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Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat

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Cherbut, Christine, Laurent Ferrier, Claude Rozé, Younès Anini, Hervé Blottière, Gérard Lecannu, and Jean-Paul Galmiche. Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. *Am. J. Physiol.* 275 (Gastrointest. Liver Physiol. 38): G1415–G1422, 1998.—Short-chain fatty acids (SCFAs) are recognized as the major anions of the large intestinal content in humans, but their effect on colonic motility is controversial. This study explores the colonic motor effect of SCFAs and their mechanisms in the rat. Colonic motility (electromyography) and transit time (plastic markers) were measured in conscious rats while SCFAs were infused into the colon, either alone or after administration of neural antagonists or immunoneutralization of circulating polypeptide YY (PYY). SCFA-induced PYY release was measured by RIA and then simulated by infusing exogenous PYY. Intracolonic infusion of 0.4 mmol/h SCFAs had no effect, whereas 2 mmol/h SCFAs reduced colonic motility (36 ± 3 vs. 57 ± 4 spike bursts/h with saline, $P < 0.05$) by decreasing the ratio of nonpropulsive to propulsive activity. This resulted in an increased transit rate ($P < 0.01$). Neither α -adrenoceptor blockade nor nitric oxide synthase inhibition prevented SCFA-induced motility reduction. Intraluminal procaine infusion suppressed the SCFA effect, indicating that a local neural mechanism was involved. SCFA colonic infusion stimulated PYY release in blood. Immunoneutralization of circulating PYY abolished the effect of SCFAs on colonic motility, whereas exogenous PYY infusion partly reproduced this effect. SCFAs modify colonic motor patterns in the rat and increase transit rate; local nerve fibers and PYY are involved in this effect.

colon; transit; fermentation

SHORT-CHAIN FATTY ACIDS (SCFAs) are produced during bacterial fermentation of carbohydrates and glycoproteins in the large intestine of monogastric mammals, including humans. The luminal colonic concentration of SCFAs, although dependent on the digestive state and nature of the diet, is usually ~ 130 mmol/l in humans (6). SCFAs, which represent the major anions of the colonic content, consist mainly of acetate, propionate, and butyrate in the molar ratio 57:21:22%, respectively, in the human right colon (6). It is now established that SCFAs influence large intestine physiology (7). They enhance water and electrolyte absorption and modulate intestinal mucosal growth. In addition, buty-

rate displays several antiproliferative and differentiating effects on neoplastic cell lines, whereas SCFA administration has been reported to relieve symptoms and to improve histological and endoscopic scores in patients with diversion or ulcerative colitis.

SCFAs have numerous biological and clinical properties, and their effect on small intestinal motility has been extensively investigated (7, 13, 15, 21); however, their effect on colonic motility remains controversial. Using isolated segments of rat colon in vitro, Yajima (39) observed that low doses of propionate caused a phasic contraction. On the contrary, Squires et al. (35) observed that infusion of physiological concentrations of SCFAs into the lumen of perfused rat colon in vitro almost completely abolished contractile activity. Finally, Flourié et al. (10) did not find any effect when SCFAs were perfused into an isolated colonic loop in the dog.

SCFAs may modulate colonic motility in three different ways. First, they may stimulate mucosal receptors connected to enteric and/or vagal nerves, as proposed by Yajima (39): epithelial SCFA-sensitive chemoreceptors, whose stimulation inhibits motility via a vago-vagal reflex, have been detected in the reticulorumen of ruminants (19). Second, SCFAs may act directly on colonic smooth muscle, which was demonstrated on isolated ileal muscle strips (4). Finally, SCFAs may release gastrointestinal regulatory peptides that modulate intestinal motility; for instance, SCFA administration into isolated perfused rabbit and rat colon increased polypeptide YY (PYY) concentration in the circulation (20, 27). PYY exerts several inhibitory effects on gastric emptying and small intestinal motility in various species, including rats, dogs, and humans (1, 25). The effects of PYY on colonic motility are still not clearly established; however, several in vitro studies show that PYY inhibits contractile activity and induces relaxation in colonic strips (18, 31, 37). Conversely, intravenous administration at a pharmacological dose increased intraluminal pressures of the duodenum and colon in anesthetized rats (38).

This work has two purposes: 1) to define the effects of SCFAs on rat colonic motility and transit time in vivo and 2) to investigate their mechanism of action. In particular, the possible implication of PYY was sought.

METHODS

Animals

Eighty-two male Wistar rats, weighing 250–300 g, were used and distributed in five groups as detailed in *Experimen-*

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tal Procedures. All rats were surgically equipped with an intracolonic catheter (1.6 mm ID, 3.2 mm OD; Tygon, Bio-block, Strasbourg-Illkirch, France) placed in the cecum, pushed through the cecocolonic junction, and fixed with its tip in the lumen of the first 2 cm of the proximal colon. This catheter was used to infuse SCFAs or saline into the colon. In addition, certain rats received an intravenous catheter (0.58 mm ID, 0.98 mm OD, polyethylene tube 3403; Guerbet Biomedical, Louvres, France) inserted in the jugular vein that was used to administrate PYY or PYY-antiserum or an intracarotidian catheter (polyethylene as described above, length 15 mm, continued by a Silastic tube, 0.64 mm ID, 1.19 mm OD, length 15 cm) for blood sampling.

