Vagal afferent responses to fatty acids of different chain length in the rat

SIMON LAL,1 ANTHONY J. KIRKUP,2 ALAN M. BRUNSDEN,2 DAVID G. THOMPSON,1 AND DAVID GRUNDY2
1Department of Gastro-Intestinal Sciences, Hope Hospital, Salford M6 8HD; and 2Department of Biomedical Science, Sheffield University, Sheffield S10 2TN, United Kingdom

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Lal, Simon, Anthony J. Kirkup, Alan M. Brunsden, David G. Thompson, and David Grundy. Vagal afferent responses to fatty acids of different chain length in the rat. Am J Physiol Gastrointest Liver Physiol 281: G907–G915, 2001.—The role of cholecystokinin (CCK) in the effect of dietary lipid on proximal gastrointestinal function and satiety is controversial. Recent work suggests that fatty acid chain length may be a determining factor. We investigated the mechanism by which long- and short-chain fatty acids activate jejunal afferent nerves in rats. Whole mesenteric afferent nerve discharge was recorded in anaesthetized male Wistar rats during luminal perfusion of saline, sodium oleate, and sodium butyrate (both 10 mM). Both fatty acids evoked characteristic afferent nerve responses, distinct from the mechanical response to saline, that were abolished in rats following chronic subdiaphragmatic vagotomy. The effect of oleate was abolished by the CCK-A receptor antagonist Devazepide (0.5 mg/kg), whereas the effect of butyrate persisted despite pretreatment with either Devazepide or a combination of the calcium channel inhibitors nifedipine (1 mg/kg) and the ω-conotoxins GVIA and SVIB (each 25 μg/kg). In summary, long- and short-chain fatty acids activate intestinal vagal afferents by different mechanisms; oleate acts via a CCK-mediated mechanism and butyrate appears to have a direct effect on afferent terminals.

cholecystokinin; vagus; small intestine; nutrients

FOLLOWING MEAL INGESTION, a complex series of mechanisms is initiated to coordinate the digestion, absorption, and further intake of food. It is clear that different macronutrients exert very specific effects, which are independent of any calorific or osmotic properties (35). The presence of lipid in the intestine, for example, leads to the inhibition of gastric emptying (14), the inhibition of gastric acid secretion (22), and a reduction in further food ingestion (40). The specificity of these effects is underlined by the findings that 1) the effective stimulus of a lipid meal is provided by its fatty acid components (11, 15) and 2) it is the carbon chain length of the fatty acid molecule that determines its efficacy (26, 29). Longer-chain lipids instilled into the cat duodenum were more effective in reducing myoelectrical recordings from the gastric antrum than their shorter-chain counterparts (29). Similarly, fatty acids with >12 carbon atoms in their chains were more effective both in inhibiting antral contractions and inducing proximal gastric relaxation in humans (26).

The processes underlying the differential effect of fatty acid chain length on gastrointestinal function are not fully understood. The vagus nerve is believed to contribute to the effect since many of the actions of lipids on gastrointestinal motor and secretory function and on feeding behavior depend on intact vagal innervation (14, 22, 44). Furthermore, direct electrophysiological recordings made from rat cervical vagal afferent fibers suggest that the presence of lipid in the intestine can stimulate neural activity (34). Although these workers did not investigate the effect of differing fatty acid length, an earlier study had identified two lipid-responsive populations of vagal afferents in the cat duodenum, one being sensitive to short-chain fatty acids and glycerol, the other selectively sensitive to long-chain fatty acids (28). However, the mechanism by which the different-chain-length fatty acids activated the vagus was not elucidated in that study.

The signaling mechanisms by which dietary fat in the intestinal lumen leads to vagal activation and then to gastrointestinal reflex control are not well defined. It is known that some of the effects of lipids on gastrointestinal function are mediated, at least in part, by the release of the hormone cholecystokinin (CCK) from endocrine cells in the intestinal mucosa. Two distinct receptors, CCK-A and CCK-B, mediate the effects of CCK in the digestive system (5, 33, 41), and the role of the former in lipid-induced gastric relaxation in humans has been demonstrated by using a selective CCK-A receptor antagonist (6, 8, 30). Moreover, since the administration of this antagonist to perivagal capsaicin-treated rats failed to further reduce the gastric relaxation induced by lipids, it would appear that these nutrients exert much of their effect via CCK-A receptor-expressing vagal afferent fibers (14).

