Acute changes in diet composition and/or origin alter gastric emptying and gastrointestinal motility. One of the hypotheses explaining these alterations involves changes in the sensitivity of duodenal vagal sensory neurons. The aim of this study was to evaluate the characteristics of multimodal duodenal vagal sensory neurons in 20 pigs feed either with milk-based or plant-based diets of identical caloric content. Twenty duodenal vagal aferents were recorded in anesthetized animal from the cervical vagus using the single fiber method. 10 pigs were fed with a milk-based diet (MD) for one month while the diet of the 10 other pigs was changed for plant-based diet (PD) the day preceding the recording session. The behavior of the receptors was tested in basal resting conditions and after challenges with duodenal intralipid and close intra-arterial injection of CCK, 5-HT or capsaicin with and without isovolumetric duodenal distensions at 20, 40 and 60 mmHg. All receptors were slowly adapting C type fiber with a receptor field located 6-7 cm distal to the pylorus. The rate of discharge during distension (20, 40 and 60 mmHg) combined with duodenal intralipid was significantly larger for MD compared with PD. Similarly, the rate of discharge observed during distensions performed with CCK and with 5-HT were greater for MD compared with PD while CCK and 5-HT without distension were equally stimulating for MD and PD. No significant difference was found between groups during capsaicin infusion irrespective of the stimulating pressure. In conclusion, a switch to plant-based diet, when compared to a milk-based diet, results in an overall decrease in mechanical sensitivity of duodenal neurons during lipid, 5HT and CCK challenges, but not in basal conditions or after capsaicin. This reduced sensitivity to distension may explain the diet-induced alteration of gastric emptying that is controlled primarily through a vago-vagal reflex.

**Key words:** vagal afferents, pig, change in diet composition, vagal recordings
INTRODUCTION

The contents of the diet influences gut motility and transit by numerous well-established local and peripheral neuro-humoral pathways (1-3). Changes in diet composition with specific reference to lipids, alter gastric emptying rate and gastro-intestinal motility (4). While these changes accounted for an immediate, meal based time frame, long lasting previous patterns of macronutrient intake affect also gut motility through a potential gastrointestinal adaptation to a specific diet. In rats, exposure to a high fat diet for one, two and eight weeks modify the response to intraduodenal fat infusion with attenuation of the inhibition of gastric emptying (5-7). Furthermore, the inhibitory effects of exogenously administered CCK on gastric emptying were also decreased following a high fat diet (8). In humans, exposure to a high fat diet for 14 days attenuates the effects of duodenal lipid on antropyloroduodenal pressures (9). The origin of these diet-induced changes in sensitivity has not been assessed in animals and in humans.

The effect of duodenal lipids on gastric motility and emptying is though to be primarily depending of their perception by vagal afferents located in the duodenal wall. Indeed, vagal cooling, in conscious dogs, suppresses proximal gastric relaxation induced by intestinal nutrients (10). Similarly, in conscious pigs, the dorsal vagal trunk has a preponderant role to control gastric emptying (11) and intragastric distribution of the food (12). Raybould and Holzer (13) have shown that vagal exposure to the selective unmyelinated afferent neurotoxin capsaicin attenuates the inhibition of gastric emptying produced by duodenal carbohydrate. Yox and colleagues (14) have shown that the suppression of food intake produced by duodenal infusions of carbohydrate, fat, and amino acids is blocked by vagotomy. In addition, Walls and colleagues (15) have shown that surgical transection of afferent components of the vagus nerve that partially supply the proximal duodenum block the suppression of sham feeding produced by duodenal nutrients.

Although vagal afferent fibers appear to carry important signals in the negative feedback control of ingestion arising from duodenal nutrient infusions (16-18), it is not clear how previous duodenal nutrient exposure is altering vagal sensing. The aim of this study is to evaluate the mechano and chemo sensitivity of duodenal vagal afferents on two populations of animals; one of which has received milk-based diet while the remaining received a plant-based diet. To cancel a possible role of the energy contents of the diet (19, 20), both diets were isocaloric differing only in fat and dietary fiber contents.

MATERIAL AND METHODS

Animals and diets

The experimental protocol was approved by the Local Ethical Committee. Twenty female large white pigs weighting 38.2 ± 5.4 kg were used. They were assigned to two experimental groups. The
first one (10 animals) received for one month a milk-based diet while the remaining 10 animals were switched from this milk-based diet to a plant-based diet one day before the experiment. The diets differed by their composition in fat and dietary fibers (Table 1). Both diets were isocaloric.