Myoelectrical Activity and Transit Time

Nichrome electrodes (diameter 0.8 mm; Microfil Industrie, Renens, Switzerland) were implanted in groups of three, 2 mm apart, in the large intestine of the rats of groups 1, 3 and 5 (see *Experimental Procedures* for definition of groups), using a previously described technique (29). Five electrode groups were implanted in the colon 3 cm from each other. The first group was placed near the tip of the infusion catheter, i.e., ~2 cm after the cecocolonic junction.

In group 2 rats, a rigid plastic cannula (5 mm ID, 8 mm OD) was inserted into the cecum 5 cm from the cecocolonic junction to introduce transit markers into the large intestine.

After at least 8 days of postoperative recovery, motility and transit time were measured in the conscious and free-moving animals as described in Ref. 5. Myoelectrical activity was recorded using an electroencephalograph (mini-Reega VII, Alvar, Paris, France) set at a short time constant (0.01 s) to eliminate the low-frequency components of the electromyogram. Electromyogram analysis consisted of determining the number of colonic spike bursts (phasic contractions) per hour, for 1 h before beginning the colonic infusion and for 1–3 h thereafter. Three types of spike bursts were distinguished: those not propagating >2 cm ("stationary"), those propagating >2 cm and <10 cm ("shortly propagated"), and those propagating under all the recording electrodes, i.e., >14 cm ("totally propagated"). The number of each type of spike burst was counted at each recording site. Because the first analysis showed no significant difference in myoelectrical activity between the different recording sites, the results were finally expressed in each rat as the mean values corresponding to all recording sites. A motility index, expressed as the percentage of basal activity, was calculated by dividing the number of total spike bursts recorded per hour during colonic infusion by the number of total spike bursts recorded per hour immediately before the infusion (basal activity).

Twenty transit markers were introduced through the cannula into the cecum of each rat. The markers consisted of plastic pellets (specific gravity 1.05, diameter 2 mm; BP Chimie, Lavera, France), which were totally recovered in the colonic digesta. The geometric center (GC) method was used to quantify the transit of markers as follows (22): $GC = \sum ROI_i \cdot i/n$, where ROI_i is the number of markers in the region of interest, i is the region of interest, and n is the total number of markers. This method gives a number that reflects the center of gravity of the overall progression of the markers. A GC of 1 means that all markers were found in the first segment (i.e., the cecum), whereas a GC of 5 means that all markers had been expelled from the colon.

Colonic Infusions

Four different SCFA solutions were tested: three of them contained a single SCFA, namely, acetic or propionic or

butyric acid, whereas the fourth one was a mixture of 65% acetic acid, 20% propionic acid, and 15% butyric acid, which are the mean proportions found in rat colonic contents (17). A 0.15 mol/l NaCl solution (saline) was also infused in each rat as control. Colonic infusions were performed at 4 ml/h for 1 h. Two concentrations of SCFAs were used: 100 mmol/l and 500 mmol/l, which led to administration of doses of 0.4 and 2 mmol SCFAs, respectively, over 1 h. The osmolarity of 0.15 mol/l NaCl, 100 mmol/l SCFAs, and 500 mmol/l SCFAs was 285, 301 and 996 mosmol/l, respectively. To test the effect of hyperosmolarity on colonic myoelectrical activity, a solution of 0.5 mol/l NaCl (1,012 mosmol/l) was infused (4 ml/h for 1 h) into the colon of four rats. The pH of all the solutions was adjusted to 6.2–6.4 with HCl or NaOH.

Experimental Procedures

For colonic motility or transit measurement experiments, the rats were deprived of food every day from 8:00 AM until 5:00 PM, and allowed food (standard meal for rats, A04, UAR, Epinay sur Orge, France) ad libitum during the rest of the nycthemera.

Group 1: Effect of SCFAs on colonic myoelectrical activity. All SCFA solutions and saline were infused in random order in each rat (one solution per day; $n = 8$ rats). Colonic myoelectrical activity was recorded for 3 h from the beginning of the infusion of saline, 0.4 and 2 mmol/h SCFA mixtures, and for 1 h during the infusion of the individual SCFAs.

Group 2: Effect of SCFAs on colonic transit time. A 1-h colonic infusion of saline (8 rats), 0.4 mmol/h SCFA mixture (8 rats), 2 mmol/h SCFA mixture (8 rats), or 2 mmol/h butyrate (8 rats) began 4 h after cecal administration of the transit markers ($n = 32$ rats total for group 2). At the end of the infusion, the rats were killed by intracardiac injection of pentobarbital sodium and the large intestine was smoothly exteriorized and divided into five compartments: cecum (compartment 1), three colonic segments of equal length (compartments 2 to 4), and feces expelled during the experiment (compartment 5). Markers were counted in each compartment, and the GC of distribution was calculated as mentioned above. Digesta collected in the proximal part of the colon (compartment 2) were weighed. Their water content was determined by freeze-drying, and SCFA concentrations were measured by gas chromatography as previously described (14). In addition, water content of feces expelled during the experiment was determined.