Recently, we have demonstrated that fatty acids require a minimum acyl chain length of 12 carbon atoms to induce CCK release both in human (26) and in...
cell culture studies (27). Thus one can postulate that, following recognition by enteroendocrine cells, the longer-chain fatty acids release CCK into the mucosa, where the peptide is capable of activating vagal afferent fibers in a paracrine manner. This hypothesis is supported by previous electrophysiological studies in our laboratory (4, 37) that have revealed direct sensitivity of vagal afferents to exogenous CCK and by the recent demonstration (32) of CCK-A receptors in rat and human nodose ganglia. A paracrine mode of action is further in keeping with morphological studies that have demonstrated close proximity between vagal afferent fibers and the basolateral membrane of CCK-immunoreactive epithelial cells (2). However, despite such evidence, there has been, as yet, no electrophysiological study of vagal afferent stimulation by endogenous CCK released by lipids.

It was therefore the aim of this study to investigate the transduction mechanisms of fatty acids of different chain lengths in the rat intestine. Specifically, we examined the effect of a representative long-chain fatty acid (oleic acid) and a short-chain fatty acid (butyric acid) on the activity of extrinsic nerves innervating the jejunal mucosa of anaesthetized rats and assessed whether the effect of either was mediated via the release of endogenous CCK. We also aimed to determine whether fatty acids could activate afferent nerves independently of CCK release and, if so, by what mechanism.

METHODS

Animals. Experiments were performed on male Wistar rats (350–450 g), which were housed under standardized conditions (regular rat chow, free access to water, 12:12-h light/dark cycle, lights on at 6 AM). All experiments conformed to United Kingdom Home Office Guidelines for the use and care of animals. General anesthesia was established with a single intraperitoneal injection of pentobarbital sodium (60 mg/kg) and maintained by intravenous infusion (0.5–1 mg·kg⁻¹·min⁻¹). Full surgical anesthesia was maintained by regularly assessing the depth of anesthesia every 30 min by observing whether the animal’s blood pressure was maintained at a constant level and/or whether it was perturbed by pinching a forepaw; the infusion rate of anesthetic was adjusted accordingly. Animals were killed at the end of an experiment by an anesthetic overdose.

Vagotomized animals. Abdominal vagotomy was performed to eliminate vagal afferent fibers in the mesenteric nerve bundles. A midline laparotomy was performed in pentobarbital-anæsthetized rats (60 mg/kg ip). The dorsal and ventral vagal trunks were freed along the subdiaphragmatic esophagus, ligated, and sectioned. The abdominal wall and skin were stitched with a polyglycolic acid suture (Dexon, Davis & Geck). Following recovery, animals were returned to the housing unit and fed on the liquid diet (Complan Original in full-fat pasteurized milk) to which they had been accustomed before surgery and which was continued for a further 5–10 days before afferent recording experiments were performed. To confirm that the subdiaphragmatic vagotomies had been effective in removing the vagal innervation of the jejunal, the peripheral ends of the right and left cervical vagus nerves were stimulated at the end of each experiment. In nonvagotomized animals, vagal stimulation caused a profound bradycardia and an increase in intestinal pressure, typically of 1.5 mmHg. However, in vagotomized animals, although the bradycardia was still observed, vagal stimulation had no effect on jejunal pressure.

Afferent recordings. The surgical techniques and other experimental methods are documented extensively elsewhere (18). An incision was made in the neck, and the trachea was intubated to maintain a patent airway. The right jugular vein was cannulated to enable anesthetic infusion and for the systemic administration of drugs. The left carotid artery was cannulated with a heparinized catheter (200 U/ml heparin in 9% wt/vol NaCl solution) to facilitate arterial pressure recording (Neurolog NL108; Digitimer, Welwyn Garden City, UK). Body temperature was maintained at 37°C by a rectal thermistor-controlled heating blanket.

A midline laparotomy was performed, and the cecum was excised to create space in the abdominal cavity. A 10-cm length of proximal jejunum was located (typically 1–5 cm from the ligament of Trietz). This was ligated, and incisions were made on the anterior mesenteric border at each end. The jejunal loop was intubated with saline-filled Portex tubing (PP30) that was passed through the right abdominal wall via two small incisions. The saline-filled abdominal cannula contained two smaller-bore saline-filled Portex tubes (PP30), one for the infusion of the test solution (applications were drained via the oral cannula), the other for intrajejunal pressure recordings (Neurolog NL108). The small abdominal wall incisions were sutured, and the muscle and skin of the large abdominal incision were sewn to a steel ring. The resulting well was filled with prewarmed (37°C) liquid paraffin.

