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Effects of chronic abdominal vagal stimulation of small-diameter neurons on brain metabolism and food intake

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ABSTRACT

Background: Abdominal bilateral vagal stimulation reduces food intake in animals. However, the classical square wave, mA range current generator is poorly effective to evoke action potentials on Aϑ and C neurons that represent the majority of vagal neurons at the abdominal level.
Objective/Hypothesis:

(i) To ascertain the capability of very high-frequency stimulation schemes (pulsons) to trigger action potentials in abdominal vagal neurons in anaesthetized pigs. (ii) To compare these stimulation schemes with classical ones using PET imaging of brain metabolism and food intake behaviour in conscious pigs.

Methods: The current thresholds for pulsons (S2 & S3) and millisecond pulses (S1) required to trigger action potentials were calculated in 5 anaesthetized pigs using single fibre recording. Similar stimulation protocols were compared chronically to sham stimulation in 24 pigs. After two weeks of chronic stimulation, food intake and brain metabolism were investigated. The electrical characteristics and histology of the vagus nerve were also studied.
Results: S3 stimulation required a lower amount of charges to trigger an action potential. Chronically applied S2 & S3 activated the dorsal vagal complex and increased the metabolism of its afferent cortical structures. They also reduced energy intake together with a reduced ingestion of high fat and high sugar diets. All these effects were not observed for the S1 group. The vagal histology for the S1, S2 and S3 groups was not different from that of the sham.
Conclusions: These findings demonstrate that pulsons applied bilaterally on the abdominal vagus reduced food intake as a consequence of the activation of the brainstem and higher-order brain areas.

Introduction

Obesity is an international public health issue that affects quality of life, increases the risk of illness, and raises health-care costs. Bariatric surgery remains the most effective treatment for obese patients [1]. However, these procedures could be associated with major adverse-effects [2]. There is an urgent need for alternative therapeutics.

Chronic vagal stimulation has the potential for weight control [3], but the animal and human data are ambiguous [4–6]. This might relate to the different modalities of stimulation, including the location of the stimulating electrodes [7], the number of electrodes for bilateral or unilateral stimulation [8], the stimulation profile and current intensity [9,10]. In addition, body mass index [11] and diets [12] are confounding factors. Retrospectively, the most efficient stimulation strategy was close to that used for

Abbreviations: DVC, Dorsal vagal complex; VNS, Vagal nerve stimulation; PET – FDG, Positron emission tomography with 18Fluorodeoxyglucose; μC, microcoulombs; HFAC, High frequency alternating current.

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epilepsy, e.g., 500 ms pulses of 2.5–5 mA for 30 s every 5 min [13], applied on both abdominal or thoracic vagal trunks. However, the optimal stimulation parameters need to be investigated [3].

At the abdominal level, most vagal neurons located either in the dorsal or ventral vagal trunks are small diameter myelinated and un-myelinated neurons, i.e., Aϑ or C type [14]. Therefore, large current pulses are required for depolarizing the axon membrane and thus generating an action potential. To do so, more than 20 mA may be needed at this level [15]. While they proved to be effective in an acute set-up, these currents are unrealistic in a chronic implant since they generate damages both to the electrode and to the tissues [16]. Recently, Qing et al. [17] proposed to chop rectangular based waveforms into shorter rectangular waves lasting between 40 and 80 μsec, called pulsons. While individual pulsons were unable to trigger an action potential, they appear collectively to be effective in anaesthetized rats for stimulating C fibres at the cervical level. Furthermore, they achieved this with less charge injection than with a long lasting rectangular waveform. However, the efficacy of this method has never been tested in chronic conditions, and its capability to trigger action potentials in small diameter neurons at the abdominal or thoracic level remains to be proven.

Our hypothesis was that the classical stimulation scheme using low intensity, long-lasting current pulses (1–5 mA, 500–1000 μs) is unable to activate a large number of abdominal vagal neurons [15]. This, in turn, might recruit only a limited number of neurons in the dorsal vagal complex (DVC). The extend of activated DVC neurons might be so small that it impossible to identify a DVC activation (versus background noise) because of the intrinsic limitations of our present imaging methods [18]. Hence the reported absence of activation of the DVC in individuals with vagal stimulation irrespective of the brain imaging method used [15,19–22]. Conversely, using pulsons, large intensity currents could be applied to the nerve trunk, allowing effective triggering of a neuronal response through a larger amount of small diameter neurons being recruited.