Group 3: Effect of pharmacological agents on SCFA-induced inhibition of myoelectrical activity. Colonic myoelectrical activity was recorded before and during the infusion of either saline or 2 mmol/h SCFAs over 1 h ($n = 8$ rats). The following compounds were administered 10–30 min before the SCFA infusion: a local anesthetic (3% procaine hydrochloride, 1 ml in the proximal colon), an α -adrenergic antagonist (phentolamine hydrochloride at 3 and 5 mg/kg im), an α -1 antagonist (prazosin hydrochloride at 0.5 and 1 mg/kg sc), an α -2 antagonist (yohimbine hydrochloride at 1 and 2 mg/kg ip), and two nitric oxide synthase inhibitors [N^w -nitro-L-arginine methyl ester (L-NAME) at 10 mg/kg ip and N^w -nitro-L-arginine (L-NNA) at 10 mg/kg ip].

Group 4: Measurement of plasma concentrations of PYY after SCFA infusion and simulation with exogenous PYY. Under Urethane anesthesia (1.25 g/kg im), a catheter was inserted into the carotid artery and blood was sampled (0.6 or 1 ml on EDTA + aprotinine) just before beginning the 1-h colonic infusion of saline ($n = 4$) or 2 mmol/h SCFAs ($n = 6$) and 15, 30, 60, and 120 min thereafter. After a 3-min centrifugation at 7,000 g , the plasma samples were collected and stored at -20°C until the PYY RIA, which was performed

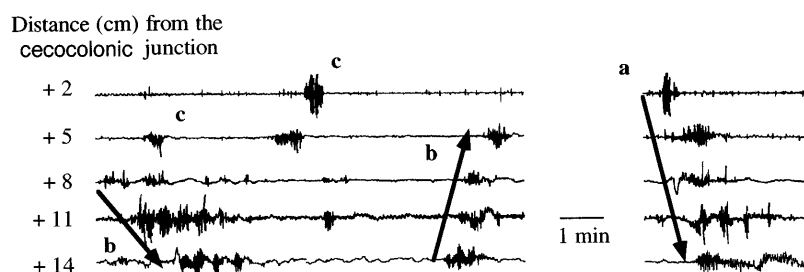


Fig. 1. Typical patterns of the electromyogram of the rat colon. *a*: Totally propagated bursts, recorded under all the electrode sites (>14 cm). *b*: Shortly propagated bursts, propagating over short distances either in the aborad or orad direction. *c*: Stationary bursts.

as previously described (11). To determine the plasma concentrations of PYY achieved by intravenous infusion of exogenous PYY, six rats received an intravenous step-dose infusion of synthetic porcine PYY at 24, 80, and 240 pmol $\cdot$ kg $^{-1}$ $\cdot$ h $^{-1}$ (30 min each). At the end of each infusion period, blood was sampled as described above and analyzed for PYY.

Group 5: Effect of PYY immunoneutralization and exogenous PYY on colonic myoelectrical activity. Six rats, equipped with colonic electrodes, received one of the following treatments every day: 1) 50 μ l iv normal rabbit serum and intracolonic infusion of 0.15 mol/l NaCl for 1 h (IC-saline), 2) 50 μ l iv normal rabbit serum and intracolonic infusion of 2 mmol/h SCFAs (IC-SCFA), 3) intravenous infusion of 25 pmol $\cdot$ kg $^{-1}$ $\cdot$ h $^{-1}$ PYY for 1 h and IC-saline, or 4) 50 μ l iv PYY antiserum and IC-SCFAs. Colonic myoelectrical activity was recorded for 1 h before and 1 h during the colonic infusions.

Drugs

SCFAs (acetic acid, propionic acid, and butyric acid), NaCl, phentolamine, prazosin, L-NAME, and synthetic porcine PYY were purchased from Sigma (L'Isle d'Abeau, St. Quentin Fallavier, France), procaine hydrochloride was from Merck (Grosseron, St. Herblain, France), and yohimbine was from Janssen Chimica (Noisy Le Grand, France). Rabbit PYY antiserum was a generous gift of Dr. G. H. Greeley and was prepared against synthetic porcine PYY; it cross-reacted $<1\%$ with neuropeptide Y, and 5 μ l of this PYY antiserum suppressed the effect of 10 pmol/kg exogenous PYY on vasoactive intestinal polypeptide-stimulated rat jejunal water secretion, in the conditions stated in Ref. 34.

Statistics

Results are expressed as means $\pm$ SE. In most cases, the results were compared by ANOVA, followed when significant by a multigroup comparison test (Scheffé's). The effects of saline and SCFAs on the distribution of transit markers in the large intestine and on the composition of digesta were compared by the Student's *t*-test for unpaired data. A *P* value < 0.05 was considered significant.

RESULTS

Myoelectrical Activity of the Rat Colon in Control Conditions and the Effect of Hyperosmolarity

Spike bursts were recorded at all electrode sites in the first 10 cm of the rat colon in the basal state and under saline infusion. They occurred as contractile periods separated by quiescent periods during which no activity was recorded. During the contractile periods, the spike bursts were organized in three distinct patterns: 1) bursts propagating over all the recording sites at a high velocity (30.7 ± 0.9 cm/min, Fig. 1*a*, totally propagated bursts), 2) bursts propagating >2 cm and

<10 cm either in the orad or aborad direction (Fig. 1*b*, shortly propagated bursts), and 3) stationary bursts, which did not propagate >3 cm (Fig. 1*c*). The totally propagated bursts did not appear frequently and amounted to only $9.3 \pm 3.5\%$ of the overall spike bursts. The occurrence of the shortly propagated bursts was $29.0 \pm 6.2\%$ ($17.5 \pm 5.3\%$ and $10.5 \pm 4.2\%$ in the aborad and orad directions, respectively), whereas the stationary bursts accounted for $61.7 \pm 11.8\%$ of overall spike bursts.

