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Desensitization of ileal vagal receptors by short-chain fatty acids in pigs

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Cuche, G., S. Blat, and C. H. Malbert. Desensitization of ileal vagal receptors by short-chain fatty acids in pigs. *Am J Physiol Gastrointest Liver Physiol* 280: G1013–G1021, 2001.— Coloileal reflux episodes trigger specialized ileal motor activities and inhibit gastric motility in pigs. The initiation of these events requires the detection by the distal ileum of the invading colonic contents that differ from the ileal chyme primarily in short-chain fatty acid (SCFA) concentrations. In addition to the already described humoral pathway, this detection might also involve ileal vagal afferents. Sensitivity to SCFA of 12 ileal vagal units was investigated in anesthetized pigs with single-unit recording at the left cervical vagus. SCFA mixtures (0.35, 0.7, and 1.4 mol/l) containing acetic, propionic, and butyric acids in proportions identical to that in the porcine cecocolon were compared with isotonic and hypertonic saline. All units behaved as slowly adapting mechanoreceptors (half-adaptation time = 35.4 ± 15.89 s), and their sensitivity to local mechanical probing was suppressed by local anesthesia; 7 units significantly decreased their spontaneous firing with 0.7 and 1.4 but not 0.35 mol/l SCFA infusion compared with hypertonic or isotonic saline. Similarly, the response induced by distension in the same seven units was reduced (5 neurons) or abolished (2 neurons) after infusion of 0.7 (22.8 ± 2.39 impulses/s) and 1.4 (30.3 ± 2.12 impulses/s) mol/l SCFA solutions compared with isotonic saline (38.6 ± 4.09 impulses/s). These differences in discharge were not the result of changes in ileal compliance, which remained constant after SCFA. In conclusion, SCFA, at concentrations near those found during coloileal reflux episodes, reduced or abolished mechanical sensitivity of ileal vagal afferents.

vagal afferents; lipids; coloileal reflux; ileal brake

SHORT-CHAIN FATTY ACIDS (SCFA) are produced during bacterial fermentation of carbohydrates and glycoproteins in the large intestine of animals with simple stomachs. When present at the ileal level as a result of a coloileal reflux episode (9), they trigger local and remote activities. Locally, they are potent stimulants of prolonged propagated contractions and discrete clustered contractions (25). At a distance, they are able to produce an ileal brake toward gastric motility and emptying (10, 11). Whereas the pathways for the ileal brake are mainly of an endocrine nature (8), those

modulating ileal motility are still not resolved. Experimental data suggest that activation of vagal afferents sensitive to SCFA might be involved, as proposed by Yajima (47) for the colon. Furthermore, epithelial SCFA-sensitive chemoreceptors were described in the reticulorumen of ruminants (7), and mucosal SCFA-sensitive chemoreceptors were reported in the proximal duodenum of sheep (5). Propionate was also shown to activate some duodenal vagal afferents of nonruminant animals (32). More recently, vagal afferents sensitive to medium-length fatty acids were demonstrated in the rat ileum (36).

The effects of SCFA on vagal afferents are controversial. In the adult sheep duodenum, SCFA exposure produces desensitization of the unit without change in mechanosensitivity (5). An opposite result, that is, increase in basal activity, was observed in the cat duodenum after administration of propionate (32). Similarly, acetate in the esophagus also increased neuronal background activity (15, 41). A region-dependent dose threshold above which desensitization occurred can be hypothesized to explain these differences. However, data from duodenal or esophageal receptors obtained in other species cannot be extrapolated to ileal receptors because ileal concentration of SCFA is ~ 50 times that at the duodenal level in the pig (14).

The aim of this study was to assess the sensitivity of ileal vagal afferents to SCFA at concentrations close to those occurring during coloileal reflux episodes in pigs. This was achieved by the measurement of single vagal afferent activity at the cervical vagus *in vivo*. The motility of the ileum was assessed concurrently with the activity of vagal neurons to discriminate the effects related to SCFA on ileal compliance from the direct effects of SCFA on nerve terminals.

MATERIALS AND METHODS

Twelve female Large White pigs (34 ± 3 kg) were used during this study. Experiments conform to European and French legislation and guidelines on experimental animal care (Agreement no. A35–622). Only one receptive unit was recorded in each animal because repetitive administrations of SCFA were suspected to generate long-lasting changes in the unit characteristics (5).

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Anesthesia. The animals were preanesthetized with ketamine (5 mg/kg im; Rhone Mérieux). Suppression of the pharyngotracheal reflex was obtained by inhalation of halothane (5% vol/vol by face mask) immediately before intubation. A venous cannula was inserted into the marginal vein of the ear to infuse a mixture of α -chloralose (60 mg/kg; Sigma) and urethane (500 mg/kg, Sigma), the primary anesthetic agent. At the completion of the abdominal and cervical surgical procedures, the surgical anesthesia level was maintained by continuous intravenous infusion of pentobarbital sodium (20 mg·kg⁻¹·h⁻¹; Sanofi Santé Animale). Motion artifacts were canceled by supplemental slow intravenous bolus injections of D-tubocurarine (0.2 mg/kg; Sigma) every 2 h. The surgical level of anesthesia was continuously assessed by arterial blood pressure measurements obtained from a catheter located in the right carotid artery.

The animals were artificially ventilated by a positive-pressure ventilator (SAL 900; Siemens) connected to the

tracheal cannula. Estimated PCO₂ and O₂ saturation were controlled for normocapnia and estimated PO₂ at 98% or above using a capnometer connected to the ventilator and a pulse oximeter placed on the tail of the animal. Fractional inspired O₂ concentration 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. The animals were killed by an intravenous overdose of thiopental sodium (Nesdonal; Merial SAS) at the end of the experiment.

