Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway

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Cuche, G., J. C. Cuber, and C. H. Malbert. Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway. Am J Physiol Gastrointest Liver Physiol 279: G925–G930, 2000.—The aim of this study was to evaluate the nervous and humoral pathways involved in short-chain fatty acid (SCFA)-induced ileal brake in conscious pigs. The role of extrinsic ileal innervation was evaluated after SCFA infusion in innervated and denervated Babkin’s ileal loops, and gastric motility was measured with strain gauges. Peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) concentrations were evaluated in both situations. The possible involvement of absorbed SCFA was tested by using intravenous infusion of acetate. Ileal SCFA infusion in the intact terminal ileum decreased the amplitude of distal and terminal antral contractions (33 ± 1.2 vs. 49 ± 1.2% of the maximal amplitude recorded before infusion) and increased their frequency (1.5 ± 0.11 vs. 1.3 ± 0.10/min). Similar effects were observed during SCFA infusion in ileal innervated and denervated loops (amplitude, 35 ± 1.0 and 34 ± 0.8 vs. 47 ± 1.3 and 43 ± 1.2%; frequency, 1.4 ± 0.07 and 1.6 ± 0.06 vs. 1.1 ± 0.14 and 1.0 ± 0.12/min). Intravenous acetate did not modify the amplitude and frequency of antral contractions. PYY but not GLP-1 concentrations were increased during SCFA infusion in innervated and denervated loops. In conclusion, ileal SCFA inhibit distal gastric motility by a humoral pathway involving the release of an inhibiting factor, which is likely PYY.

Babkin loops; peptide YY; glucagon-like peptide-1.

NUTRIENTS IN THE ILEUM INHIBIT gastric motility and emptying, a phenomenon called the “ileal brake” (25, 28). Gastroparesia was also triggered by short-chain fatty acids (SCFA) infused in the distal ileum in pigs (7, 9). Unlike nonabsorbed nutrients originating from the upper part of the gut, SCFA were spontaneously present in the distal ileum as a result of frequent coloileal reflux episodes (8). These events, more frequent after a meal, supply SCFA concentration large enough to initiate an ileal brake.

The mechanisms of the ileal brake are still controversial (21). The role of a nervous pathway between the ileum and the stomach has been identified in gastric emptying (26) and proximal gastric tone (2) for carbohydrates. However, in these models, ileal or jejunal infusion will eventually reach the colon, for which clear evidence of a nervous pathway inhibiting gastric motility has been established (4, 6, 12, 31). This, together with data obtained in humans and in dogs indicating that peptide YY (PYY) and possibly glucagon-like peptide-1 (GLP-1) increase during ileal infusion of nutrients, suggests that the ileal brake might be primarily of a humoral nature.

The aim of this study was to evaluate the roles of nervous and humoral pathways between the ileum and the stomach that are activated by the presence of SCFA in the distal ileum as a result of spontaneously occurring coloileal reflux. This was achieved by monitoring gastric motor activity during SCFA infusion in innervated and denervated ileal Babkin loops. The amount of SCFA infused was identical to that present during postprandial reflux episodes. Since SCFA infused in the loops might be absorbed and act directly on gastric motility, we also tested the effect of intravenous infusion of SCFA. Finally, the concentrations of the candidate peptides for a humoral modulation of nutrients inducing ileal brake (PYY and GLP-1) were measured in the same experimental conditions.

MATERIALS AND METHODS

Experimental design. Fifteen female Large White pigs (41 ± 1.0 kg, 3 mo old) were divided into three groups of equal size. Group I, with an intact small bowel, received intravenous or ileal infusions of SCFA. Innervated ileal Babkin loops (3) were constructed in group II to prevent SCFA passage into the cecocolonic segment. Denervation of a similarly created loop was performed in group III. Four animals taken at random in groups II (2 animals) and III (2 animals) were also used, in the interval between motility experiments, for blood sampling and subsequent PYY and GLP-1 assays. Surgical preparation. Under aseptic conditions and general anesthesia, a midline abdominal laparotomy was performed in the three groups. The anesthesia protocol has previously been described in detail (8). Briefly, preanaesthesia was induced by ketamine (5 mg/kg im). Administration of halothane (5% vol/vol) by a face mask suppressed pharyngotracheal reflex, allowing intubation. Anesthesia was main-
tained with inhaled halothane (3% vol/vol). Three strain
gauge force transducers (Vishay Measurements) were su-
tured 8 (proximal antrum), 5 (distal antrum), and 3 (terminal
antrum) cm proximal to the pylorus. Wires were brought
subcutaneously to exit between the shoulders. A silicon cath-
eter (ID = 0.76 mm) was introduced into the external jugular
vein and brought subcutaneously between the shoulders.