Nerve preparation. A single paravascular nerve bundle (~1 cm in length) was dissected out from a mesenteric arcade supplying the loop of jejunum, severed distally from the jejunal serosa (to eliminate the recording of efferent nerve activity) and cleared. It was then attached to one of a pair of platinum recording electrodes, with a length of connective tissue wrapped around the other to act as a reference. The electrodes were connected to a head stage (Cambridge Electronic Design, CED, Cambridge, UK), and the signal was differentially amplified 10,000 times and filtered with a bandwidth of 100 Hz to 1 kHz (CED single channel 1902 preamplifier/filter). The output from the 1902, together with the signals from the pressure transducers, were passed into a Micro 1401 interface system (CED), captured, and viewed online by a PC running Spike 2 software (version 3.15; CED).

Single-unit analysis. Multunit recordings containing only a few units often displayed action potentials of sufficiently different amplitude and waveform to allow accurate discrimination and hence identification of single units by using computerized waveform analysis as described previously (13). This offline analysis was performed using a PC running Spike 2 software. The nerve signal was digitally sampled at 25 kHz, which was sufficient to allow accurate spike discrimination. Each spike above a given amplitude was used to set up templates for the individual action potentials. Action potential waveforms were automatically averaged, DC offset arising from noise was removed, and the resulting spike shapes were assigned to different waveform templates. The afferent recording was subsequently analyzed such that each action potential was compared with the waveform template and either matched to one or left unclassified. The timing of each template-matched spike was then used to calculate firing frequency and to plot discharge frequency against time. Tolerance was variable, but typically the allowed amplitude error was set at between 1% and 2%, and for a spike to be matched at least 85% of the data points had to fall within the template shape. These relatively rigid parameters were shown empirically to discriminate action potentials accu-
rately, but at high firing frequencies a small proportion of individual spikes, typically <5%, could be missed because of summation. However, this underestimate of spike frequency was obviously preferable to less rigorous discrimination configurations in which “cross-contamination” could occur. The software allowed as many as eight templates to be simultaneously sampled, but in our experience the system was most reliable dealing with fewer (typically 1–4 templates) of the relatively larger-amplitude spikes. Once stored in computer, spike discrimination was checked manually by overlaying templates and comparing the spikes by eye. Ambiguous spikes could be reassigned to a different template or ignored.

**Experimental protocols.** After a 30-min baseline recording for signal stabilization, the viability of the preparation was assessed with bolus doses of CCK (10 and 100 pmol iv) and, after a further 5–10 min, the intestinal loop was distended for 10 s with isothermic saline. Afferent nerve preparations that failed to respond to the test stimuli were discarded.

The effects of intraluminal administration of 10 mM sodium oleate and 10 mM sodium butyrate on jejunal afferent nerve discharge were investigated in control, nifedipine-treated, and vagotomized rats. This concentration of fatty acid was chosen since it yielded a robust and reproducible jejunal afferent fiber discharge response in pilot studies. Since the procedure of infusion also stimulated intestinal mechanoreceptors, the response to fatty acids was compared with the infusion of saline. The pH of the sodium oleate solution was 8.9, and that of sodium butyrate was 6.6. Pilot experiments had shown no difference in the effect on nerve discharge of a high-pH saline solution (pH 9) compared with that of one with pH 7, and so, in all experiments, a pH 7 saline solution was used as the control. All solutions were of similar measured osmolality (295–310 mosmol/kgH2O) and were administered at the same temperature (37°C).

In seven control rats and six vagotomized animals, stimuli were applied as follows: CCK (10 then 100 pmol iv), 1.0 ml intrajejunal saline, 1.0 ml intrajejunal sodium oleate, and 1.0 ml intrajejunal sodium butyrate. The solutions for jejunal distension were introduced (~5 s) via the distal jejunal cannula, maintained for 10 s, then drained via the proximal end of the loop. The particular test solution so infused was then left in contact with the mucosa for 10 min. This methodological design allowed any mechanical effect to be readily distinguished from any chemical effect that the intestinally perfused fatty acid may have on afferent nerve discharge. The loop was then flushed with 10 ml of saline before application of the next test solution. Each dose of CCK was administered intravenously as a bolus, and full recovery was allowed before proceeding to the next treatment. Preliminary experiments had revealed that 1) the mesenteric afferent fiber response to a particular fatty acid was unaffected by prior administration of the other fatty acid, 2) repeated administration of the same fatty acid led to desensitization of the response, and 3) there was no cross-desensitization.

