 2.83</td>
<td>32.24 ± 1.32</td>
</tr>
<tr>
<td>2 weeks</td>
<td>35.52 ± 2.34</td>
<td>37.18 ± 0.81</td>
</tr>
<tr>
<td>3 weeks</td>
<td>42.61 ± 0.79</td>
<td>40.70 ± 2.22</td>
</tr>
<tr>
<td>4 weeks</td>
<td>42.57 ± 2.80</td>
<td>44.45 ± 3.03</td>
</tr>
</tbody>
</table>


**TABLE 2. Anesthesia Parameters (Mean ± SEM) Measured in Group C. BIS Shows an Increase Secondary to Stimulation (P2) Whereas Heart Rate Is Seen to Decrease**

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane end-tidal (%)(^\circ)</td>
<td>2.28 ± 0.20</td>
<td>2.52 ± 0.14</td>
<td>2.72 ± 0.14</td>
</tr>
<tr>
<td>Systolic arterial pressure (mmHg)(^\circ)</td>
<td>86.13 ± 2.68</td>
<td>75.77 ± 1.36</td>
<td>71.55 ± 1.45</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)(^\circ)</td>
<td>64.50 ± 2.52</td>
<td>54.97 ± 0.70</td>
<td>54.76 ± 0.97</td>
</tr>
<tr>
<td>Diastolic arterial pressure (mmHg)(^\circ)</td>
<td>49.42 ± 1.46</td>
<td>42.47 ± 0.75</td>
<td>42.45 ± 1.04</td>
</tr>
<tr>
<td>Central venous pressure (mmHg)(^\circ)</td>
<td>1.79 ± 0.35</td>
<td>1.13 ± 0.27</td>
<td>0.83 ± 0.29</td>
</tr>
<tr>
<td>BIS</td>
<td>57.13 ± 3.41</td>
<td>54.03 ± 3.31</td>
<td>56.66 ± 3.41</td>
</tr>
<tr>
<td>Heart rate (b.p.m.)</td>
<td>94.21 ± 3.03</td>
<td>136.77 ± 38.79</td>
<td>98.76 ± 2.63</td>
</tr>
</tbody>
</table>

P1: 30 min before implantation; P2: 30 min while the stimulator was on; P3: 30 min during which the stimulator was off. b.p.m.: beats per minute.

\(^\circ\)Statistically different between periods.

**TABLE 3. Lesion Score (Mean ± SEM) of the Ventral Vagal Trunk of Both Groups. In Group A Most Prominent Lesion Found Was Inflammation, Whereas in Group B Marked Fibrosis Was Seen**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>1.20 ± 0.73</td>
<td>2.10 ± 0.56</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.80 ± 0.51</td>
<td>0.40 ± 0.19</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0.20 ± 0.20</td>
<td>2.20 ± 0.56</td>
</tr>
<tr>
<td>Vascularization</td>
<td>0 ± 0</td>
<td>0.30 ± 0.20</td>
</tr>
<tr>
<td>Fiber degeneration</td>
<td>0.40 ± 0.24</td>
<td>0.50 ± 0.22</td>
</tr>
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</table>


**FIGURE 1.** Food intake variations (mean ± SEM) during postoperative period. (A) continuous line. (B) Dotted line. No significant differences were found in food intake between both groups.
that studying the effects of VNS on the food intake pattern in swine is a necessary initial step toward applying VNS for the management of morbid obesity in humans. From the results obtained in this study, it appears that stimulation of the vagus nerve using the same stimulation parameters that were used in our previous study in rabbits (11) does not change food intake pattern or body weight in swine.

Roslin et al. (8) employed constant current stimulation in their study and did find a change in food intake, whereas we, using constant voltage, did not. We believe that the type of stimulation that we used, which differed from the stimulation that Roslin et al. used, most probably stimulated different types of fibers than those stimulated by Roslin et al., thereby stimulating different functions. This difference in type of stimulation might explain the different effects on food intake and body weight.