The surgical preparation at the ileal level differed among
groups. In group I, with the use of a right lateral laparotomy,
a silicon catheter (ID = 1.5 mm) used for ileal SCFA infusions
was inserted in the distal ileum 15 cm proximal to the
ileocecal sphincter. In groups II and III, ileal Babkin loops (3)
were created. The last 20 cm of the ileum were isolated from
the terminal ileum and placed under the skin. The two ends
of the loop were passed through the skin to create two
stomies. Bowel continuity was restored by an end-to-end
anastomosis. A fine silicon sheet was wrapped loose around
the loop’s mesenteric arcade to help locate the arcade during
the following surgical procedure, which was performed in

Animals were allowed 1 wk to recover from surgery before
the start of the recordings. Once a day and when experiments
were not performed, the loops were rinsed using Sustacal (1
ml/min for 1 h; Mead Johnson).

At the end of the experiments, the animals were killed
with an overdose of pentobarbital sodium, and tissues were
sampled from the loops for histopathological evaluation. No
major alteration could be observed in all loop samples, but
the height of the villi and the thickness of the muscular layer
were less compared with intact ileum collected in pigs of identical
age and weight at the slaughterhouse.

**Experimental protocol.** Studies were performed in con-
scious pigs at 2-day intervals and after at least 14 h of
fasting. Pigs did not have access to water during experi-
ments.

In groups I, II, and III, the effects of an ileal infusion of
a mixture containing 60% acetate (C1), 30% propionate (C2),
and 10% butyrate (C4) (pH 6.8; 0.5 M) administered at a rate
of 2.4 ml/min for 1 h were evaluated compared with the
infusion at the same rate and duration of saline. This mix-
ture has the same SCFA chemical composition as the colonic
refluxate in pigs. In group I only, the effect of intravenous
acetic acid (pH 6.8; 1.2 M, 1 ml/min for 1 h), the only SCFA
found in the blood after intestinal infusion of C2, C3, and C4
mixture (15), was tested vs. an identical intravenous infusion
of saline.

All infusions were started 5 min after the onset of an
antral phase I of a migrating motor complex (MMC). Motility
measurements were performed for 3 h before and after the
infusion.

Blood samples were collected twice before the start of the
ileal infusion. Afterwards, blood was sampled at 0, 15, 30, 45,
60, 75, 90, 120, 150, and 180 min relative to the onset of
SCFA infusion. Plasma was immediately separated (4,500
rpm, 5 min) and stored at −20°C for later analysis.

**Recordings.** Strain gauge signals were recorded on a mul-
tichannel chart recorder (Gould 4042 and Beckman R611)
using a half bridge coupler (1B31; Analog Devices) and si-
multaneously digitized (15 Hz) on a computer (Macintosh II;
Apple Computer) using an A/D card (NB M1016; National
Instruments) after low-pass filtration at 10 Hz. Data were
stored digitally for subsequent analysis.

**Peptides assay.** RIA for PYY was performed as previously
described with antiseraum A4D obtained from a rabbit after
repeated injection of synthetic porcine PYY conjugated to
BSA through ethylcarbodiimide condensation (5). This anti-
serum, which cross-reacted <0.1% with porcine pancreatic
polypeptide and NPY, was used in the assay at a final
dilution of 1:800,000. The synthetic peptide was iodinated
with carrier-free Na125I by means of the chloramine-T re-
agent and was purified by reverse-phase HPLC as previously
described (5). The minimum detectable amount of PYY and
the ID50 of the assay were 1 and 7 fmol/tube, respectively.
Plasma samples run on a Sephadex G-50 column revealed a
single immunoreactive peak coeluting with the synthetic
peptide.