The effect of the CCK-A receptor antagonist, Devazepide, and the calcium antagonist and toxins was used to attenuate smooth muscle activity, neurotransmitter release, and enteroendocrine cell release of peptides (7, 17, 18, 24, 25, 39, 43, 45). The rats were initially challenged with luminal sodium butyrate as described above, and the combination of calcium channel antagonists was administered 5 min before a second challenge with the fatty acid; because of desensitization of the butyrate response, the effects were compared in four time-matched controls.

**Drugs.** Pentobarbital sodium (Sagatal) was obtained from Rhône Mérieux (Harlow, UK). ω-conotoxin GVIA was obtained from Latoxan (Rosans, France), and ω-conotoxin SVIB was purchased from Alomone Laboratories (Jerusalem, Israel). Devazepide was a kind donation from SmithKline Beecham, Tween 80, bovine serum albumin, sodium oleate, sodium butyrate, DMSO, CCK (sulfated octapeptide), and nifedipine were each purchased from Sigma Chemical (Poole, UK).

With the exception of CCK, Devazepide, and nifedipine, all of the drugs and chemicals were dissolved and diluted in saline to the required concentration. Stock solutions of CCK were prepared in 2% wt/vol bovine serum albumin in saline and diluted in saline. Devazepide was dissolved in a mixture of DMSO and Tween 80 (1:1, 5 g/l) as a stock solution; this was diluted with saline to 1 g Devazepide/l for use. Nifedipine was made up in 25% DMSO as a stock solution of 1 mg/ml and screened in aluminum foil to prevent photodegradation.

**Data analysis.** Baseline impulse discharge frequency (imp/s) and the response discharge frequency (imp/s) were obtained by averaging afferent activity for the 30-s period immediately before the intraluminal application of the test solution (sodium, sodium oleate, or sodium butyrate) and for the 30-s period immediately following the end of the distension period. The response to a particular test solution is expressed as the change in afferent activity during the latter period expressed as a percentage of the baseline value. Corresponding changes in intraluminal pressure (mmHg) were expressed similarly. The afferent discharge response to the systemic administration of CCK is expressed as the change in peak afferent discharge expressed as a percentage of the basal firing rate measured for 30 s before CCK administration.

The data are presented as means ± SE (n = number of animals) and compared by using the Student's paired or unpaired t-test for parametric data and the Mann-Whitney rank sum test for nonparametric data, as appropriate. Significant differences between a series of treatments within a single group of animals (%changes in afferent discharge and intraluminal pressure) were determined by using repeated-measures ANOVA (RM ANOVA) on ranks followed by Dunn's multiple comparison method (for analysis of the responses of each fatty acid vs. the response to saline) and Student-Newman-Keuls test (for pairwise comparisons made between the response to saline, the response to the first application of the particular fatty acid, and the response to the second application of the particular fatty acid). Spearman rank-order correlation was used to compare whole nerve responsiveness to CCK and sodium oleate. A probability of P < 0.05 was considered statistically significant.

**RESULTS**

Whole mesenteric nerve responses to intraluminal administration of fatty acids. Both sodium oleate and sodium butyrate evoked an afferent nerve response but with markedly different latencies and time courses of activation, which could be distinguished from the pri-
discharge responses

Table 1. Effect of intrajejunal stimulation on afferent discharge responses

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<th>n</th>
<th>Saline</th>
<th>Oleate</th>
<th>Butyrate</th>
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<tr>
<td>Control animals</td>
<td>7</td>
<td>114 ± 5</td>
<td>143 ± 13*</td>
<td>524 ± 144*</td>
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<tr>
<td>Devazepide-treated animals</td>
<td>5</td>
<td>110 ± 2</td>
<td>111 ± 4</td>
<td>337 ± 67*</td>
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<td>Vagotomized animals</td>
<td>6</td>
<td>108 ± 6</td>
<td>102 ± 9</td>
<td>102 ± 4</td>
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Values are mean ± SE % changes (expressed as %baseline) in whole afferent nerve discharge in the 30-s period immediately following the intraluminal administration of saline, sodium oleate, and sodium butyrate; n = no. of animals. (*P < 0.05 vs. saline by repeated-measures ANOVA on ranks followed by Dunnett’s test).