Table 1. Composition of diets

<table>
<thead>
<tr>
<th>Ingredients (g/kg as fed)</th>
<th>Milk-based</th>
<th>Plant-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skimmed milk powder</td>
<td>260</td>
<td>-</td>
</tr>
<tr>
<td>Wheat</td>
<td>-</td>
<td>805</td>
</tr>
<tr>
<td>Maltodextrine</td>
<td>595.5</td>
<td>50</td>
</tr>
<tr>
<td>Soluble Fish Protein Concentrate</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Vegetal oil</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin-mineral mixture</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>CaHPO&lt;sub&gt;4&lt;/sub&gt;, 2H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>NaHCO&lt;sub&gt;3&lt;/sub&gt;-3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>-</td>
<td>2.2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1.9</td>
<td>-</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>-</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Chemical composition

| Dry Matter (%)                           | 91.1       | 87.4         |
| Crude protein (N´6.25, % DM)             | 17.9       | 17.6         |
| Crude fat (% DM)                         | 3.4        | 2.9          |
| Total dietary fiber (% DM)               | 0          | 12.4         |
| Net Energy (MJ/kg DM) <sup>a</sup>       | 9.88       | 9.50         |
| Calcium (g/kg DM) <sup>b</sup>           | 14.5       | 14.9         |
| Sodium (g/kg DM) <sup>b</sup>            | 2.2        | 2.1          |
| Chloride (g/kg DM) <sup>b</sup>          | 3.3        | 2.2          |
| Potassium (g/kg DM) <sup>b</sup>         | 4.7        | 3.7          |
| Phosphorus (g/kg DM) <sup>b</sup>        | 10         | 10.5         |
| WHC <sup>c</sup> (g/g)                   | N.D.       | 0.68         |

<sup>a</sup> calculated from digestible energy and elemental analysis

<sup>b</sup> estimated from tables (INRA 1989)

<sup>c</sup> water holding capacity

supplying 9.8 and 9.5 MJ kg<sup>-1</sup> dry matter. The meal were offered at 100 g/kg body weight<sup>0.75</sup> and were supplied as a mash (45% dry matter), irrespective of the diet.

**Anesthesia**

The animals were pre-anesthetized with Ketamine (5 mg kg<sup>-1</sup> intramuscularly, Rhone Mérieux). Suppression of the pharyngo-tracheal reflex was obtained by inhalation of halothane (5% v/v by a face mask) immediately before intubation. A venous cannula was inserted into the marginal vein of the ear to infuse a mixture of a chloralose (60 mg kg<sup>-1</sup>, Sigma) and urethane (500 mg kg<sup>-1</sup>, Sigma): the primary anesthetic agent. At the completion of the abdominal and cervical surgical procedures, the surgical anesthesia level was maintained by continuous IV infusion of pentobarbital.
(20 mg kg hr⁻¹, Sanofi Santé Animale). Motion artifacts were cancelled by supplemental slow IV bolus injections of D-tubocurarine (0.2 mg kg⁻¹, Sigma) every two hours. The surgical level of anesthesia was continuously assessed by arterial blood pressure measurement obtained from a catheter located in the right carotid artery. The animals were artificially ventilated by a positive pressure ventilator (Siemens, SAL 900) connected to the tracheal cannula. SpCO₂ and O₂ saturation were controlled for normocapnia and Sapo₂ at 98 % or above using a capnometer connected to the ventilator and a pulse oxymeter placed on the tail of the animal. FiO₂ ranged from 30 to 45%. Body temperature was kept at 38.5 ± 0.5°C by a self-regulating heating element placed under the animal.

Surgery

A duodenal segment starting 1 cm distal the pylorus and 20 cm long was isolated by two Foley catheters (18 F). Within this segment, a probe consisting in a balloon and a side-hole open tip manometry catheter was inserted in a retrograde manner. The proximal end of the probe was secured to the duodenal wall using a transparietal stitch. An additional Foley catheter was also inserted in the stomach to allow drainage of the stomach contents. Before abdominal closure, a arterial catheter was inserted into the right epiploic artery with its tip oriented towards the pylorus (Fig. 1).