Ileal surgery. Ileal surgery was performed under aseptic conditions because previous nonaseptic attempts failed to identify ileal receptors, probably as a consequence of the rapid deterioration of the ileal mucosa caused by a proliferation of exogenous or/and colonic bacteria. A left retrocostal laparotomy was performed to access the distal ileum. Two Foley catheters (CH18; Vygon) were inserted in the ileal lumen and secured in position with a purse suture to isolate an ileal loop 15 cm in length located 5 cm proximal to the

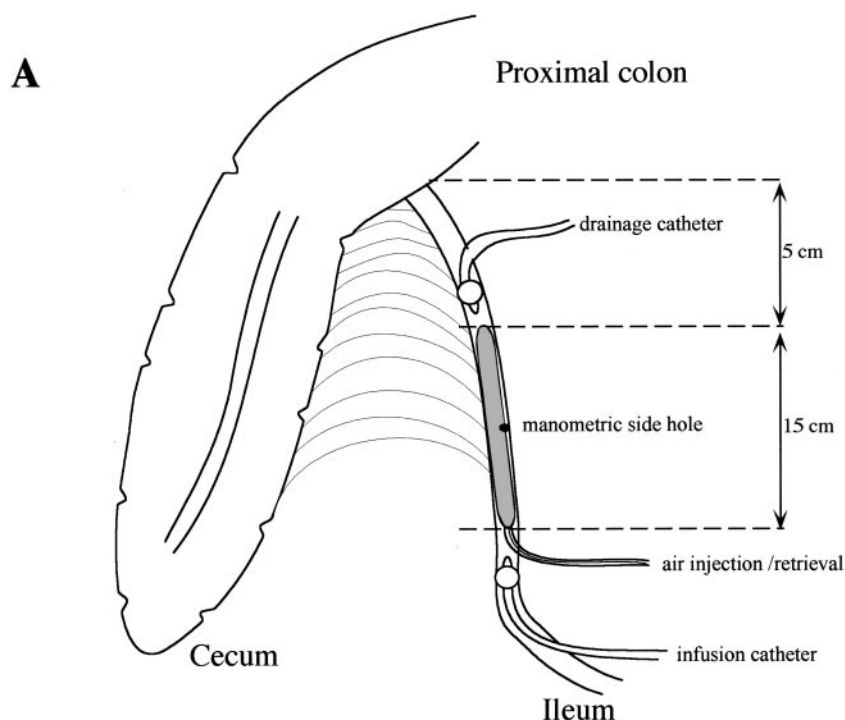
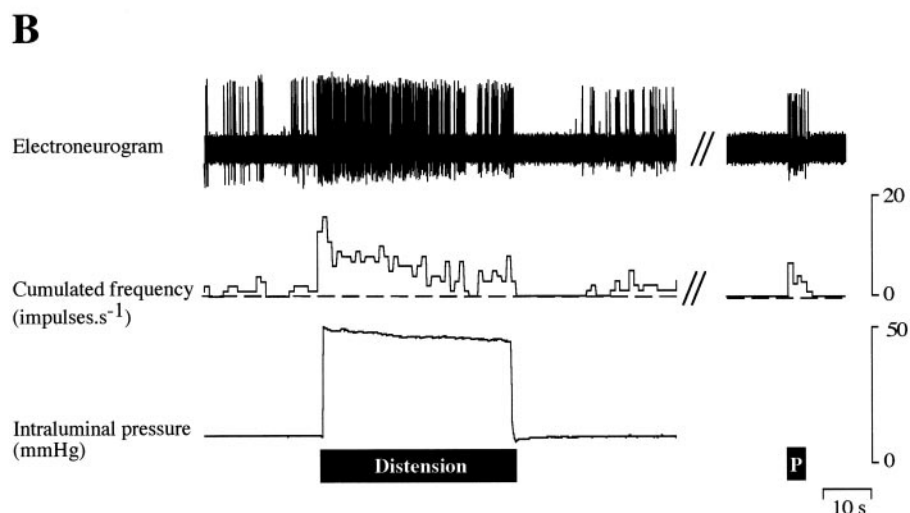


Fig. 1. Diagram of the ileal surgical preparation and response of an ileal vagal afferent to distension. **A:** a 15-cm-long cylindrical balloon was inserted in the distal ileum so that its distal tip was located 5 cm proximal to the ileocecal sphincter. An open-tip catheter was also placed along the balloon for intraluminal pressure recording (tubing not shown). Two Foley catheters isolated the ileal segment so that it was possible to infuse various solutions into the ileal segment. **B:** Single-unit recording of an ileal afferent (conduction velocity 4.9 m/s) during rapid distension. The unit behaved as a slowly adapting unit with an exponential decay of the discharge starting 5 s after inflation completion. Mucosal probing (P) with a 500-mg von Frey hair on the open ileum elicited a burst of firing that stopped abruptly when the stimulus was removed.



ileocecal sphincter (Fig. 1). A double-lumen catheter (ID 3.5 mm for air injection/retrieval and 1 mm for pressure sensing) incorporating a latex balloon 15 cm in length was placed in the middle of the ileal loop. The larger-bore opening was used for air injection and retrieval, allowing inflation and deflation of the latex balloon. The smaller-diameter opening was connected to a pressure transducer (PX23; Gould) to record the static air pressure within the balloon in the absence of artifacts related to dynamic pressure changes during inflation and deflation. A polyvinyl chloride (PVC) catheter (OD 1 mm) incorporating one side hole was also placed in the ileal lumen to record the intraluminal pressure of the ileum (see *Recordings*). The oral end of the manometric catheter was attached to the oral end of the balloon. Distally, at the entrance into the gut lumen of the balloon and the manometric catheter, both catheters were secured in position by a purse suture. At the completion of the abdominal surgery, the laparotomy was closed and the cervical vagal dissection was performed.

Recordings. Electrical activity from single vagal afferent neurons was recorded by classic neurophysiological methods (30) adapted to the pig. Briefly, the left vagus was made free from surrounding connective tissue. The skin and cervical muscles were sutured to a metallic frame to create a pool filled with warm paraffin oil. Monopolar recordings of vagal bundles were performed after sectioning of the cervical vagus and microdissection of its distal end. Adequate amplification of the signal was provided by a homemade amplifier (gain 50,000, impedance 20 M Ω) placed near the recording electrodes (tungsten, 50 μ m; WPI). After low- and high-pass filtration (300–6,000 Hz), the raw electroneurogram was stored on a digital tape (Biologic) for postprocessing 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 (19, 33). Instantaneous and cumulative frequency histograms were constructed after detection of the adequate ileal unit. The conduction velocity was measured by a modified peripheral stimulus technique (22). Briefly, 4 cm 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 ileal segment was obtained by low-compliance manometry using a side hole PVC catheter (34). 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 and a pressure rise rate in excess of 1,000 mmHg/s while occluded. Compliance of the ileum was assessed using pressure-volume data obtained during graded step distensions lasting 30 s (2-mmHg increments; 2–30 mmHg range). This procedure allowed us to build a pressure-volume curve from which the compliance was extracted as previously described (31, 46). Briefly, distension was achieved with a computer-controlled barostat (Visceral Stimulator; Synectics) connected to the balloon used for ileal stimulation.