Infusion of 0.5 mol/l NaCl did not significantly modify these patterns of activity compared with infusion of 0.15 mol/l NaCl ($P = 0.924$, 0.845 , and 0.836 for totally propagated, shortly propagated, and stationary bursts, respectively).

Dose-Dependent Effect of SCFAs on Myoelectrical Activity and Transit Time

Infusion of 0.4 mmol/h SCFAs had no effect on colonic myoelectrical activity (Table 1), whereas infusion of 2 mmol/h SCFAs significantly decreased the number of shortly propagated bursts by 56% ($P < 0.05$) and of stationary bursts by 38% ($P < 0.05$) compared with saline (Table 1). They also decreased the ratio of the orally propagated (antiperistaltic) to aborally propagated (peristaltic) bursts: this ratio was 0.64 with saline vs. 0.49 with 2 mmol/h SCFAs. The number of totally propagated bursts was not changed. The inhibitory effect of SCFAs on colonic motility remained significant over 2 h, i.e., 1 h after completion of the colonic infusion of SCFAs (Fig. 2).

The GC of distribution of transit markers in the colon did not change significantly with 0.4 mmol/h SCFAs but increased from 1.84 ± 0.35 with saline to 3.64 ± 0.44 ($P < 0.01$) with 2 mmol/h SCFAs. Thus 2 mmol/l SCFAs

Table 1. Effect of intracolonic infusion of either saline or SCFAs on the number of spike bursts per hour in the rat colon, counted during a 1-h infusion

Activity Patterns	0.6 mmol/h Saline	0.4 mmol/h SCFA	2 mmol/h SCFA
Totally propagated	5.3 ± 0.6	6.6 ± 1.2 (NS)	6.6 ± 1.4 (NS)
Shortly propagated	16.6 ± 1.4	19.7 ± 1.7 (NS)	$7.3 \pm 0.8^*$
Aborally	10.1 ± 1.1	12.8 ± 1.5 (NS)	$4.9 \pm 0.6^*$
Orally	6.5 ± 1.0	6.8 ± 1.6 (NS)	$2.4 \pm 0.7^*$
Stationary	35.4 ± 3.5	36.6 ± 5.3 (NS)	$22.0 \pm 2.7^*$
Total	57.4 ± 4.2	63.1 ± 5.1 (NS)	$35.9 \pm 3.0^*$

Values are means $\pm$ SE of 8 rats. SCFA, short-chain fatty acid. * $P < 0.05$ vs. saline. NS, not significant.

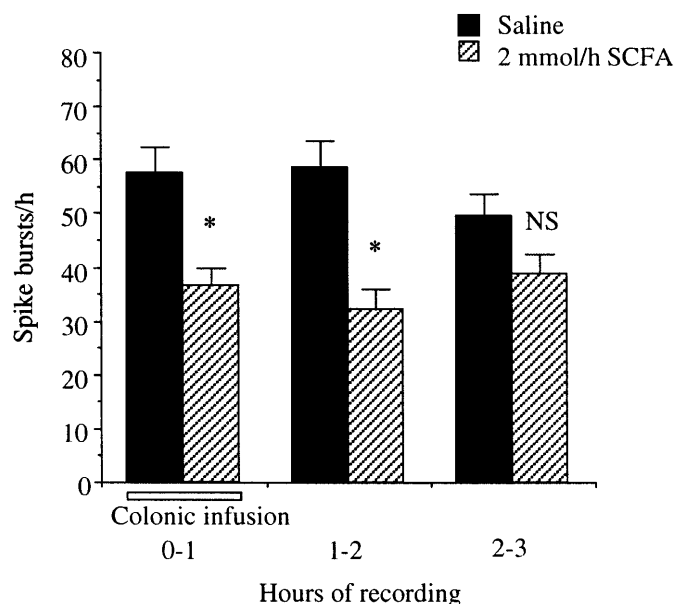


Fig. 2. Total number of spike bursts recorded per hour during and after a 1-h infusion of saline or short-chain fatty acids (2 mmol/h SCFAs) into the rat colon. Values are means $\pm$ SE of 8 animals. * P < 0.05 vs. saline. NS, not significant.

decreased colonic myoelectrical activity and increased colonic transit of solid markers.

Intraluminal SCFA Concentration After Infusion

The SCFA concentration measured in the proximal colonic contents was 76 ± 13 μ mol/g of wet weight after saline infusion. It was not changed by 0.4 mmol/h SCFAs (75 ± 12 μ mol/g), whereas it doubled after administration of 2 mmol/h SCFAs (150 ± 13 μ mol/g). Neither the intraluminal pH (6.4 ± 0.4 with saline vs. 6.5 ± 0.4 with 0.4 mmol/h SCFAs and 6.3 ± 0.2 with 2 mmol/h SCFAs) nor the percentage of water contained in the digesta (84.3 ± 3.2 with saline vs. 81.8 ± 2.7 with 0.4 mmol/h SCFAs and $83.8 \pm 1.5\%$ with 2 mmol/h SCFAs) was significantly affected by SCFA infusions. Furthermore, dry matter content of stools expelled during the intracolonic infusion was similar with saline ($53.2 \pm 2.1\%$) and with SCFAs ($55.7 \pm 1.8\%$ with 0.4 mmol/h and $48.7 \pm 3.2\%$ with 2 mmol/h).