Recordings

Electrical activity from single vagal afferent neurons was recorded by classical neurophysiological methods adapted to the pig (21). Briefly, the left vagus was made free from surrounding connective tissue. The skin and cervical muscles were sutured to a metallic frame to

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**Fig. 1. Left panel** - schematic of the surgical preparation allowing mechanical and chemical stimulation of the proximal duodenum. The balloon inserted in the duodenum served to distend the organ while local intra arterial infusion was performed by an arterial access in the right epiploic artery. Gastric tube served to avoid over distension of the stomach due to acid secretions. **Right panel** - increased in spiking activity of a slow adapting mechanoreceptor located in the duodenum during intra-arterial administration of CCK (1 × 10⁻⁹ M in toto in 60 sec).
create a pool filled with warm paraffin oil. Monopolar recordings of vagal bundles were performed after section of the cervical vagus and micro-dissection of its distal end. Adequate amplification of the signal was provided by a homemade amplifier (gain 50000, impedance 20 Mohms), placed near the recording electrodes (tungsten, 50 μm, WPI USA). After low and high pass filtration (300-6000 Hz), the raw electroneurogram was stored on a digital tape (Biologic, France) for post-processing at 20 KHz together with lower frequency pressure and volume signals (see below). Unitary vagal activity was discriminated off-line using adaptive shape matching criteria. Instantaneous and cumulative frequency histograms were constructed after detection of the adequate unit. The conduction velocity was measured by a modified peripheral stimulus technique. Briefly, four centimeters distal to the recording site, the entire vagus nerve was lying on a pair of platinum electrodes connected to a homemade isolated voltage stimulator. A "Wagner ground" electrode was also inserted between the recording and stimulating electrodes to reduce stimulation artifact.

The intraluminal pressure at the middle of the duodenal segment was obtained by low compliance manometry using a side-hole PCV catheter. Briefly, the open tip catheter was continuously perfused with degassed water using a low compliance pneumatic pump at a constant pressure of 375 mmHg achieving a perfusion rate of 0.1 ml min\(^{-1}\) and a pressure rise rate in excess of 1000 mmHg sec\(^{-1}\) while occluded.

**Experimental protocol**

Duodenal compliance was evaluated prior the identification of duodenal unit. Compliance of the duodenum was assessed using pressure-volume data (22) obtained during graded step distensions lasting 30 s (2 mmHg increment; 2-30 mmHg range). Distension steps were achieved with a computer-controlled barostat (Visceral Stimulator, Synectics) connected to the balloon used for duodenal distension. Balloon distension was performed irrespective of the migrating motor complex (MMC) period. Indeed, pentobarbital anesthesia suppressed the occurrence of MMC on the duodenum.

Rapid balloon distension of the duodenum was used to identify mechanosensitive duodenal units. This was achieved by connecting the balloon to a compressed air source (750 mmHg) through a computer-controlled valve until the pressure within the balloon equaled 20 mmHg. Thereafter, the balloon was deflated by computer-controlled connection of the balloon to a vacuum source (-75 mmHg). Once duodenal vagal units were identified, their adaptation to distension was characterized using the procedure described by (23) resulting in the definition of half adaptation time for each unit. Afterwards, mean spiking activity was calculated (i) before (Control) and after (ii) intraduodenal infusion of emulsified lipids (Intralipid, 100 ml.min\(^{-1}\), 2 min infusion) (iii) intra-arterial (IA) injections of 5HT (0.05 10\(^{-6}\) M in toto) and CCK (1 10\(^{-9}\) M in toto). Finally, the response of the duodenal unit to capsaicin (IA 0.5 10\(^{-6}\) M in toto) was evaluated. Successive injections or infusions were separated from each other by a washout period of 30 minutes.

To analyze the response of the units to distension, the following experimental sequence was used. After a 5 minutes basal recording, a 30 seconds isovolumetric distension (initial pressure = 20 mmHg) was performed. Upon completion of the distension and after one minute rest, a 30 seconds isovolumetric distension using an initial pressure of 40 mmHg was achieved. Finally, a third distension was performed using an initial pressure of 60 mmHg. Distension procedures were performed using a computer controlled compressed air source associated with a pressure transducer that was used to stop the inflation of air within the duodenal balloon once the requested pressure was reached. The three distensions levels were repeated for each single experimental conditions i.e. control and after administration of lipids, 5HT, CCK and capsaicin.
At the completion of the previous protocol, the abdominal wall stitches were removed, the duodenal loop was exteriorized, and the gut was cut along the anti mesenteric border to access the duodenal mucosa. The receptive field of the unit was localized using a calibrated von Frey hair (500 mg perpendicular). Finally, the units were classified on the basis of their conduction velocity using electrical stimulation (1 Hz, 20 V, 1 ms with current limitation set at 5 mA) of the distal vagal trunk while the duodenal unit was still being recorded. Conduction velocity was calculated using the latency of averaged evoked potentials (24).