Experimental protocol. Computer-controlled rapid (rise rate \geq 100 mmHg/s) distension of the ileum was used to identify mechanosensitive ileal 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 30 mmHg. Thereafter, the balloon was deflated by computer-controlled connection of the balloon to a vacuum source (–75 mmHg).

Once ileal vagal units were identified, their behaviors were analyzed before and after ileal infusions of isotonic and hypertonic saline versus the SCFA mixture. The SCFA mixture contained 60% acetic, 30% propionic, and 10% butyric acids, a ratio identical that found in porcine cecocolonic fluids (11). Solution pH was adjusted with 7 N NaOH to 6.5, which corresponds to the mean pH of cecocolonic fluids in pigs (14). Increasing SCFA concentrations (0.35, 0.70, and 1.40 mol/l) were administered in random order at the oral side of the ileal loop at a rate of 150 ml/min. A green dye (E102) was also added to SCFA and hypertonic saline solutions to assess their passage throughout the ileal loop. A preliminary experiment ($n = 2$ pigs) had shown that direct application on the mucosa of the dye alone has no effect on the activity of ileal vagal unit. The osmolalities of SCFA solutions were 509 mosmol/kg for 0.35 mol/l, 1,054 mosmol/kg for 0.70 mol/l, and 1,963 mosmol/kg for 1.4 mol/l. The osmolality of the hypertonic saline solution equaled that of the highest SCFA solution concentration, that is, 1,963 mosmol/kg. Isotonic saline corresponded to 0.9% saline, and its osmolality equaled that of the plasma.

The following experimental sequence was used in all 12 animals/units. First, after 10-min basal recording, a slow (40 ml/s) distension was performed until the volume of the balloon equaled 300 ml. Once this volume was reached, it was maintained for 60 s. The balloon was then deflated to –1 mmHg. The inflation phase of the distension was used afterward to evaluate the firing threshold of the ileal unit. Second, after a 5-min rest, a gradual step distension sequence was initiated to obtain the basal compliance of the ileum. Third, isotonic and hypertonic saline and SCFA solutions were infused at random within the loop. The time required for the solutions to travel through the loop was variable, ranging from 1 to 3 min. Because response latency of SCFA-sensitive units found elsewhere in the gut is large because of diffusion time (23), the test solutions were left in contact with the mucosa for 2 min after the end of the infusion itself. A distension similar to that performed at the beginning of the experimental sequence was then achieved. An ~3-min isotonic saline rinse was performed afterward until the green dye was not observed in the ileal effluent. Before saline rinse and for SCFA 1.4 mol/l only, gradual step mode distension was performed to calculate compliance. This was followed by a rapid distension 5 min after the completion of the former sequence.

At the completion of the previous protocols, the abdominal wall stitches were removed, the loop was exteriorized, and the gut was cut along the antimesenteric border to access the ileal mucosa. The receptive field of the unit was localized using a calibrated von Frey hair (500 mg perpendicular, homemade). Topically applied lignocaine cream (10%) was used to test suppression of the firing activity during von Frey hair mechanical stimulation. At the completion of this test, the ileal mucosa was washed with saline and the same mechanical stimulation was performed 5–10 min later to evaluate the reversibility of the local anesthetic-induced inhibition. Finally, the units were classified on the basis of their conduction velocity (1) using electrical stimulation (1 Hz, 20 V, 1 ms with current limitation set at 5 mA) of the distal vagal trunk while the ileal unit was still being recorded. Conduction velocity was calculated using the latency of averaged evoked potentials (3).

Data analysis. Receptor mechanical threshold was calculated for each solution using firing rate data obtained during the inflation phase of the slow distension episodes as previously described (42). Briefly, a linear fitting between firing rate and pressure was calculated. Threshold was then ex-

trapolated from the pressure required for the firing rate to be nullified. Half-adaptation time (in s) was calculated according to a previously described method (6).

Data are presented as means \pm SE. Comparisons between treatments (isotonic and hypertonic saline and the 3 SCFA mixture concentrations) during infusions and during distensions were performed using one-way analysis of variance (StatView 5.0; SAS Institute). $P < 0.05$ indicated a significant difference.

RESULTS

Twelve units that responded to rapid ileal distension were recorded. Six of them were spontaneously active and as a consequence were not taken into account for the calculation of mechanical threshold. Of the 12 neurons, 8 were classified as C fibers (2.5 ± 0.93 m/s) and 4 as A δ fibers (5.1 ± 0.94 m/s). A δ fibers did not show mechanical or chemical sensitivity significantly different from that of C fibers. In addition, there was no significant difference in conduction velocities between neurons that were desensitized by SCFA compared with those that were not. Therefore, A δ and C neurons were analyzed together. All units responded to von Frey hair stimulation. Topically applied lignocaine reversibly inhibited the response to mucosal probing for all units. The receptive fields of units were of ellipsoidal shape with an area ranging from 3 to 8 mm².

All units behaved as slowly adapting receptors with half-adaptation time obtained from the rapid inflation data equal to 35.4 ± 15.89 s. No significant change in half-adaptation time between 1.4 mol/l SCFA infusion and isotonic saline could be noticed (40.6 ± 17.37 vs. 35.4 ± 15.89 s for 1.4 mol/l SCFA vs. isotonic saline; $P > 0.05$).

Spontaneous activity. All units increased their firing rate or became active during infusion of isotonic or hypertonic saline and SCFA solutions, probably as a result of increased intraluminal pressure during infusion (see below). Those that were classified initially as quiescent became active when the pump was turned on. No significant difference was found in the increased firing rate during isotonic versus hypertonic saline infusions ($P > 0.05$).

Seven of twelve neurons were found to decrease their firing rate significantly during SCFA infusions compared with isotonic and hypertonic saline (Figs. 2 and 3A). Of these seven neurons, five were spontaneously active and two were quiescent during basal (no perfusion) period. The most potent inhibitor was 1.4 mol/l SCFA. Conversely, 0.35 mol/l SCFA did not significantly modify firing rate compared with isotonic or hypertonic saline. For two neurons spiking activity was abolished during SCFA infusions irrespective of the SCFA concentrations, whereas their mean firing rates were 5.9 ± 0.45 and 5.8 ± 0.52 spikes/10 s during isotonic and hypertonic saline infusions, respectively. No significant difference could be observed in mean intraluminal pressure during SCFA and hypertonic or isotonic saline infusions (15.2 ± 4.12 , 15.1 ± 5.27 , 15.0 ± 4.92 , 14.8 ± 4.92 , and 14.8 ± 4.56 mmHg for

0.35, 0.7, and 1.4 mol/l SCFA, hypertonic saline, and isotonic saline, respectively; $P > 0.05$).