It should be kept in mind that different branches of the vagus nerve have been stimulated by different groups. Krolczyk et al. (9) and Sobocki et al. (11) stimulated the dorsal vagus nerve that distributes differently along the gastrointestinal tract. Roslin et al. (8) and Laskiewicz et al. (10), on the other hand, used bilateral stimulation of both vagus nerves that were simultaneously stimulated. Laskiewicz et al. showed a greater resultant decrease in rat food intake and body weight: Food intake fell between 12% and 26% when compared to baseline and body weight suffered a decrease of between 14% and 30%. Laskiewicz et al. (14) in another study hypothesized that this increased effect on satiety was because unilateral stimulation of the vagus nerve induces a compensatory response from the nonstimulated vagus nerve to the central nervous system (CNS), and this effect is eliminated by the simultaneous stimulation of both nerves.

To assess the direction of the current, we studied anesthesia parameters in group C. The vagus nerve is a mixed nerve consisting of motor and sensory fibers; most the sensory fibers are afferent nonmyelinated fibers (15). Ninety percent of the total abdominal vagus is made up of afferent fibers (16). The motor component of the vagus is involved in gastric secretion and empying and adaptative relaxation.

The sensory compound action potential comprises mechanoreceptors and chemoreceptors, the former report gastric and intestinal distension to the CNS and the alter, the presence of nutrients, cholecystokinin, etc. The sensory portion of the vagus connects with the CNS through the nucleus of the solitary tract and area postrema, from which the information is directed mainly to regions of the brain implicated in food intake and energetic homeostasis such as the hypothalamus (17), amygdale (18), and even the cerebral cortex (19).

The vagus nerve is mainly composed of afferent fibers (15), so it is logical that stimulation of the nerve may provoke changes in heart rate and CNS activity.

Central nervous system activity was evaluated indirectly by means of the BIS. BIS value is obtained from an algorithm empirically derived from EEG studies in anesthetized humans (20). VNS has been shown to cause synchronization or desynchronization of the EEG (21). Although the BIS was developed to document the degree of sedation and hypnosis under general anesthesia, it is likely that changes caused by the stimulation in the EEG could affect BIS values. BIS is inversely related to the depth of anesthesia (22). Furthermore, a positive relationship between the BIS and CNS metabolic activity has been reported, implying that an increase in cerebral cortex metabolism leads to higher values of BIS (23,24). We used the BIS as a measurement of depth of anesthesia, but, in turn, it also measures cerebral cortex activity, which is important data for our study. In line with this, our findings suggest that vagal stimulation increases CNS activity, deducible from the increased BIS value during the stimulation period.

As far as cardiovascular parameters are concerned, we believe that the decrease in arterial and venous pressure seen during the VNS period was due to an increase in the depth of anesthesia represented by the increase of EtISO; it has already been reported that volatile anesthetics cause a dose-dependent reduction in blood pressure (25).
On the other hand, the parasympathetic nervous system stimulation hardly affects it (26). We believe that the heart rate could have increased both because of this decrease in arterial pressure and also as a direct effect of stimulation. If it is a direct effect of stimulation, it seems as if low frequency stimulation inhibits vagus nerve function, at least where heart rate is involved. Regarding the effect of the vagus nerve on gastrointestinal physiology, we cannot state with certainty that low frequency stimulation has an inhibitory effect because we did not perform a gastric pH study or gastric emptying study. The stimulation could also stimulate sympathetic fibers that accompany the vagus nerve, as has been previously described by the finding of catecholamines in the vagus nerve of the cat (27) and humans (28) and the presence of adrenergic fibers in the dog (29).

Regarding histopathology, as expected, the severity of lesions found was greater in group B (stimulated) than in group A (nonstimulated). Those observed in a stimulated nerve are related to the mechanical and electrical effects of the stimulator (30), thus it is logical that group B presented a greater lesion score in inflammation, fibrosis, vascularization, and fiber degeneration. However, we have no explanation for the finding that necrosis was more severe in group A, when compared to group B. Fibrosis has been principally attributed to the mechanical effects of the implanted stimulator (30), but, in fact, our results suggest that the electrical field generated also increases connective tissue growth as we had described in our previous study (31).

Conclusion

The stimulation protocol applied in this study did not cause changes in feeding behavior in swine; however, it did increase CNS activity. Nevertheless, we believe that further studies, using different parameters and different protocols, should be performed before we can conclusively state that VNS, in swine, has no effect on food intake or weight gain. Other future paths for research include a long-term study, determination of the mechanism of action using a direct method such as measuring vagus nerve activity via a neurogram, and detailed evaluation of the microscopic features of the vagus nerve by means of electron microscopy.

Acknowledgment

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References