The GLP-1 assay was performed as described earlier (1,
23). Briefly, antiserum against GLP-1-(7–36) amide was ob-
tained in a rabbit by immunization with synthetic GLP-1-(7–
36) amide conjugated to BSA and was used at a final dilution
of 1:300,000. The reactivity of the antiserum 199D was 100%
for GLP-1-(7–36) amide, 84% for GLP-1-(1–36) amide, and,<0.1% for GLP-1-(1–37), GLP-2, glucagon,
secretin, vasoactive intestinal peptide, and gastrointestinal
inhibitory peptide. The synthetic GLP-1-(7–36) amide was
radioiodinated using the chloramine-T method and purified
by reverse HPLC. The detection limit and ID50 were 0.6 and
4.5 fmol/tube, respectively. The plasma samples (1 ml) were
treated with 2 volumes of ethanol. The ethanol extracts were
dried and kept at −30°C. The plasma ethanol extracts were
reconstituted in assay buffer (50 mM phosphate, pH 7.5,
containing 5 mM EDTA and 2% horse serum) on the day of
the assay.

**Data analysis.** Strain gauge data were automatically ana-
alyzed using MAD 3.2 software to evaluate the amplitude and
frequency of antral contractions (7, 9). The amplitude of
contractions was expressed as the percentage of the maximal
amplitude recorded before the infusion. Motility index was
calculated, for the distal antral strain gauge only, as the sum
of amplitude multiplied by frequency of all contractions re-
corded during 1 h. It was expressed as a percentage of the
maximum amplitude recorded before the infusion per
minute. Propagation profiles of antral contractions were ex-
pressed as the percentage of antral contractions occurring in
sequence on three adjacent recording sites. The time window
during which contraction had to occur to be part of a propa-
gated pattern was ±5 s (for adjacent recording locations).
Contractions that did not fulfill this criteria were considered
stationary contractions.

Statistical analysis was performed using Statview soft-
ware (SAS Institute). Data are expressed as means ± SE.
Comparisons between SCFA and saline infusions or between
acetate and saline infusions within the same experimental
group were achieved by one-way analysis of variance. Com-
parisons between experimental groups were performed by
two-way analysis of variance. Fischer protected least signif-
cant differences test was used in both situations to test the
level of significance of P < 0.05. Comparisons for PYY and
GLP-1 profiles were achieved with repeated-measures analy-
sis of variance.

**RESULTS**

**Parenteral acetate.** The amplitude and frequency of
antral contractions were not significantly modified
during intravenous acetate compared with saline infu-
sion (Figs. 1 and 2). Distal antral motility index was
also similar for acetate and saline infusions (3,225 ±
476.4 vs. 3,899 ± 440.1%/min for saline and acetate
infusions, respectively; P > 0.05). Recurrence and du-
ration of the MMC phases were identical (P > 0.05) for
both treatments; hence the MMC duration was not significantly different (69 ± 8 vs. 62 ± 4 min). Antral contraction propagation patterns were identical for saline and acetate infusions (stationary contractions, 17 ± 3.7 vs. 18 ± 2.3%; contractions propagated over the 3 recording sites, 46 ± 6.2 vs. 41 ± 5.2%, respectively, during saline and C₂ infused; P > 0.05).

**Enteral SCFA.** SCFA mixture infused in the intact terminal ileum (group I) decreased the amplitude and increased the frequency of antral contractions irrespective of the recording site (Figs. 3 and 4). The motility index recorded at the distal antral level was reduced by 35% compared with saline (2,624 ± 503.4 vs. 4,077 ± 388.2%/min; P < 0.05). Gastric MMC duration was unchanged during SCFA infusion compared with saline (74 ± 4 vs. 82 ± 4 min; P > 0.05). However, the first MMC recorded at the completion of the infusion lasted significantly longer for SCFA than saline (94 ± 5 vs. 67 ± 4 min; P < 0.05). Afterwards, the characteristics of the MMC were similar to preinfusion ones. The percentage of stationary contractions was greater during ileal SCFA infusion compared with saline, whereas that of propagated ones was reduced (Table 1).

Antral motility was also modified by SCFA mixture compared with saline infusion within innervated ileal loop (group II), the amplitude of antral contractions being decreased and their frequency being increased by SCFA. Similarly, SCFA infusion decreased distal antral motility index compared with saline (2,343 ± 273.4 vs. 3,294 ± 254.2%/min; P < 0.05). This reduced antral motility was within the range of that found for group I (2,343 ± 273.4 vs. 2,624 ± 503.4%/min; P > 0.05). In the same way as for group I, MMC duration was not modified during SCFA infusion (73 ± 6 vs. 74 ± 8 min for SCFA vs. saline; P > 0.05) and was significantly increased at the completion of infusion (90 ± 5 vs. 78 ± 4 min for SCFA vs. saline; P < 0.05). Antral contraction propagation patterns were altered by SCFA compared with saline as for group I (Table 1).