Distension-elicited activity. Ileal distension was significantly less effective in increasing firing rate after SCFA infusions compared with isotonic or hypertonic saline (Fig. 3B). For the same seven neurons that were inhibited by SCFA infusions, 0.7 and 1.4 mol/l SCFA were also able to reduce the firing rate significantly during slow distension (Fig. 4). Furthermore, the reduction in firing exhibited a dose relationship pattern, the highest SCFA concentration being the most potent inhibitor of unit activity (22.8 ± 2.39 , 30.3 ± 2.12 , and 38.6 ± 4.09 spikes/s for 1.4 and 0.7 mol/l SCFA and saline, respectively). The five remaining neurons that did not change their firing rate during SCFA infusion were also insensitive to SCFA during slow distension ($P > 0.05$). The increased intraluminal pressure recorded during distension was not significantly modified by SCFA or hypertonic or isotonic saline (44.2 ± 1.36 vs. 42.8 ± 1.45 mmHg for 1.4 mol/l SCFA vs. isotonic saline; $P > 0.05$).

The mechanical threshold, calculated from slow distension data, was modified by 1.4 mol/l SCFA infusion. Of the six quiescent units for which a valid mechanical threshold could be calculated, only two reduced their activity during SCFA infusion. The calculated threshold of these two neurons was increased after SCFA infusion (19.2 vs. 17.0 mmHg and 18.6 vs. 16.1 mmHg for 0.7 and 1.4 mol/l SCFA, respectively, vs. isotonic saline).

Ileal compliance. Pressure-volume curves recorded during graded isobaric distension showed a typical S shape irrespective of the solution infused. The larger slope, indicative of ileal compliance, was always found between 14 and 22 mmHg. It was unchanged by the composition of the infused solution (21.9 ± 3.75 vs. 21.4 ± 4.84 mmHg/ml for 1.4 mol/l SCFA vs. isotonic saline; $P > 0.05$).

DISCUSSION

The results of this study indicate that vagal ileal units are inhibited by SCFAs. Furthermore, the mechanical sensitivity of ileal units was severely impaired after SCFA contact. This desensitization occurred with SCFA concentrations in the range of those occurring during coloileal reflux episodes.

Ileal afferents described in this study are all of the mucosal type because their mechanical sensitivity was reversibly inhibited by topical lignocaine application (20). However, they differed significantly from vagal mucosal afferents described elsewhere in the gut. All units found in our study were slowly adapting to mechanical stimulus, whereas Leek (28) described duodenal mucosal receptors with an on-off response to mechanical distension. In contrast, Cottrell and Iggo (5) showed mucosal afferent fibers in the adult sheep duodenum with persistent responses to mechanical distension. The adaptation time of these duodenal mucosal receptors was within the range of that found for ileal units. More surprisingly, none of the 40 duodenal

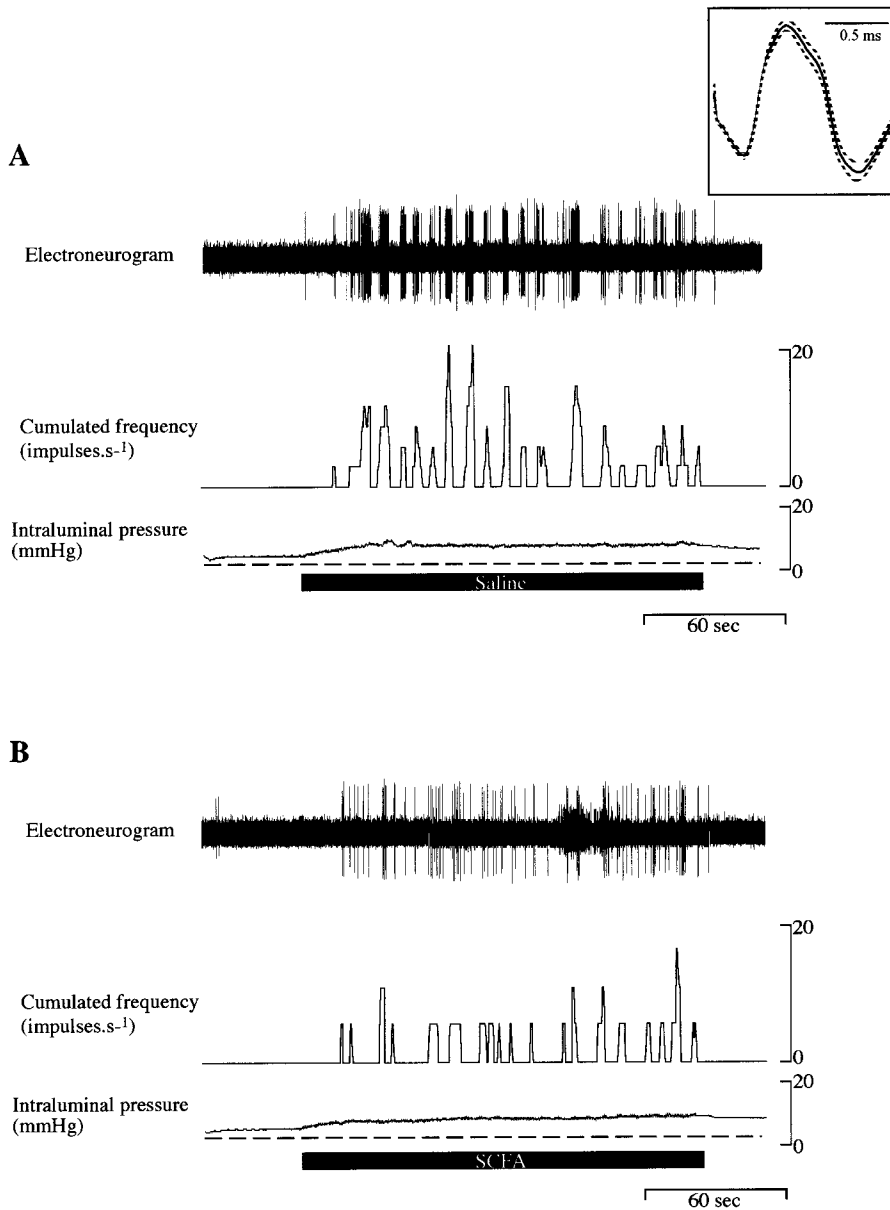


Fig. 2. Inhibition of spontaneous activity of an ileal vagal afferent (conduction velocity 3.2 m/s) during short-chain fatty acid (SCFA) vs. hypertonic saline infusion. SCFA infusion (1.4 mol/l, B) was associated with a significant decrease in firing compared with hypertonic saline (A). Cumulative frequency histogram (1-s interval) did not show a staircase shape because of the large time scale. Two units were active in this strand; the discriminator template for the ileal unit is shown in the *inset*. The second unit that was more active in the last 60 s of SCFA infusion was not projecting in the ileum.