mary mechanical response to saline distension. With saline, the afferent nerve response closely paralleled the corresponding rise and fall in intraluminal pressure as the lumen was first distended and then drained (Fig. 1A); these fibers so activated are mechanosensitive. When sodium oleate was infused, the afferent nerve response was initially similar to that of saline, reflecting stimulation of these mechanosensitive fibers (Fig. 1B). However, once luminal pressure had returned to baseline, afferent discharge continued to increase with a second peak; this secondary rise reflects activation of chemosensitive fibers. A similar response was seen in seven such experiments. There was a latency of onset to this secondary rise in afferent discharge of 3 ± 0.4 s from the end of distension, and it persisted for 35 ± 6 s before returning to basal levels. In contrast, the effect of sodium butyrate was more immediate and superimposed on the mechanical response to distension; the overall response to butyrate reflects stimulation of chemosensitive fibers. A similar response was seen in seven such experiments. There was a latency of onset to this secondary rise in afferent discharge of 3 ± 0.4 s from the end of distension, and it persisted for 35 ± 6 s before returning to basal levels. In contrast, the effect of sodium butyrate was more immediate and superimposed on the mechanical response to distension; the overall response to butyrate was also more prolonged, lasting for 238 ± 54 s (Fig. 1C) before returning to baseline. The group data representing all seven experiments are given in Table 1; the mean percentages of increase in afferent discharge for the 30 s immediately following distension with sodium oleate and sodium butyrate are both significantly greater than that with saline (P < 0.05; RM ANOVA on ranks followed by Dunnett’s test). Because there was no significant difference in the corresponding changes in intraluminal pressure on administering these solutions in these animals (P = 0.31 by RM ANOVA on ranks; Table 2), any differences observed in afferent discharge cannot be explained by a differential effect of the test solution on intraluminal pressure.

Desensitization. Reapplication of sodium oleate 15–30 min after a first administration did not evoke a chemosensitive component to the afferent firing response [Fig. 2; n = 4; mean increase in afferent discharge in response to the first application of sodium oleate = 164 ± 15%; P < 0.05 vs. second application of oleate and P < 0.05 vs. saline (Student-Newman-Keuls multiple comparison test); mean increase in afferent discharge in response to the second application of sodium oleate = 109 ± 6%; nonsignificant vs. saline (Student-Newman-Keuls multiple comparison test); mean increase in afferent discharge in response to saline = 116 ± 5%], although the mesenteric nerve bundle still remained responsive to intravenous CCK. In contrast, the afferent nerve response to sodium butyrate was maintained on second and subsequent applications, albeit at a lower level [Fig. 3; n = 5; mean increase in afferent discharge in response to sodium butyrate = 472 ± 163% (P < 0.05 vs. second application of butyrate and P < 0.05 vs. saline; Student-Newman-Keuls multiple comparison test); mean increase in afferent discharge in response to the second application of sodium butyrate = 110 ± 4% (P < 0.05 vs. saline; Student-Newman-Keuls multiple comparison test); mean increase in afferent discharge in response to saline = 116 ± 5%].

Table 2. Changes in intraluminal pressure following luminal distension

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Values are mean ± SE % changes (expressed as %baseline) in intraluminal pressure in the 30-s period immediately following intraluminal administration of saline, sodium oleate, and sodium butyrate; n = no. of animals. There was no significant difference in the % changes in intraluminal pressure on administering saline, oleate, or butyrate in either the control or Devazepide-treated animals (P = 0.31 by repeated-measures ANOVA on ranks).
to the second application of sodium butyrate = 166 ± 17% \((P < 0.05\) vs. saline; Student-Newman-Keuls multiple comparison test); mean increase in afferent discharge in response to saline in these animals = 109 ± 5%].

**Effect of Devazepide on the afferent nerve responses to fatty acids.** Whole mesenteric nerve responses to the intraluminal administration of saline, sodium oleate, and sodium butyrate were assessed following the intravenous administration of Devazepide (0.5 mg/kg). This dose of Devazepide abolished the afferent response to exogenous CCK (10–100 pmol iv). Devazepide also attenuated the secondary rise in afferent discharge in response to sodium oleate \((P < 0.05\) vs. control rats by Mann-Whitney rank sum test; Table 1).