Data analysis

Due to the variability between resting discharge frequency of single unit vagal afferents, it is necessary to perform data normalization using a well-defined stimulus in a standardized condition as done previously by others (25, 26). Spiking activity of vagal afferents recording obtained during 20, 40 and 60 mmHg distensions while the animal was in the control period i.e. the period before the administration of lipids, 5HT, CCK or capsaicin was used for reference in the normalization procedure. Hence, results were expressed as % of the control response for the same type of mechanical stimulation obtained during the control period. The normalization procedure explained the almost identical figures of spiking irrespective of the distending pressures. Comparison between groups (milk-based versus plant-based) in control condition cannot be performed in a similar way. Hence we select to use, for within control condition comparison only, the spiking activity recorded during 20 mmHg distension as a reference for normalization. Spiking activity data without units corresponded to normalized spiking activity while data expressed in spikes sec-1 were not normalized.

Statistical analysis

Differences between conditions were tested using repeated measures ANOVA model performed on JMP 5.0 (SAS Institute). Data were expressed as mean ± SE. p<0.05 indicated a significant difference.

RESULTS

A total of 20 duodenal vagal afferent was tested. All units were C type fibers with conduction speed equaled to 5.6 ± 1.23 m s⁻¹. All units were spontaneously quiescent in the absence of duodenal distension or before the administration of the challenging drugs. All units behaved as slowly adapting receptors with half-adapting time of 20.2 ± 2.42 s. No significant change in half-adaptation time before and after intralipid, 5HT, CCK and capsaicin could be noticed. All units responded to Von Frey hair stimulation. The units' receptive fields were of ellipsoidal shape with an area ranging from 3 to 8 mm² and were located 6-7 cm distal to the pylorus.

1. Distension elicited activity

There was no significant difference in spiking activity recorded during 40 and 60 mmHg distensions between the milk based and the plant based group (137 ± 29.4 versus 161 ± 50.9 for 40 mmHg distension and 170 ± 64.3 versus 116 ± 16, milk-based and plant-based diet respectively, p>0.05). This demonstrated that in
unchallenged conditions, vagal afferents sensitivity was not altered by the nature of the diet.

2. Mechanical sensitivity after chemical challenge

In challenged situation using intralipid duodenal infusion, the mechanical sensitivity to distension is reduced in the plant-based diet group compared to the milk-based diet group indicated by a reduced spiking activity in plant-based group during distension (Fig. 2). For instance, during 20 mmHg pressure distension, discharge rate was 104 ± 18.2 for milk-based diet compared to 49 ± 15.8 for plant-based diet. This reduction in spiking for plant-based versus milk-based group was statistically significant for all levels of distension pressure.

CCK and 5HT IA injections resulted in an increase in basal discharge rate, 5HT producing a clustered type discharge whereas CCK generated a sustained discharge of the unit (Fig. 1). Similar to the duodenal administration of lipids, 5HT and CCK injections were associated with a decreased mechanical sensitivity of duodenal vagal afferents irrespective of the applied pressure except for CCK during 40 mmHg distension (Fig. 3). The largest reduction in discharge rate was observed with CCK during 20 mmHg distension (168 ± 45.0 versus 43 ± 14.9 for milk-based diet versus plant-based diet respectively, p<0.01). There was no significant difference in mechanical sensitivity for 40 mmHg distension after CCK. No significant changes in discharge rate between milk-based and plant-based diet groups was found after the IA administration of capsaicin irrespective of the distending pressure. For instance, afferent discharge was 103 ± 12.0 for milk-based diet compared to 106 ± 14.3 for plant-based diet during 20 mmHg distension (p>0.05). Furthermore, while capsaicin in resting pressure condition