receptors sensitive to lipids (including propionate) described by Méloné (32) responded to mechanical stimulation. On the contrary, the ileal receptors sensitive to medium-chain fatty acids described by Randich et al. (36) were all sensitive to mechanical stimulus, a feature also found in the *in vitro* ileal preparation of Cervero and Sharkey (3) and in our *in vivo* preparation. Hence, it is likely that porcine ileal vagal units sensitive to SCFA had the same sensitivity to mechanical stimulus as rat ileum.

The sensitivity to SCFA demonstrated in our study cannot be called chemosensitivity as such. Indeed, duodenal SCFA-sensitive units increase their firing rate in contact with SCFA and in relation to the molecular weight of the acid (29), whereas we observed reduced basal and stimulated firing after contact with SCFA. However, units sensitive to organic or nonorganic acids are commonly desensitized after the initial application

of acids (5). During this period, they are “turned off” for 5–15 min (37) but their mechanical sensitivity is unchanged, unlike the situation observed for ileal units. In contrast, the desensitization observed with SCFA was very similar to that demonstrated after acute application of capsaicin: desensitization to the actual compound itself plus cross-desensitization to mechanical stimuli observed in 37% of the afferents (2).

Unlike nonabsorbed nutrients present within the ileum during pathological conditions that have been shown to trigger the ileal brake (44), SCFA represent a stimulus of physiological relevance, at least for the pig. We previously demonstrated (9) that SCFA were present in the porcine ileum as a result of cecoileal reflux episodes occurring about six times per hour. These reflux episodes last more than 10 min and deliver 10–20 mmol of SCFA into the distal ileum. This amount is within the range delivered by the 0.35 mol/l

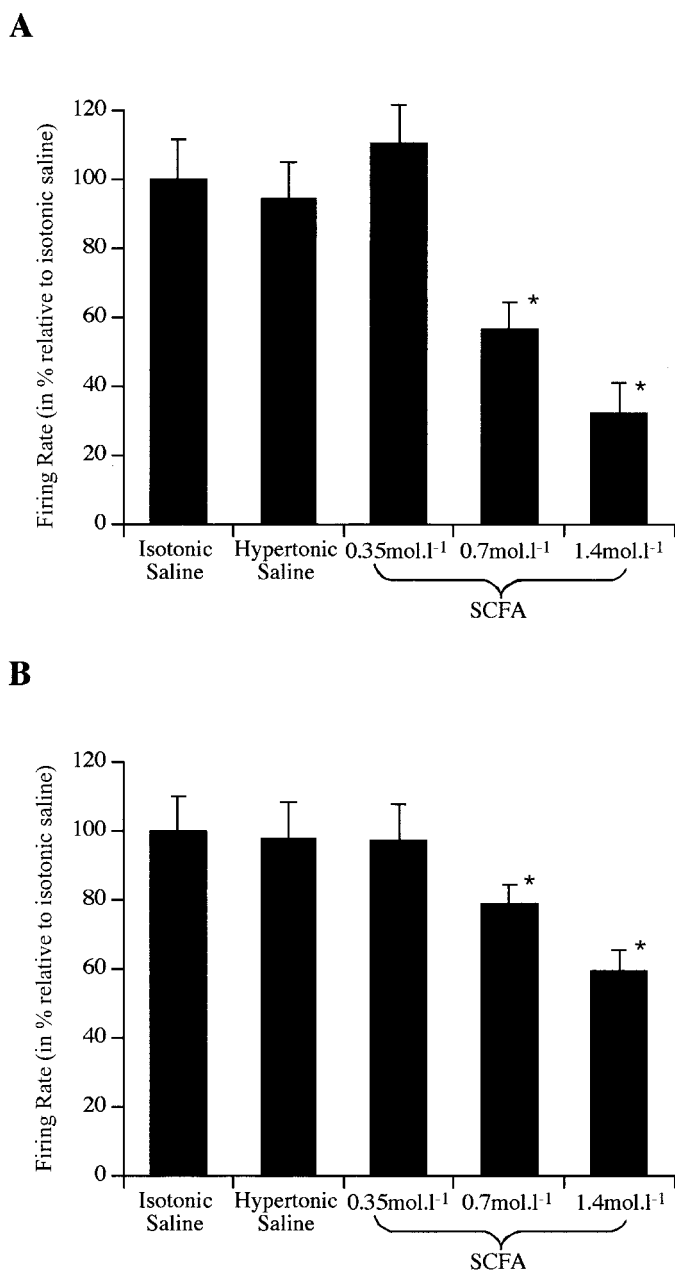


Fig. 3. Firing rate of ileal afferents relative to isotonic saline after SCFA or hypertonic saline infusions. *A*: relative firing rate during 60-s infusions of hypertonic saline or SCFA solutions at 0.35, 0.7, or 1.4 mol/l. The mean firing rate recorded during the 60-s infusion period was normalized relative to isotonic saline, which was considered as 100%. Note the absence of effect of hypertonic saline and SCFA solution at the dose of 0.35 mol/l. On the contrary, the firing rate was significantly and dose-dependently decreased for 0.7 and 1.4 mol/l SCFA solutions. *B*: relative firing rate during 60-s distension (300 ml) after ileal mucosal contact with hypertonic saline or SCFA solutions at 0.35, 0.7, or 1.4 mol/l. The method used for normalization was identical to that described in *A*. *Significant difference at $P < 0.05$ compared with isotonic saline; $n = 7$ units selected for their SCFA-sensitive behavior. Infusions were performed in a randomized order.

solution infused at 150 ml/min over 1 min. Using the same logic, the 0.75 mol/l solution supplied about the same amount of SCFA as three ileocecal refluxes and the 1.4 mol/l solution was equivalent to six reflux

episodes. It is unlikely that vagal afferent desensitization induced by SCFA was related to a volume, osmolarity, or pH effect of the SCFA solution. Indeed, the pH of the solution was adjusted to neutrality. Distension- and osmolarity-related effects were tested by infusing isotonic and hypertonic saline used as volume and osmotic controls, respectively.