Effect of Individual SCFAs on Myoelectrical Activity

Infusion of 0.4 mmol/h propionate or butyrate had no significant effect, whereas 2 mmol/h propionate or butyrate (but not acetate) significantly decreased the number of myoelectrical events (Fig. 3). Propionate and butyrate (2 mmol/h) reduced the number of shortly propagated bursts ($P = 0.011$), but they did not significantly affect stationary and totally propagated activity ($P = 0.081$ and 0.233 , respectively). Butyrate (2 mmol/h) also significantly increased the progression of colonic transit markers compared with saline (GC = 2.80 ± 0.26 with butyrate vs. 1.84 ± 0.35 with saline, $P < 0.05$), an effect that was not significantly different from that of the SCFA mixture.

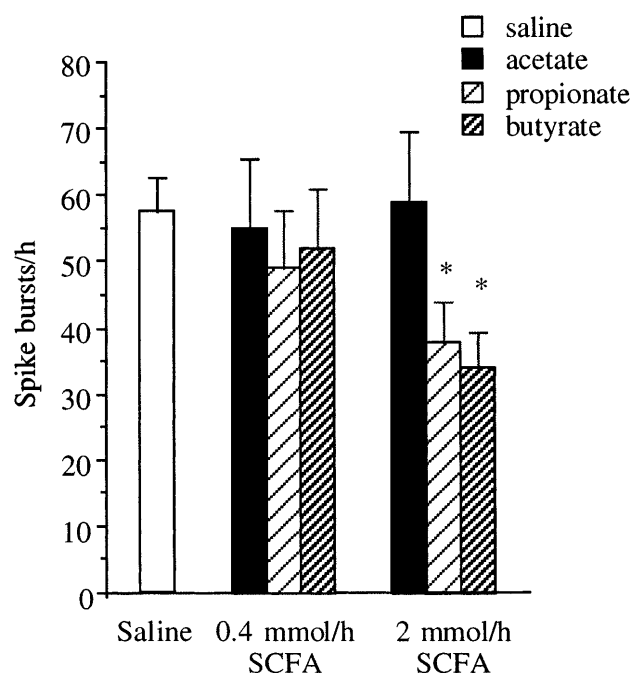


Fig. 3. Total number of spike bursts recorded per hour during a 1-h intracolonic infusion of saline or 0.4 or 2 mmol/h of individual SCFAs (acetate, propionate, or butyrate). Values are means $\pm$ SE of 8 animals. * P < 0.05 vs. saline.

Effect of Pharmacological Agents on SCFA-Reduced Colonic Motility

Procaine alone did not significantly modify colonic myoelectrical activity but suppressed most of the inhibitory effect of SCFAs, which changed from -42% of basal with SCFAs alone to -11% of basal with procaine + SCFAs ($P < 0.05$; Table 2). Pretreatment with either phentolamine or yohimbine tended to increase basal motility (although not significantly), whereas prazosin did not change it (Table 2). Nevertheless, previous administration of these α -adrenoceptor blocking agents did not suppress the inhibitory effects of SCFAs, which decreased the myoelectrical activity by 41% (phentol-

Table 2. Effects of different pharmacological compounds on colonic motility in response to infusion of either saline or SCFAs into rat colon

	Colonic Infusions	
	Saline	2 mmol/h SCFA
Control (0.15 mol/l NaCl, 1 ml ip)	101.2 $\pm$ 3.1	58.3 $\pm$ 4.2*
Procaine (30 mg/kg ic)	104.8 $\pm$ 4.2	89.5 $\pm$ 3.8†
Phentolamine (3 mg/kg im)	126.1 $\pm$ 6.4	74 $\pm$ 5.7*
Phentolamine (5 mg/kg im)	134.2 $\pm$ 5.2	81.3 $\pm$ 4.8*†
Prazosin (0.5 mg/kg sc)	107.1 $\pm$ 4.6	60.1 $\pm$ 4*
Prazosin (1 mg/kg sc)	102.4 $\pm$ 3.7	61.5 $\pm$ 4.1*
Yohimbine (1 mg/kg ip)	123.3 $\pm$ 5.2	75.8 $\pm$ 5.3*
Yohimbine (2 mg/kg ip)	132.8 $\pm$ 6.1	84.2 $\pm$ 5.8*†
L-NAME (10 mg/kg ip)	159.2 $\pm$ 6.8†	84.8 $\pm$ 5.4*†
L-NNA (10 mg/kg ip)	147.1 $\pm$ 5.5†	89.2 $\pm$ 4.7*†

Values are means $\pm$ SE of 8 rats, expressed as percentage of basal activity (no. of total spike bursts) during 1 h preceding colonic infusion. L-NAME, *N*^ω-nitro-L-arginine methyl ester; L-NNA, *N*^ω-nitro-L-arginine. * P < 0.05 vs. saline; † P < 0.05 vs. control.

amine 3 mg/kg im), 38% (yohimbine 1 mg/kg ip), and 44% (prazosin 0.5 mg/kg sc), respectively, compared with the effect of α -blockers alone. Finally, L-NAME alone significantly enhanced colonic motility but did not abolish the effect of SCFAs, which still reduced myoelectrical activity by 47% compared with the effect of L-NAME alone (Table 2). Similarly, L-NNA did not affect the inhibitory effect of SCFAs though increasing myoelectrical activity of the colon (Table 2).