Fig. 2. Spiking activity during 20, 40 and 60 mmHg isovolumetric distensions after infusion of intraduodenal lipids in milk based (black bars) and plant based (open bars) groups. Data were expressed after normalization with similar distensions performed during the control period i.e. before the inraduodenal administration of lipids. The responses to distension were reduced in the plant-based group irrespective of the distention pressures. Asterisks showed a significant difference between groups at p<0.05.
increased significantly the afferent discharge, this increase is not significantly different between groups (55 ± 5.3 versus 3 ± 0.9 spikes sec⁻¹ for milk based group and 51 ± 4.9 versus 2 ± 1.2 spikes sec⁻¹ for plant-based group respectively)

3. Duodenal compliance

Pressure-volume curves recorded during graded distension showed a typical S-shape irrespective of the group. The larger slope, indicative of duodenal compliance, was always found between 14 and 20 mmHg. It was similar in milk-based and plant-based group (0.15 ± 0.014 versus 0.18 ± 0.016 mmHg ml⁻¹ for milk-based versus plant-based group).

**DISCUSSION**

This is the first demonstration that introducing a new diet alters the mechanical sensitivity of duodenal vagal afferents in challenged conditions. The absence of changes in mechanical sensitivity in unchallenged conditions suggests that such mechanism is of importance in the pathophysiology of gut function occurring at weaning (27) or after introduction of a new diet (28, 29).

The mechanical properties of vagal afferent neurons were tested by the measurement of the spiking activity during isovolumetric distensions at three
distending pressures. We were uncertain of a detectable difference between the plant and milk based diets groups in resting basal conditions. Therefore, the same procedure was repeated in conditions that are known to challenge the enterochromaffin cell-vagal afferent terminal complex. Lipids challenge was selected over others macronutrients since hyperlipidic diets are the only nutrient known to alter durably gastro-duodenal functions (4). CCK and 5-HT were selected since these neurotransmitters are the only ones known to be present on vagal afferents (30, 31). Furthermore, their role towards the transmission of mechanical and chemical stimuli between the enterochromaffin cell and the afferent terminal is well documented (32). Finally, we used capsaicin also as a challenging compound because of (i) the intricate relationship of VR1 receptor and CCK receptor (30) and (ii) the association between VR1 receptor and C type neurons (33).

The changes observed in the mechanical sensitivity were not associated with changes in gut compliance suggesting that the alteration was located at the level of the afferent terminal itself or at the signal transduction level i.e. the enterochromaffin cell (31, 32). It cannot be excluded that the phenotype of the enterochromaffin cell was modified by the arrival of a new type of diet since such alteration have been already demonstrated for the enterocyte itself (34, 35) or, on the contrary, inappropriate to this new diet. Nevertheless, the absence of changes in mechanosensitivity observed after capsaicin that acts as a non specific neurostimulant at the dose used in our study (36-38), suggest that the changes induced by the alimentary switch was located distally of the afferent i.e at the enterochromaffin cell level. Indeed, within the gut wall, VR1 receptors are located only at the afferent terminals and are absent more distally (39).

The nature of the diet used in our study excludes the involvement of energy specific changes since both diets have the same energy contents. The only differences between the two diets were in the amount of fat and dietary fibers. Dietary fibers are known to alter colonocytes behavior through the production of SCFA but this process is unlikely to occur at the proximal gut level (40). However, we have previously demonstrated that dietary fibers are able to alter gastric emptying, proximal gastric filling and intragastric repartition of the meal (41). Such changes might modify the pattern of availability of nutrients at the duodenal level after a meal. CCK and GLP1 are surprisingly not affected by high fat diet in humans and cannot explain the difference in mechanosensitivity (9, 42). On the contrary, it could not be excluded that modifications in fat contents, even limited induced long lasting changes in hormonal secretion such as ghrelin, peptide YY and GIP.

CCK and 5HT injections induced both a reduction in the mechanosensitivity for plant-based versus milk-based diet. While the intensity of this reduction was not equal, it was surprising since 5HT is not primarily involved in the response to fat unlike CCK while both neuropeptides have been demonstrated to act as transmitters between the enterochromaffin cells and the afferent terminals (31, 43, 44). As such the absence of specific response is in favor of a non-specific
reduction in afferent sensitivity, a phenomenon re-enforced by a similar effect produced by intraduodenal lipids infusion.

In conclusion, introduction of a new diet with different fat and fiber contents but similar energy content, reduced non specifically the sensitivity of vagal afferent probably by modifications occurring at the enterochromaffin cell level.

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