Our primary objective was to demonstrate that, in anaesthetized animals, pulsons of large current intensity applied bilaterally on both abdominal vagal trunks are able to trigger action potentials in small diameter neurons (C and Aϑ types), while their activities were recorded at the cervical level using the single fibre method. Our secondary objective was to investigate the long-lasting consequences on brain metabolism of chronic vagal stimulation using pulsons. Our third objective was to evaluate the capability of this stimulation pattern to modify food intake and to correlate these changes with the alterations in brain metabolism measured by PET imaging. These objectives were carried out using the large white pig as a large animal model of normal human physiology [23]. Furthermore, the size of the animal is well suited for laparoscopic surgery and for brain imaging using high end clinical PET scanners [24].

In this study, we assessed, first in an acute set-up, the capability of three vagal stimulation schemes to trigger action potentials on slow adapting gastric and intestinal mechanosensitive vagal neurons (Aϑ and C types). One stimulation pattern reproduced the classical long lasting pulse (S1) while the others (S2 and S3) integrated pulsons. The same stimulation patterns were also compared, in a chronic experiment, for their capabilities to modify brain metabolism and food intake behaviour.

Methods

The experimental procedures were conducted in accordance with the current ethical standards of the European and French legislation (Agreement number A35-622 and Authorization number 01894, Ethical approval R-2012-CHM-03 and 00341.01).

Experimental paradigm

Acute experiment

The experiment consisted of recording evoked action potentials at the cervical level on the left vagal nerve after careful microdissection of the nerve bundle to obtain single neuron action potentials. Evoked action potentials were generated by applying current pulses on two cuff electrodes implanted on the anterior and posterior vagus nerve at the entrance of the diaphragm using a lateral thoracic approach. This terminal procedure was performed on 5 animals. Two to three neurons per animal were analysed for the entire range of stimulation, while 4 to 8 neurons were unable to be studied for all current/stimulation scheme combinations, as a consequence of mechanical stress on the bundle or perineurium bleeding.

The stimulation pulses are presented in Fig. 1 and consisted of a long lasting 1000 μsec pulse (S1), 14 constant amplitude pulsons forming a pattern lasting 1000 μsec (S2) and 14 pulsons of rising amplitude (S3) with a total duration identical to the two former patterns. Current increments in amplitude used in the last stimulation pattern were following a one-fourth sinusoidal series. The stimulating patterns were applied in a sequential manner, but the order was randomly selected. One minute without stimulation was allowed between each stimulation pattern.

Chronic experiment

The procedure was performed on 24 growing pigs (50% sex ratio and age-matched) distributed in 4 experimental groups consisting of sham, S1, S2 or S3 stimulation patterns (Fig. 1). The experiment started with the application of one of the three stimulation patterns (Fig. 1) or a sham stimulation immediately after the surgical procedure used to insert the stimulating electrodes (see supplementary material). The animals were placed for the entire experimental period in a robotic feed dispenser to evaluate their food intake. At the end of this period, the brain metabolism was mapped using PET-FDG imaging and the animal was euthanized afterward. The vagal trunks were sampled for histological assessment of potential damage due to the stimulation.

We selected purposely different current amplitudes for S1 and for S2/S3 with the aim to inject the same amount of electrical charges per unit of time irrespective of the stimulation pattern. As a consequence, the S1 current was set to the maximal current used during unilateral vagal stimulation at the cervical level for epilepsy therapy, i.e., 5 mA supplying 5 μC. This value was also proven to be effective when applied bilaterally at the juxta-diaphragmatic level to alter food intake in pigs [6,13]. For the S2 stimulation pattern, the current required to inject the same 5 μC charge was 15 mA. Therefore, this current was selected for the S2 and S3 stimulation patterns. Consequently, S3 stimulation pattern injected 3.52 μC, i.e., approximately one third less charges than the S2 or S1 stimulation patterns.

Animals

All experiments were performed on young Large-White pigs weighing 32 ± 4 kg at the time of surgery. The animals were housed in individual cages, one week before the beginning of the experiment to allow adaptation to their new environment, and subjected to standard 12: 12 h light/dark cycle. During this period, the animals used in the acute experiment were fed daily between 08.00 h and 09.00 h with 1 kg of granulated porcine meal (3.63 kcal/g). The animals used for the chronic experiment were placed in special cages that include a robotic feeder, during the same time period