In contrast, sodium butyrate still evoked an increase in discharge rate over and above that of saline despite Devazepide pretreatment \((P < 0.05\) vs. saline by RM ANOVA on ranks followed by Dunnett’s test; Table 1). There was no significant difference between the magnitude of the afferent nerve response to butyrate in control and Devazepide-treated animals \((P = 0.6\) by Mann-Whitney rank sum test; Fig. 4, A and B).

Thus it would appear that sodium oleate activated mesenteric afferents via CCK, whereas the effect of butyrate was CCK independent. There was a degree of variability in the afferent nerve response to oleate between individual animals. This is demonstrated in Fig. 5A, in which the absolute values of mean afferent discharges over 30 s are shown both before and after luminal infusion of the fatty acid in individual control rats. It can be seen that this variability existed primarily in the baseline firing rate before administration of the fatty acid but that there was a clear increase in discharge following oleate administration in all seven animals. Additionally, there was no correlation between the magnitude of the response of the whole mesenteric nerve bundle to oleate and its ability to respond to exogenous CCK \((P > 0.05\) by Spearman rank-order correlation). The latter observation would suggest that, whereas the activation of mesenteric afferent fibers by sodium oleate is mediated (at least in part) by CCK, additional factors determine the magnitude of the afferent response to the fatty acid.

**Single-unit analysis of whole nerve responses.** Waveform discrimination was used to identify single units within multiunit mesenteric nerve recordings. With the use of the rigid selection criteria set for the identification of single units, it was not possible to identify any single units activated by sodium oleate in the seven control animals. In contrast, a total of 29 sodium butyrate-sensitive single units were identified. Of these, 10 were also sensitive to CCK. Thus two distinct populations of sodium butyrate-responsive fibers were identified: those that responded only to the fatty acid and those that responded to both the fatty acid and CCK (Fig. 6).

**Afferent nerve responses in vagotomized animals.** The afferent nerve responses to the intraluminal administration of sodium butyrate and oleate were absent in animals following chronic subdiaphragmatic vagotomy (Table 1). Thus the population of mesenteric nerve responses to sodium butyrate = 166 ± 17% \((P < 0.05\) vs. saline; Student-Newman-Keuls multiple comparison test); mean increase in afferent discharge in response to saline in these animals = 109 ± 5%].

**Fig. 2.** Representative recording of mesenteric afferent discharge displayed as a sequential rate histogram following repeated luminal perfusion with sodium oleate (10 mM) and repeated intravenous application of cholecystokinin (CCK; 10 pmol). Note that the mesenteric nerve response to sodium oleate is absent on a second application, whereas the nerve maintains its responsiveness to exogenous CCK.

**Fig. 3.** Representative recording of mesenteric afferent discharge displayed as a sequential rate histogram following luminal perfusion with saline and repeated perfusion with sodium butyrate (10 mM). Note that, although the mesenteric nerve response to sodium butyrate is diminished on the second application, a chemosensitive component to the response can still be distinguished.
afferent fibers activated by the fatty acids in control animals would appear to be exclusively vagal in origin. Afferent nerve responses to sodium butyrate following coadministration of L-, N-, P-, and Q-type calcium antagonists. To investigate the possibility that the responses evoked by sodium butyrate in jejunal vagal afferents may have been by an indirect effect on smooth muscle cells, secondary to the excitation of enteric neuronal circuits or via the release of an intermediary peptide other than CCK, sodium butyrate was administered before and after treatment with the combination of nifedipine and the omega-conotoxins GVIA and SVIB in four animals. The afferent response to the second application of sodium butyrate following treatment with the calcium channel blockers was reduced compared with the first in each of the four rats; however, the magnitude of the reduction was comparable to that seen in time-matched controls (mean increase in afferent discharge in response to sodium butyrate following calcium antagonists = 189 ± 22% \((n = 4)\); mean increase in time-matched control rats = 166 ± 17% \((n = 5)\); \(P = 0.56\) by Mann-Whitney rank sum test). This would be in keeping with sodium butyrate having a direct stimulatory effect on vagal afferent terminals.