The SCFA-induced motility changes described for groups I and II were not abolished in animals with a denervated ileal loop (group III). Distal antral motility index was decreased by SCFA mixture infusion within the denervated ileal loop (2,210 ± 342.4 vs. 3,081 ± 283.8%/min for SCFA vs. saline; P < 0.05). This reduction was comparable to that observed for groups I and II (P > 0.05). Changes in MMC duration similar to those described in groups I and II were also found in group III (77 ± 4 vs. 63 ± 4 min for SCFA vs. saline; P < 0.05). Contraction propagation patterns were changed by SCFA vs. saline, and these changes were not significantly different between groups I and III (Table 1).

**PYY concentrations.** PYY plasma concentration was increased from 15 to 60 min after the onset of ileal saline or SCFA infusion compared with preinfusion values (414 ± 57.0 vs. 294 ± 51.3 pg/ml). Nevertheless, this increase was significantly greater for SCFA infusion at 30 and 45 min compared with saline (Fig. 5). Plasma PYY concentrations recovered values similar to basal level 15 min after the completion of infusion. The area under the curve of PYY response to ileal SCFA was increased compared with saline infusion (6,648 ± 2,932.6 vs. -285 ± 2,281.8 pg·ml⁻¹·h; P < 0.05). The effect of SCFA infusion on PYY concentration was not significantly different (P > 0.05) for groups II and III.

**GLP-1 concentrations.** Plasma GLP-1 concentration increased during SCFA and saline infusions compared with preinfusion values. Nevertheless, no significant differences were noticed between saline and SCFA infusions irrespective of sampling time (Fig. 5). Similarly, the area under the curve was identical for both situations (111 ± 98.7 vs. 299 ± 107.9 pg·ml⁻¹·h, respectively, for saline and SCFA; P > 0.05). Finally,
Fig. 3. Antral motility during saline or short-chain fatty acid (SCFA) mixture infusion in intact terminal ileum (group I; A), innervated ileal loop (group II; B), and denervated ileal loop (group III; C). Gastric motor inhibitions induced by SCFA were similar for all groups. Data shown were obtained in 1 representative animal for each experimental group.

Fig. 4. Characteristics of antral contractions during saline or SCFA mixture infusion in intact terminal ileum (group I; A), innervated ileal loops (group II; B), and denervated ileal loops (group III; C). For each group, the amplitude of antral contractions was significantly ($P < 0.05$) decreased and their frequency was increased by ileal SCFA compared with saline. *$P < 0.05$ compared with saline.
there was no difference between GLP-1 responses in groups II and III (P > 0.05).

DISCUSSION

Using innervated and denervated ileal loops, we have demonstrated that, in conscious pigs, extrinsic ileal innervation was not necessary for ileal SCFA to inhibit gastric motility. PYY but not GLP-1 was released in the bloodstream during SCFA ileal infusion and might be responsible for gastric motility inhibition.

There is considerable evidence that the distal intestine participates in the regulation of proximal gut function (19). In physiological situations, stimulation of the ileum occurs in the late postprandial regulation of gastrointestinal response (17), whereas in malabsorptive states, impaired gastric emptying and intestinal transit are adaptive answers to compensate for the absorption deficit (27). The ileal brake consists of a variety of motor, secretory, and behavioral responses, including gastric motor inhibition (10), reduction of gastric emptying rate (30), lower interdigestive gastric acid output (18), and suppression of short-term food intake (29). The chemical nature of the nutrients triggering the ileal brake is species dependent and controversial since in dogs glucose, lipids, and proteins have been found effective (27), whereas in humans proteins and glucose are not (30). Nevertheless, lipids are the most potent class of compounds. Whereas their mechanism of action toward ileal mucosa is not well understood, Lin et al. (21) suggested that they might involve the end product of lipid digestion i.e., SCFA. This, together with the presence of SCFA within the ileum as a result of coloic reflux (8), might explain the exquisite sensitivity of the ileal brake to SCFA in pigs.