SCFA-Induced PYY Release and Simulation by Exogenous PYY

Infusion of 2 mmol/h SCFAs into the proximal colon increased plasma PYY concentration up to a plateau (~ 30 pmol/l), which remained steady for at least 1 h after the end of infusion, whereas saline infusion did not change plasma PYY (Fig. 4A). Accordingly, the area under the PYY response to SCFAs vs. time (1.69 ± 0.20 nmol $\cdot$ l $^{-1}$ $\cdot$ 120 min $^{-1}$) was significantly higher than that induced by saline (0.14 ± 0.20 nmol $\cdot$ l $^{-1}$ $\cdot$ 120 min $^{-1}$, $P < 0.05$).

Intravenous infusion of synthetic porcine PYY at three different doses produced a dose-dependent increase in plasma PYY levels (Fig. 4B). The dose of

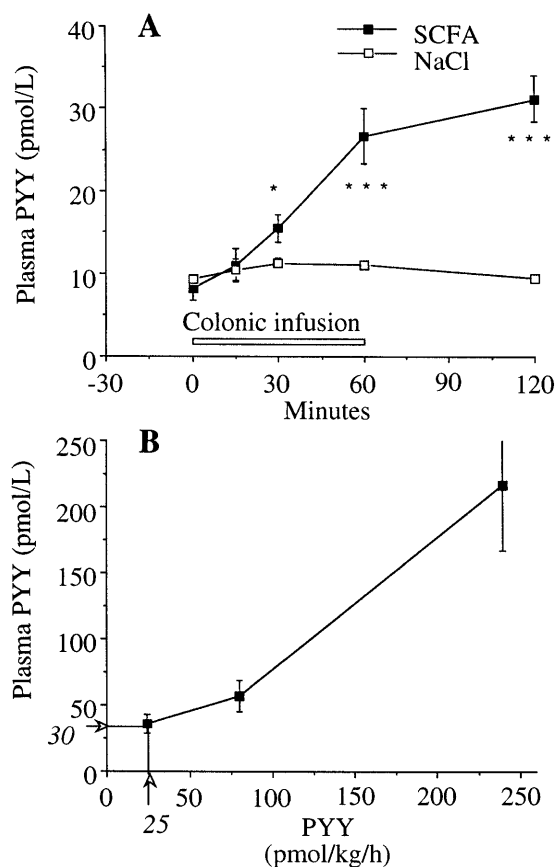


Fig. 4. A: plasma polypeptide YY (PYY) concentrations during and after a 1-h infusion of saline (NaCl) or 2 mmol/h SCFAs into the rat colon. Values are means $\pm$ SE of 6 animals. * $P < 0.05$, *** $P < 0.001$ vs. saline. B: plasma PYY concentrations induced by the intravenous infusion of 24, 80, and 240 pmol $\cdot$ kg $^{-1}$ $\cdot$ h $^{-1}$ of synthetic porcine PYY in the rat. Values are means $\pm$ SE of 6 animals.

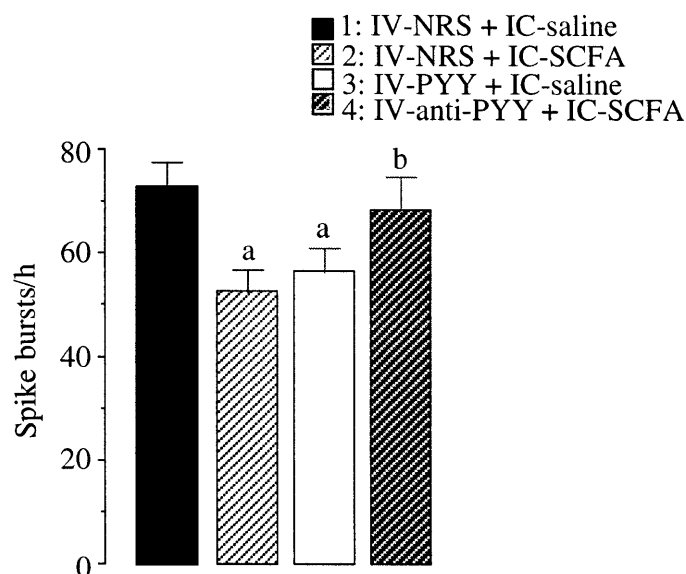


Fig. 5. Total number of spike bursts recorded per hour during each of the following treatments: 1) 50 μ l intravenous normal rabbit serum and 1 h intracolonic infusion of saline (IV-NRS + IC-saline), 2) 50 μ l intravenous normal rabbit serum and 1 h intracolonic infusion of 2 mmol/h SCFAs (IV-NRS + IC-SCFA), 3) 1 h intravenous infusion of 25 pmol $\cdot$ kg $^{-1}$ $\cdot$ h $^{-1}$ PYY and 1 h intracolonic infusion of saline (IV-PYY + IC-saline), and 4) 50 μ l intravenous PYY antiserum and 1 h intracolonic infusion of 2 mmol/h SCFAs (IV-anti-PYY + IC-SCFA). Values are means $\pm$ SE of 6 animals. ^a $P < 0.05$ vs. IV-NRS + IC-saline, ^b $P < 0.05$ vs. IV-NRS + IC-SCFA.

exogenous PYY that achieved the SCFA-induced plasma concentration of PYY was ~ 25 pmol $\cdot$ kg $^{-1}$ $\cdot$ h $^{-1}$.