DISCUSSION

We have demonstrated that long- and short-chain fatty acids activate rat jejunal mesenteric afferent nerve fibers by different mechanisms. The long-chain fatty acid, oleic acid, activated vagal afferents via a CCK-mediated mechanism. In contrast, butyric acid, a short-chain fatty acid, appeared to have a direct effect on vagal afferent terminals.

By demonstrating that the afferent response to luminal oleate is absent following chronic subdiaphragmatic vagotomy and also following pretreatment with the CCK-A receptor antagonist, we have shown that endogenous CCK plays a role in the transduction of signals elicited by a long-chain fatty acid into extrinsic vagal afferent discharge. Previous work in our department has shown this population of CCK-responsive vagal fibers to be exclusively mucosal in nature (37). Sternini and colleagues (38), however, recently failed to demonstrate CCK-A receptor immunoreactivity on such fibers. They also failed to visualize the CCK-A receptor in other putative CCK targets, such as smooth muscle cells, D cells, and chief cells, as would have been expected from functional studies (9, 10, 23, 31). As the authors point out, their inability to detect the CCK-A receptor in these structures and in vagal afferents may be due to levels of receptor expression that cannot be detected with immunohistochemistry.

It would appear that longer-chain fatty acids exert an indirect effect on vagal afferents by releasing CCK, which then acts in a paracrine manner. The relatively short duration of the afferent response to oleate is consistent with release of preformed CCK that is stored in vesicles within mucosal enteroendocrine cells, activation of nerve endings, and then diffusion of the peptide away from the lamina propria. In contrast, intestinal administration of the fatty acid has a more prolonged effect on circulating plasma CCK levels (20, 26); it is therefore unlikely that release of the peptide into the bloodstream is required before it can induce afferent firing. Moreover, the afferent response to oleate was not repeatable on reapplication of the fatty acid. This could be explained by the release of preformed CCK from stores that require longer than a 15-
to 30-min period to replenish. Alternatively, the enteroendocrine cell itself may become refractory to subsequent fatty acid stimulation. Both explanations would concur with results of cell culture experiments, whereby readministration of medium-chain fatty acids to a CCK-secreting cell line did not induce rerelease of CCK for up to 4 h after the first administration (27).

Our findings would thus be in keeping with the original hypothesis that the longer-chain fatty acids have an indirect action on afferent discharge: recognition by enteroendocrine cells within the mucosa releases CCK, which in turn activates vagal mucosal afferents in a paracrine fashion. Two additional observations support this view. First, there was latency to the secondary rise in afferent discharge in response to luminal application of sodium oleate. Second, we failed to find a direct correlation between the responsiveness of the nerve bundle to exogenous CCK and its responsiveness to sodium oleate infusion. Both observations would suggest the following: 1) there is no fixed relation between afferent nerve endings and enteroendocrine cells (2). With the administration of exogenous CCK, an immediate neural response is seen due to flooding of the lamina propria with the peptide. However, the response to endogenous CCK is largely dependent on the proximity of the CCK-producing enteroendocrine cell to the nerve ending. 2) Factors other than neural responsiveness to CCK are important in determining the magnitude of the afferent response to luminal administration of the long-chain fatty acid. Intermediate factors involved in the absorption and processing of these fatty acids in the intestinal wall may be involved before the release of CCK. For example, the ability of a lipid emulsion to inhibit gastric emptying and to increase plasma levels of CCK in rats has been shown to be dependent on the formation of chylomicrons within the intestinal wall (36). Since chylomicron formation is only required for the absorption of fatty acids with >12 carbons in their chain, it is perhaps this process that explains the differential properties of the long- and short-chain fatty acids in activating vagal afferent fibers.

In contrast to the CCK-mediated effect of the long-chain fatty acid, sodium butyrate had an immediate, direct, and “CCK-independent” effect in stimulating vagal afferent discharge. Intrajejunal application of sodium butyrate failed to induce changes in luminal pressure, and the effect on afferent discharge persisted despite pretreatment with the L-type calcium channel antagonist; both of these findings would suggest that the short-chain fatty acid activated vagal afferents independently of an effect on smooth muscle (25). Similarly, not only does the effect of sodium butyrate appear to be CCK independent, but it is also unlikely to be mediated by other neuroexcitatory agents (e.g., 5-HT), because the L-type and N-type calcium channel antagonists together would have attenuated the release of such agents from intestinal enterochromaffin cells (39, 45). In addition, the response to sodium butyrate was unaffected by both conotoxins, which together inhibited N-, P-, and Q-type calcium channels; this suggests that the action of sodium butyrate on extrinsic fibers was not secondary to the activation of or release of neurotransmitters from enteric neuronal