It is unlikely that the inhibitory effect of SCFA infusion was related to changes in the compliance of the ileum wall as already observed at the gastric level during impaired relaxation of the organ wall (13). Indeed, neither the compliance nor the intraluminal ileal pressure was significantly modified by SCFA versus saline. The reduced sensitivity to mechanical distension after SCFA may be related to an increased firing threshold of the unit, whereas the adaptation characteristics remained constant. Indeed, we found a slight reduction (2 mmHg) in the threshold for the two units responsive to SCFA and for which a valid threshold could be calculated. The magnitude of this reduction must be evaluated with care because it is unlikely that the relationship of discharge rate to stimulus intensity was linear at or near the threshold level.

The amount of SCFA required to inhibit mechanical sensitivity is also physiologically relevant for species other than the pig. Indeed, the amount of SCFA able to desensitize ileal afferent is theoretically (for 0.7 mol/l SCFA solution) yielded by the fermentation of 13 g of unabsorbed carbohydrate (such as resistant starch or dietary fiber). Because no quantitative value for the volume of coloileal refluxate exists in the literature aside from the porcine model (9, 10), it is difficult to evaluate the proportion of these SCFA synthesized in the colon that can be found in the distal ileum. Nevertheless, motility data for the Mayo group (16, 25, 27) suggest that, for the dog model, it might be of the magnitude of that found in pig.

About 65% of the ileal neurons were classified as C fibers with conduction velocities within the range already described in sheep (6) and ferrets (35). This percentage is more than double that found in the esophagus (35) but is similar to that observed in the extensive study of Cervero and Sharkey (3) in the rat intestine. Because the methods used to evaluate mechanical threshold were different (42), it is difficult to draw conclusions about a potential lower threshold of A δ fibers (17). However, in our experimental conditions, mechanical thresholds of C versus A δ fibers were not statistically different.

The physiological significance of ileal vagal afferent desensitization by SCFA is still hypothetical. Whereas the pathways for the ileal brake are mainly endocrine (8), those modulating ileal motility are likely of a nervous nature (12). However, the motor response of the distal ileum to mechanical distension and the neuronal network controlling this response are complex. Indeed, a classic peristaltic event will be elicited by distension if it occurs in an oral-to-aboral direction (18). In contrast, a single, broad-based contraction (prolonged propagated contraction) or brief bursts of phasic contractions (discrete clustered contractions) must be triggered after a

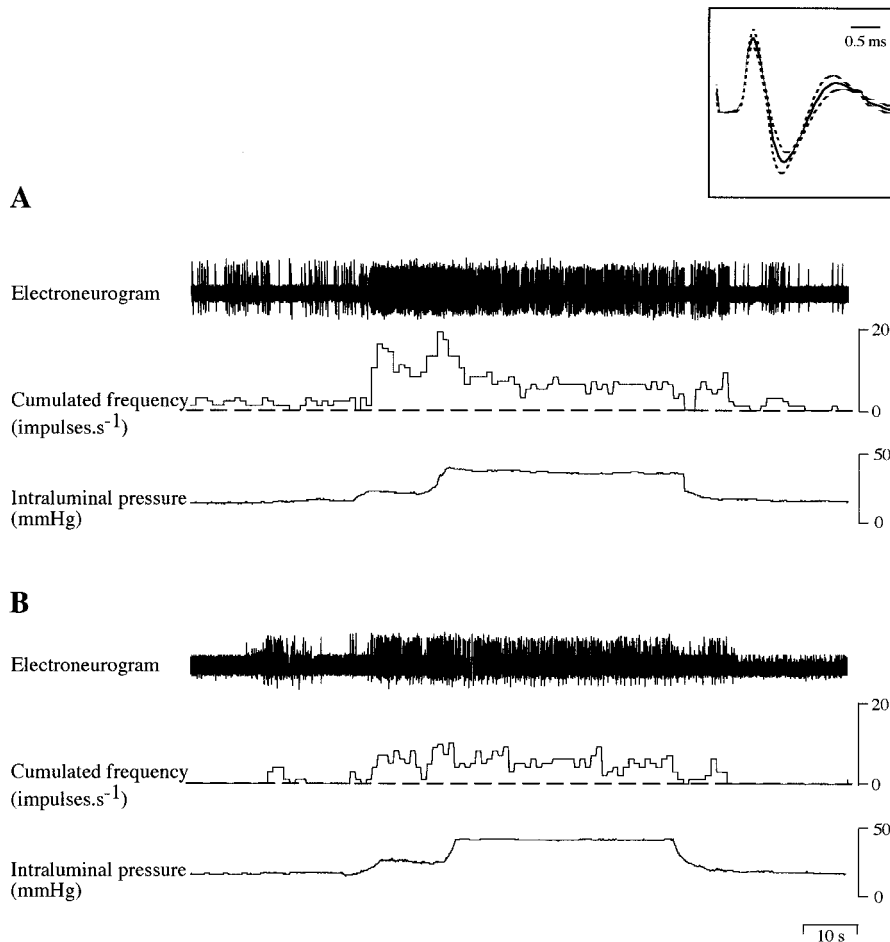


Fig. 4. Reduced mechanical sensitivity of an ileal vagal afferent (conduction velocity 5.6 m/s) to distension before (A) and after (B) mucosal contact with 1.4 mol/l SCFA. Distension (300 ml) of the ileum was less efficient at generating afferent activity after infusion of 1.4 mol/l SCFA than after isotonic saline infusion, indicating a reduced sensitivity to mechanical distension elicited by SCFA. Infusions were stopped 60 s before distension. In both cases, the rate of distension (change in volume over time) was similar (40 ml/s). *Inset* corresponds to the discriminator template for the ileal unit. Infusions were performed in a randomized order.