The results from parenteral acetate infusion invalidate the possibility that absorbed SCFA per se might represent an effective humoral inhibitory agent toward gastric motility. Indeed, 30 μmol·kg⁻¹·min⁻¹ intravenous acetate, the sole SCFA present in the blood after SCFA mixture ileal infusion (15), was ineffective in triggering significant antral motility changes, whereas a similar amount infused within the ileum inhibited gastric motility. Similarly, the antral inhibition was not related to colonic stimulation by SCFA transported together with the intestinal fluid from the ileum to the colon, since gastroparesis was also present while SCFA were perfused in the ileal loop (group II).

Our demonstration of a pure humoral pathway at the origin of an ileal brake in pigs is in accordance with the reversion of lipid-induced ileal brake with the use of PYY immunoneutralization in dogs (21). Nevertheless, the previous experiments are partially in contradiction with an extrinsic nervous pathway triggered either by carbohydrate (26) or a carbohydrate-containing mixture (2) demonstrated in dogs. Although the characteristics of the antral contractions have not been evaluated in the former studies, they clearly demonstrated that undigested nutrients effectively inhibit gastric tone and total gastric emptying rate by a nervous pathway. Hence, it could be speculated that the ileal brake triggered by fat is of humoral origin, whereas the one triggered by carbohydrate is of nervous origin.

Whereas our experiment univocally demonstrates the humoral nature of the SCFA-induced ileal brake, the inhibitory substance released by the ileum wall is still putative. GLP-1 was not involved since its plasmatic concentrations were unchanged by SCFA infusion. This confirms the secondary role of GLP-1 already suggested in humans (20). On the contrary, the increased plasmatic PYY concentration after SCFA infusion would suggest a strong involvement of PYY in ileal brake mechanism.

Table 1. Propagation profile of antral contractions during ileal saline or SCFA infusions in intact terminal ileum, innervated loops, and denervated loops.

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
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<tbody>
<tr>
<td>% of stationary contractions</td>
<td>19±4.5</td>
<td>17±2.0</td>
<td>19±1.8</td>
</tr>
<tr>
<td>SCFA</td>
<td>27±4.2*</td>
<td>26±3.3*</td>
<td>27±3.1*</td>
</tr>
<tr>
<td>% of contractions propagated over the 3 recording sites</td>
<td>40±6.9</td>
<td>39±3.0</td>
<td>37±4.6</td>
</tr>
<tr>
<td>saline</td>
<td>26±6.0*</td>
<td>29±4.7*</td>
<td>27±5.7*</td>
</tr>
<tr>
<td>SCFA</td>
<td>26±6.0*</td>
<td>29±4.7*</td>
<td>27±5.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 pigs/group. SCFA, short-chain fatty acids; group I, intact terminal ileum; group II, innervated loops; group III, denervated loops. *P < 0.05 for saline vs. SCFA mixture.

Fig. 5. Plasma concentrations of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) during ileal infusion of SCFA or saline in group II (innervated loops). Plasma PYY levels were about doubled at 30 and 45 min after the onset of SCFA infusion. Plasma GLP-1 levels were not significantly modified by ileal SCFA infusion irrespective of sampling time. Values are means ± SE. *P < 0.05 for saline vs. SCFA infusions.
sion within the innervated or denervated ileal loop is indicative of a possible role for this peptide. Unlike in previous experiments in rats and rabbits (11, 22, 24), PYY was actually released by the ileum and not as a result of an SCFA-induced colonic stimulation. Surprisingly, PYY concentrations were about doubled during ileal SCFA infusion in pigs, whereas in rats, PYY concentrations were unchanged under the same circumstances (11). However, major interspecies differences in PYY responses have already been mentioned in response to intraluminal nutrient infusions (14). In addition to an overall increase in PYY concentration, supplementary events seem to support a role for PYY in SCFA-induced ileal brake. PYY concentration after SCFA was significantly different from saline 30 min after the onset of infusion, a delay consistent with the onset of gastric inhibition. Similarly, whereas gastric inhibition lasted 15 min after the completion of the SCFA infusion, PYY peak also extended for a similar duration. Finally, the concept of PYY participation in the ileal brake has been suggested by several groups (13, 16, 20) and has received major additional support from the finding that immunoneutralization of PYY reversed the inhibitory consequences of ileal infusion of lipids (21).

In conclusion, we have demonstrated that gastric inhibition triggered by SCFA infused in the ileum in quantities similar to those observed during physiological coloileal reflex episodes is of humoral nature. It likely involves PYY release from the ileal mucosa. An extrinsic neuronal pathway, if present, is not mandatory for the reflex to occur.

REFERENCES