Effect of Exogenous PYY and of PYY Immunoneutralization on Myoelectrical Activity

Administration of exogenous PYY at a dose that mimicked the effect of 2 mmol/l SCFAs (25 pmol $\cdot$ kg $^{-1}$ $\cdot$ h $^{-1}$ for 1 h) significantly decreased colonic motility by 22% (Fig. 5). This inhibition was not significantly different from the effect of intracolonic SCFAs (2 mmol/h) measured in the same rats, which decreased basal motility by 28% when SCFAs were infused after the previous injection of 50 μ l of normal rabbit serum as a control (Fig. 5). Like SCFAs, PYY decreased the ratio of nonpropulsive (stationary plus shortly propagated spike bursts) to propulsive (totally propagated spike bursts) activity: 7.8 ± 0.6 with PYY vs. 5.3 ± 0.4 with SCFAs vs. 9.3 ± 0.4 with saline ($P < 0.05$). When the colonic infusion of 2 mmol/h SCFAs was administered after the previous injection of 50 μ l of PYY antiserum, the inhibitory effect of SCFAs totally disappeared (Fig. 5).

DISCUSSION

In this study, SCFAs, the main anions of colonic contents, modified myoelectrical activity of the rat colon by reducing shortly propagated and stationary activities and shortened the colonic transit time of undigestible markers. These effects depended on the intraluminal concentration of SCFAs and on their chemical nature and were abolished by local anesthe-

sia. In addition, SCFAs released PYY in the systemic circulation, a PYY antiserum suppressed the SCFA-induced decrease in motility, and exogenous PYY inhibited colonic myoelectrical activity, thus suggesting that PYY could mediate the inhibitory effect of SCFAs on the rat colonic motility.

As previously reported by Ferré and Ruckebusch (9), myoelectrical activity of the rat large intestine comprises long spike bursts that are associated with phasic contractions. These activities may propagate or not, corresponding in the rat to the colon migrating and nonmigrating motor complexes described by Sarna et al. (30) in the dog. In addition to the motor complexes, the rat colon also exhibits totally propagated bursts that move aborally over all the recording sites (more than 14 cm) at a very high speed and that resemble the giant migrating contractions described in the colon of dogs and humans (16, 23). These contractions are thought to be ultrapropulsive and to produce mass movements that precede defecation (16).

Certain patterns of activity were reduced by infusing SCFAs into the proximal colon, a finding consistent with the stimulation of colonic activity that was previously found in the rat after SCFA production was suppressed by oral antibiotic administration (5). A decreased motility was also induced by SCFA in the reticulorumen and large intestine of sheep (12, 36). Opposite effects of SCFAs on the colon were reported in the literature: Squires et al. (35) observed that physiological concentrations of SCFAs abolished contractile activity of the isolated rat colon, whereas Yajima (39) reported that low concentrations of SCFAs stimulate contractions of rat colonic muscle strips *in vitro*. Moreover, Flourié et al. (10) found that SCFAs had no effect on an isolated colonic loop in the dog. These conflicting results may be related to the different species and experimental models used and to the form and concentration of SCFAs applied (i.e., acids vs. sodium salts, very low vs. physiological concentrations). It is, however, worth mentioning that the results obtained in the whole rat colon (35) are consistent with our data in the conscious rat, i.e., in an experimental model more closely reflecting physiological conditions.

The effect of SCFAs on myoelectrical activity depended on the intraluminal concentration: 0.4 mmol SCFAs infused over 1 h did not change the intraluminal concentration (they were probably immediately absorbed) and had no effect on motility, and 2 mmol/h SCFAs increased the intraluminal concentration, which remained, however, in the physiological range (17), and altered motility. This amount remains moderate, since we calculated that 2 mmol SCFAs are theoretically yielded by the fermentation of 200 mg of unabsorbed carbohydrate (such as resistant starch or dietary fiber), which represents ~1% of the daily food intake of a rat. Nevertheless, the SCFA-induced reduction of electrical activity seems discordant with the increase in motility index observed in the rat proximal colon ~5 h after feeding by Ferré and Ruckebusch (9). In addition to the different experimental procedures used in the two studies, this discrepancy could come from the feeding

state of the rats, which received only ~17 g of chow · kg of body wt⁻¹ · day⁻¹ in the previous study, whereas they were fed *ad libitum* in our study (i.e., ~65 g · kg of body wt⁻¹ · day⁻¹). This calculation indicates that the effect of the meal was measured in a nearly empty colon in the work by Ferré and Ruckebusch (9), whereas the effect of SCFAs was estimated in a "normally" filled colon in our study.

The 500 mmol/l SCFA solution was hyperosmolar, but it is unlikely that the osmolarity explains the SCFA effect, since a 0.5 mol/l NaCl solution (1,012 mosmol/l) did not change myoelectrical activity. In addition, although the very slow rate of infusion (4 ml/h) should allow immediate dilution of the solution into the colonic contents, no change in the water concentration of digesta was found, and, when testing individual SCFAs, acetate had no effect, whereas propionate and butyrate modified motility, although all three solutions had the same osmolarity.

The lower ability of acetate to affect contractile activity was already observed *in vitro* in the rat colon (35, 39) as well as in the sheep cecum and rumen *in vivo* (12, 36). Actually, butyrate and to a lesser extent propionate were more efficient than acetate in influencing other biological processes, such as vascular relaxation (24) and intestinal epithelial cell proliferation (33).