distension that occurs in an aboral-to-oral direction (26). It is worth noting that two key parameters, the chemical nature of the intraluminal contents and the direction of the distension, control the amplitude and velocity but not direction of the contractile events. A similar situation exists at the lower esophageal level with primary and secondary peristalsis associated with gastroesophageal acidic reflux episodes. There are numerous similarities in the chemical sensitivity to acid (HCl and SCFA) of esophageal and ileal vagal afferents. First, acid-sensitive esophageal units are scant (40). Second, *in vitro*, a second application of acid induces a response that is either greatly reduced or nonexistent (35). Third, there is also a general desensitization to mechanical stimulation after application of HCl (35). Fourth, the chemical sensitivity is not related to the conduction velocity of the neurons (35). All of these similarities suggest that the hypothesis postulated for esophageal vagal afferents is also valid for ileal units, that is, a role in inflammatory processes but a limited contribution in physiological conditions (43). However, it is equally possible that the inhibition of vagal units sensitive to SCFA is a prerequisite for the switch from peristalsis to fast propulsion, the initial stimulus triggering motor events being identical, that is, distension of the distal ileum.

The mechanisms of SCFA-induced desensitization were not evaluated in our study. Nevertheless, it is

likely that the mechanisms used by SCFA to interact with vagal afferents differed from those suspected for longer-chain fatty acids because most of the SCFA passage to the blood occurred outside the chylomicron route (39). Hence, it is possible to rule out a direct activation of vagal afferents by chylomicrons (38) or by chylomicron-induced release of other activating substances (24). On the contrary, on the isolated ileal muscle cell, SCFA induce contractions through an acid-sensitive, calcium-dependent mechanism (4) that could also occur at the terminal endings of vagal afferents. Similarly, SCFA in the ileum induce a large peptide YY response (8) that might also activate vagal afferents. Finally, stimulation of vanilloid receptor subtype 1 (VR1) by mild change in acidic concentration (2) induced after the absorption of SCFA was a strong candidate to explain afferent desensitization. Indeed, protons decrease the temperature threshold for VR1 activation such that even moderately acidic conditions (pH = 5.9) activate VR1 at room temperature (45). Together with the already mentioned mechanical stimuli desensitization obtained with capsaicin, it could be possible that SCFA, at the concentration used in our study, activated VR1 receptor without neurotoxic effects (21).

In conclusion, this is the first description of vagal sensory neurons that are desensitized by SCFA. These

neurons behave as slowly adapting units and exhibit mechanical desensitization while exposed to SCFA at concentrations within the range occurring during spontaneous reflux episodes.