Fig. 6. Two distinct sodium butyrate-responsive single-unit mesenteric nerves identified using waveform discrimination from the same multiunit recording. For each unit, the sequential rate histogram of afferent discharge is shown in top and corresponding changes in intraluminal pressure are shown at bottom. The unit represented in A is responsive both to intravenous CCK (100 pmol) and luminal sodium butyrate (10 mM); that at in B is responsive only to sodium butyrate. The response of each unit to luminal saline is included for reference.
circuits (7, 17, 18, 43). The latter finding is notable since an earlier study demonstrated that intrinsic primary afferent neurons in the guinea pig ileum are activated by luminal sodium acetate (3).

Together, our results suggest a direct effect of the short-chain fatty acid on extrinsic nerve terminals, which would be in keeping with anatomic studies that have demonstrated vagal afferent arborizations in the mucosa around the crypts and lamina propria of intestinal villi (1). Since no vagal fibers actually protrude into the lumen, it is likely that luminal short-chain fatty acids activate vagal afferents directly following absorption across the mucosal epithelium to the nerve terminal in the lamina propria.

Using waveform discrimination, we were unable to identify any oleate-sensitive afferent units. This is most likely due to the stringent criteria set for the selection of single units; low-amplitude spikes were excluded to minimize misrepresentation of the multiunit afferent bundle. In contrast, the more intense response to sodium butyrate yielded a greater number of clearly identifiable units that passed these rigid selection criteria.

Indeed, two populations of sodium butyrate-sensitive vagal afferents were revealed: one that responded only to the short-chain fatty acid and a second that also responded to exogenous CCK. The latter population could potentially be oleate-sensitive in view of the CCK-mediated mode of action of the long-chain fatty acid on vagal afferent firing. Thus it would appear that a complex signaling mechanism is in place that allows distinct hardwiring of afferent signals in response to different nutrients in the gastrointestinal tract. Although long- and short-chain fatty acids activate the vagus nerve by different mechanisms, both are capable of generating a convergent signal via a population of fibers characterized by CCK sensitivity. Sodium butyrate, in addition, is able to generate a unique and possibly divergent signal via a separate population of vagal fibers that are solely sensitive to the short-chain fatty acid.

It is interesting to note that both types of sodium butyrate-sensitive afferent fibers displayed a diminished response on reaplication of the fatty acid. Desensitization of the afferent nerve is unlikely since the nerve remained responsive to exogenous CCK and desensation. Thus the mechanism of desensitization may relate to a reduction in the ability of the short-chain fatty acid molecule itself to access and activate afferent terminals. One possible explanation may be exhaustion of luminal protons, which have been shown to facilitate the absorption of short-chain fatty acids by intestinal cells both in vitro (12) and in vivo (42).

CCK is believed to exert a number of physiological effects in response to a meal, including inducing satiety (21), delaying gastric emptying (30), stimulating pancreatic and bile secretion, and causing gallbladder contraction (16, 19). The ability of longer-chain fatty acids to exert their effects via CCK release may have a functional basis in humans: the longest saturated fatty acid that is liquid at human body temperature is undecanoic acid (11 carbon atoms), whereas those with 12 or more carbons in their chain require emulsification by bile to be absorbed. Thus by activating CCK-mediated responses that allow their coordinated and controlled entry into the small intestine, longer-chain fatty acids can be optimally absorbed. The ability of the gastrointestinal tract to recognize and respond differentially to fatty acid chain length may also have implications in disease. Butyric acid, which in health is absorbed completely by the proximal small intestine, is a major short-chain fatty acid produced by bacterial fermentation of undigested carbohydrates and proteins; the presence of an intrinsic recognition system within the digestive tract may have additional implications, for example, in disease processes that are characterized by overgrowth of bacteria within the small intestine.

In conclusion, our results demonstrate that the gastrointestinal tract is finely attuned first to recognize and then to respond to fatty acids of different chain lengths within the lumen. The ability of the specific nutrient components of a meal to elicit responses via different transduction pathways from the gut to the brain allows the physiological processes of digestion, secretion, and absorption to be carefully coordinated. Moreover, the ability to recognize nutrients present under pathological circumstances may allow the activation of alternative signaling pathways in disease.

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