The literature suggests that the environmental pH modulates the effect of SCFAs on intestinal motility. The effect does not depend on the proton *per se*, since introduction of acidic solutions without SCFAs into the colon does not affect contractions (35), whereas the motor effect depends on the concentration of the associated SCFAs in the sheep reticulorumen (12). At neutral pH, SCFAs are mostly dissociated, but, when pH lowers, the proportion of associated acids increases. Our results are in agreement with these findings; indeed, we delivered all SCFA solutions at the same acidic pH (6.2–6.4), and we found that the intraluminal pH was always ~6.3 after the infusion, whatever the SCFA concentration used. Therefore, the motor effect cannot result from a simple luminal acidification.

In this study, reduction of myoelectrical activity was associated with an increased transit rate, an apparently paradoxical relationship that was in fact already reported in the large intestine of patients suffering diarrhea or constipation (3). SCFAs preferentially decreased the shortly propagated and stationary activities, whereas they did significantly not affect the totally propagated bursts; they also decreased the ratio of the orally to aborally propagated activity. If the stationary bursts produce to and fro movements but no significant transit rate (32), their reduction will induce an increased transit rate. Also, if the shortly propagated bursts are involved in the propulsion and retropulsion of digesta, as shown for the colonic migrating motor complexes in the dog colon (32), decreasing the ratio of the orally (retropulsive) to aborally propagated (propulsive) bursts by SCFAs will also enhance the net distal propulsion of the markers, hence increasing the transit rate. Besides, we cannot exclude that SCFA infusion

may have induced water secretion, although it seems unlikely. Actually, SCFAs are known to stimulate water absorption rather than secretion (2). Moreover, we did not notice any change in either colonic water content or fecal output of water during and after the infusion.

It has been proposed that the motor effect of SCFAs might be due to a neural-sensitive mechanism located within the mucosa (39), and vagal enteroceptors stimulated by SCFAs have indeed been demonstrated in the ruminant stomach (19). When procaine, an agent that blocks conduction in small unmyelinated nerves, was administered locally, basal motility was not significantly altered, as previously observed in the dog ileum (14), but the inhibitory effect of SCFAs was abolished, suggesting that the SCFA effect *in vivo* involves a neural mechanism. We thus looked at the adrenergic or nonadrenergic noncholinergic inhibitory systems. Both phentolamine, a nonspecific α -adrenoceptor antagonist, and yohimbine, an α_2 -antagonist, stimulated basal colonic motility, indicating the presence of a basal α_2 -adrenergic inhibitory tone, whereas prazosin, an α_1 -antagonist, had no effect. However, none of these compounds suppressed the SCFA effect on myoelectrical activity, suggesting that SCFAs did not activate α -adrenergic inhibition. To test nitric oxide nonadrenergic noncholinergic inhibition, we blocked its synthesis by administering L-NAME or L-NNA, and basal myoelectrical activity of the colon was greatly increased. Nevertheless, SCFA infusion into the colon still decreased this activity, thus indicating that nitric oxide is probably not the mediator of SCFA inhibition in the rat colon.

We then sought to explore an endocrine or paracrine mediation. Infusing 2 mmol/h SCFAs into the rat colon increased the circulating concentration of PYY, as already found in the rabbit and rat isolated perfused colons (20, 27). Moreover, the plasma PYY concentrations remained elevated for at least 1 h after the end of SCFA infusion, a finding that paralleled the duration of colonic inhibition after SCFA infusion (Fig. 2). Thus a time concordance existed between these two biological effects of SCFAs.

PYY, a peptide synthesized and released by L cells of the ileocolonic epithelium, influences gastrointestinal motility. It slows down gastric emptying in dogs and humans (25), delays distal propagation of migrating motor complexes, inhibits myoelectrical activity in the rat small intestine (1), and has been proposed as the mediator of the "ileal brake" induced by infusion of fat into the terminal ileum (26). However, the effects of PYY on colonic motility were not clear (31, 38). Administration of exogenous PYY, at doses that mimic SCFA-induced plasma concentrations, decreased myoelectrical activity of the colon to a level not significantly different from that evoked by SCFAs. The reduction of colonic motility by PYY is consistent with previous results obtained *in vitro* on guinea pig intestinal strips, which showed that PYY induced dose-dependent relaxation of the longitudinal colonic smooth muscle (18) and inhibited the twitch contractions mediated by electrofield transmural stimulation of cholinergic neurons

in the ileum and the colon (31, 37). In addition to the similar inhibitory action of SCFAs and PYY, we also showed that immunoneutralization of circulating PYY with a PYY antiserum abolished the effect induced by SCFAs. Taken together, these data strongly suggest that PYY is involved in the colonic motor effect of SCFAs in the rat.

In conclusion, our results show that SCFAs inhibit colonic myoelectrical activity in a dose-dependent fashion. The effect depends on the chemical nature of SCFAs and appears to be mediated through a neuroendocrine mechanism involving PYY. The physiological significance of this effect deserves speculation. It may represent a self-regulatory mechanism of the large intestinal fermentation: elevated concentrations of SCFAs might reduce bacterial fermentations by decreasing mixing movements and increasing transit. Such a mechanism provides additional evidence that colonic fermentation and gastrointestinal motility are closely related functions (8, 28).

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