REFERENCES

1. **Accornero N, Bini G, Lenzi GL, and Manfredi M.** Selective activation of peripheral nerve fibre groups of different diameter by triangular shaped stimulus pulses. *J Physiol (Lond)* 273: 539–560, 1977.
2. **Blackshaw LA, Page AJ, and Partosoedarso ER.** Acute effects of capsaicin on gastrointestinal vagal afferents. *Neuroscience* 96: 407–416, 2000.
3. **Cervero F and Sharkey KA.** An electrophysiological and anatomical study of intestinal afferent fibers in the rat. *J Physiol (Lond)* 401: 381–397, 1988.
4. **Cherbut C, Aube AC, Blottiere HM, Pacaud P, Scarpignato C, and Galmiche JP.** In vitro contractile effects of short chain fatty acids in the rat terminal ileum. *Gut* 38: 53–58, 1996.
5. **Cottrell DF and Iggo A.** Mucosal enteroreceptors with vagal afferent fibres in the proximal duodenum of sheep. *J Physiol (Lond)* 354: 497–522, 1984.
6. **Cottrell DF and Iggo A.** Tension receptors with vagal afferent fibres in the proximal duodenum and pyloric sphincter of sheep. *J Physiol (Lond)* 354: 457–475, 1984.
7. **Crichlow EC and Leek BF.** The importance of pH in relation to the acid-excitation of epithelial receptors in the reticulorumen of the sheep (Abstract). *J Physiol (Lond)* 310: 60P, 1981.
8. **Cuche G, Cuber JC, and Malbert CH.** Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway. *Am J Physiol Gastrointest Liver Physiol* 279: G925–G930, 2000.
9. **Cuche G and Malbert CH.** Relationships between cecoileal reflux and ileal motor patterns in conscious pigs. *Am J Physiol Gastrointest Liver Physiol* 274: G35–G41, 1998.
10. **Cuche G and Malbert CH.** Ileal short-chain fatty acids inhibit transpyloric flow in pigs. *Scand J Gastroenterol* 34: 149–155, 1999.
11. **Cuche G and Malbert CH.** Short-chain fatty acids present in the ileum inhibit fasting gastrointestinal motility in conscious pigs. *Neurogastroenterol Motil* 11: 219–226, 1999.
12. **Dinning PG, Bampton PA, Kennedy ML, and Cook IJ.** Relationship between terminal ileal pressure waves and propagating proximal colonic pressure waves. *Am J Physiol Gastrointest Liver Physiol* 277: G983–G992, 1999.
13. **Distrutti E, Azpiroz F, Soldevilla A, and Malagelada JR.** Gastric wall tension determines perception of gastric distention. *Gastroenterology* 116: 1035–1042, 1999.
14. **Etienne M.** Premiers résultats concernant les quantités d'acides gras volatils et d'acide lactique présentes dans le tube digestif du porc. *J Rech Porcine Fr* 1: 131–136, 1969.
15. **Fass R, Naliboff B, Higa L, Johnson C, Kodner A, Munakata J, Ngo JM, and Mayer EA.** Differential effect of long-term esophageal acid exposure on mechanosensitivity and chemosensitivity in humans. *Gastroenterology* 115: 1363–1373, 1998.
16. **Fich A, Phillips S, Hakim N, Brown M, and Zinsmeister AR.** Stimulation of ileal emptying by short-chain fatty acids. *Dig Dis Sci* 34: 1516–1520, 1989.
17. **Fox AJ, Barnes PJ, Urban L, and Dray A.** An in vitro study of the properties of single vagal afferents innervating guinea-pig airways. *J Physiol (Lond)* 469: 21–35, 1993.
18. **Furness JB, Bornstein JC, Kunze WAA, Bertrand PP, Kelly H, and Thomas EA.** Experimental basis for realistic large-scale computer simulation of the enteric nervous system. *Clin Exp Pharmacol Physiol* 23: 786–792, 1996.
19. **Gadicke R and Albus K.** Real-time separation of multineuron recordings with a DSP32C signal processor. *J Neurosci Methods* 57: 187–193, 1995.
20. **Grundy D and Scratcherd T.** Sensory afferents from the gastrointestinal tract. In: *Handbook of Physiology. The Gastrointestinal System. Motility and Circulation*. Bethesda, MD: Am. Physiol. Soc., 1989, sect. 6, vol. I, pt. 1, chapt. 16, p. 593–620.
21. **Holzer P.** Neural Injury, Repair, and Adaptation in the GI Tract. II. The elusive action of capsaicin on the vagus nerve. *Am J Physiol Gastrointest Liver Physiol* 275: G8–G13, 1998.
22. **Iggo A.** The electrophysiological identification of single nerve fibres with particular reference to the slowest conducting vagal afferent fibres in the cat. *J Physiol (Lond)* 142: 110–126, 1958.
23. **Jeanningros R.** Vagal unitary responses to intestinal amino acid infusions in the anesthetized cat: a putative signal for protein induced satiety. *Physiol Behav* 28: 9–21, 1982.
24. **Kalogeris TJ, Rodriguez MD, and Tso P.** Control of synthesis and secretion of intestinal apolipoprotein A-IV by lipid. *J Nutr* 127: 537S–543S, 1997.
25. **Kamath PS, Hoepfner MT, and Phillips SF.** Volatile fatty acids stimulate ileal peristalsis (Abstract). *Gastroenterology* 90: A1482, 1987.
26. **Kamath PS and Phillips SF.** Initiation of motility in canine ileum by short chain fatty acids and inhibition by pharmacological agents. *Gut* 29: 941–948, 1988.
27. **Kohler L, Hedde R, Miedema B, Phillips S, and Kelly KA.** Response of canine ileocolonic sphincter to intraluminal acetic acid and colonic distension. *Dig Dis Sci* 36: 1594–1600, 1991.
28. **Leek BF.** Abdominal and pelvic visceral receptors. *Br Med Bull* 33: 163–168, 1977.
29. **Leek BF and Harding R.** Sensory nervous receptors in the ruminant stomach and the reflex control of reticulo-ruminal motility. In: *Digestion and Metabolism in the Ruminant*, edited by McDonald IW and Warner ACI. Armidale, Australia: Univ. of New England Publ. Unit, 1975, p. 60–76.
30. **Lepionka L and Malbert CH.** Are fundic “tension” receptors sensitive to circumferential wall tension? (Abstract). *Neurogastroenterol Motil* 10: 81, 1998.
31. **Lepionka L, Malbert CH, and Laplace JP.** Proximal gastric distension modifies ingestion rate in pigs. *Reprod Nutr Dev* 34: 449–457, 1997.
32. **Mélone J.** Vagal receptors sensitive to lipids in the small intestine of the cat. *J Auton Nerv Syst* 17: 231–241, 1986.
33. **Nordstrom MA, Mapletoft EA, and Miles TS.** Spike-train acquisition, analysis and real-time experimental control using a graphical programming language (LabView). *J Neurosci Methods* 62: 93–102, 1995.
34. **Omari T, Bakewell M, Fraser R, Malbert CH, Davison G, and Dent J.** Intraluminal micromanometry: an evaluation of the dynamic performance of micro-extrusions and sleeve sensors. *Neurogastroenterol Motil* 8: 241–245, 1996.
35. **Page AJ and Blackshaw LA.** An in vitro study of the properties of vagal afferent fibres innervating the ferret oesophagus and stomach. *J Physiol (Lond)* 512: 907–916, 1998.
36. **Randich A, Tyler WJ, Cox JE, Meller ST, Kelm GR, and Bharaj SS.** Responses of celiac and cervical vagal afferents to infusions of lipids in the jejunum or ileum of the rat. *Am J Physiol Regulatory Integrative Comp Physiol* 278: R34–R43, 2000.
37. **Roze C, Couturier R, Chariot J, and Debray C.** Inhibition of gastric electrical and mechanical activity by intraduodenal agents in pigs and the effects of vagotomy. *Digestion* 15: 526–539, 1977.
38. **Sakata Y, Fujimoto K, Ogata SI, Koyama T, Fukagawa K, Sakai T, and Tso P.** Postabsorptive factors are important for satiation in rats after a lipid meal. *Am J Physiol Gastrointest Liver Physiol* 271: G438–G442, 1996.
39. **Schmitt MG Jr, Soergel KH, Wood CM, and Steff JJ.** Absorption of short-chain fatty acids from the human ileum. *Am J Dig Dis* 22: 340–347, 1977.
40. **Sekizawa S, Ishikawa T, Sant'Ambrogio FB, and Sant'Ambrogio G.** Vagal esophageal receptors in anesthetized dogs: mechanical and chemical responsiveness. *J Appl Physiol* 86: 1231–1235, 1999.
41. **Sengupta JN and Gebhart GF.** Gastrointestinal afferent fibers and sensation. In: *Physiology of the Gastrointestinal Tract* (3rd ed.), edited by Johnson LR. New York: Raven, 1994, vol. 1, p. 483–519.
42. **Sengupta JN, Su X, and Gebhart GF.** Kappa, but not mu or delta, opioids attenuate responses to distention of afferent fibers innervating the rat colon. *Gastroenterology* 111: 968–980, 1996.

43. **Smid SD, Page AJ, O'Donnell T, Langman J, Rowland R, and Blackshaw LA.** Oesophagitis-induced changes in capsaicin-sensitive tachykininergic pathways in the ferret lower oesophageal sphincter. *Neurogastroenterol Motil* 10: 403–411, 1998.
44. **Spiller RC, Lee YC, Edge C, Ralphs DNL, Stewart JS, Bloom SR, and Silk DBA.** Delayed mouth-caecum transit of a lactulose labelled liquid test meal in patients with steatorrhoea caused by partially treated coeliac disease. *Gut* 28: 1275–1282, 1987.
45. **Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, and Julius D.** The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 21: 531–543, 1998.
46. **Whitehead WE, Delvaux M, Azpiroz F, Barlow J, Bradley L, Camilleri M, Crowell MD, Enck P, Fioramonti J, Track J, Mayer EA, Morteau O, Phillips SF, Thompson DG, and Wingate DL.** Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. *Dig Dis Sci* 42: 223–241, 1997.
47. **Yajima T.** Contractile effect of SCFA on isolated colon of the rat. *J Physiol (Lond)* 368: 667–678, 1985.

