

Effect of short-chain fatty acids on contraction of smooth muscle in the canine colon

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Objective—To determine effects of short-chain fatty acids (SCFA) on canine colonic smooth muscle.

Sample Population—Colonic tissue obtained from 14 healthy dogs.

Procedure—Short-chain fatty acid (SCFA; acetate, propionate, and butyrate; 1 to 100 mmol/L)-induced contractions were compared with responses obtained with acetylcholine (AMCh; 10^{-4} mol/L). Roles of enteric neurons, cholinergic receptors, calcium stores in the sarcoplasmic reticulum, and extracellular calcium in the SCFA-induced responses were investigated by incubating muscle strips with tetrodotoxin (1 μ mol/L), atropine (1 μ mol/L), ryanodine (10 μ mol/L), nifedipine (1 μ mol/L), ethylene glycol-bis (β -aminoethylether)-N,N,N',N'-tetra-acetate (EGTA; 0.1 mmol/L), or an extracellular calcium-depleted (zero extracellular calcium) solution prior to the addition of propionate or butyrate.

Results—Incubation with SCFA elicited isometric stress responses (0.25 to 2.15×10^4 N/m²) in colonic longitudinal smooth muscle. Maximal responses to butyrate and propionate (50 mmol/L) were 37 and 23%, respectively, of the maximal AMCh response. Acetate was least effective in stimulating contractile responses. Tetrodotoxin and atropine did not affect SCFA-induced contractions. Nifedipine and zero extracellular calcium solution abolished responses to butyrate and propionate, whereas EGTA attenuated (> 60%) but did not abolish those responses. Ryanodine did not affect SCFA-induced contractile responses. The SCFA did not affect colonic circular smooth muscle.

Conclusions and Clinical Response—The SCFA stimulate longitudinal but not circular colonic smooth muscle contractions via a direct effect on smooth muscle. The mechanism of the SCFA effect appears to involve the influx of extracellular calcium. These findings may account for some of the effects of canine colonic motility. (*Am J Vet Res* 2002;63:295–300)

Short-chain fatty acids (SCFA) are the end products of fiber fermentation in the colon of dogs. The major fermentation substrates include cellulose, hemicellulose, and pectin, substrates that typically are not

digested by pancreatic or intestinal amylases.¹ Acetate, propionate, and butyrate account for more than 85% of formed SCFA, and they accumulate in concentrations up to 150 mmol/L in the colon of dogs.^{2,4} The SCFA are rapidly absorbed by the colonic mucosa,^{2,3} are readily metabolized by colonic epithelial cells,⁴ and have various physiologic effects. Among their physiologic effects, SCFA promote differentiation and proliferation of colonocytes,⁵ stimulate absorption of water and electrolytes,⁶ provide 7 to 10% of an animal's overall energy requirements,³ and influence or modify motility of the gastrointestinal tract.⁷ The SCFA stimulate propulsive motility in the ileum of dogs,⁸⁻¹⁰ but their effects in the colon may be more complicated. Diets supplemented with α -cellulose reduce colonic spike-burst duration in the proximal portion of the colon in dogs,⁷ but SCFA perfusions do not stimulate colonic propulsive motility.¹¹ These findings may suggest that SCFA contribute to the overall muscular tone of the colon instead of propulsive motor activity.

The objective of the study reported here was to investigate *in vitro* effects of various SCFA on the proximal and distal portions of the canine colon, the major sites of fiber fermentation in the gastrointestinal tract of dogs. In addition, the study was designed to determine the physiologic mechanisms responsible for the smooth muscle responses to SCFA.

Materials and Methods

Animals—Colonic tissue was obtained from each of 14 healthy sexually intact male and female mixed-breed dogs (12 to 18 months old) that were being euthanatized (overdose of pentobarbital, IV) as part of an unrelated study^a at our university. Dogs were part of a breeding colony, did not have clinical signs or gross evidence of pathologic changes of gastrointestinal tract disease, and had not received medication other than routine administration of prophylactic anthelmintics. All dogs were fed the same commercially available diet,^b and food was withheld for 8 hours prior to euthanasia. Immediately following euthanasia, the entire colon was removed from each dog through a midline incision and placed in hydroxyethylpiperazine ethanesulfonic acid (HEPES)^c buffer solution for the subsequent determination of mechanical properties. All procedures were approved by an institutional animal care and use committee.

Preparation of colonic muscle tissue—Colonic segments were placed in silicone elastomer^d-coated dissection dishes containing HEPES buffer solution (137.3 mmol of NaCl/L, 5 mmol of KCl/L, 1 mmol of MgCl₂/L, 1.5 mmol of CaCl₂/L, 10 mmol of glucose/L, and 5 mmol of HEPES/L) at a pH 7.4 at room temperature (20 to 22 C). We selected HEPES buffer, because it is a zwitterionic buffering system that has excellent buffering capacity^{12,13}; incubation of acetate, propionate, or butyrate at concentrations ranging from 1 to 100 mM does not change pH of the buffering sys-

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tem. The colon was opened longitudinally along the mesenteric surface, and luminal contents were gently removed, using repetitive washes with HEPES buffer. To approximate in vivo conditions as closely as possible, tissue strips with intact mucosa and submucosa were prepared from the proximal (1 to 5 cm distal to the ileocecal sphincter) and distal (1 to 5 cm proximal to the pelvic inlet) portions of the colon. A set of tissue strips (1 × 10 mm) was dissected along the orientation of the longitudinal muscles, and another set was dissected along the orientation of the circular muscles at each anatomic site. Tissue strips then were transferred to continuously aerated (100% O₂) and warmed (37 C) 10-ml tissue baths^c filled with HEPES buffer solution (pH 7.4).

Measurement of isometric stress—Tissue strips were suspended in tissue baths in longitudinal or circular muscle orientation and attached to isometric force transducers,^f as described elsewhere.^{14,15} After a 30-minute equilibration period, the length for optimal development of active force (L_o) was determined by increasing the length of each muscle strip by 1-mm increments until the maximal active contractile response to 10⁻⁷M acetylcholine (AMCh)^g was achieved. Active isometric forces were recorded at each tissue length. Optimal muscle length was subsequently maintained during the duration of each experiment. Acquisition and analysis of data for active isometric forces were performed by use of transducer amplifiers,^h computer software,ⁱ and a computer,^j as described elsewhere.^{16,17}

Stimulation of muscle tissues—At L_o, tissue strips in the longitudinal or circular muscle orientation from the proximal or distal portion of the colon were stimulated with AMCh (10⁻⁴ mol/L) or with noncumulative concentrations of sodium acetate,^k sodium butyrate,^l or sodium propionate^m (1 to 100 mmol/L). The mechanism for the contractions induced by propionate and butyrate were studied in the proximal portion of the colon. Prior incubation of tissues with tetrodotoxinⁿ (1 μmol/L), a sodium-channel neural toxin, and atropine^o (1 μmol/L), a muscarinic cholinergic-receptor antagonist, for 45 minutes was used to determine the role of enteric neurons and cholinergic receptors, respectively, in the SCFA-induced contractile response. To determine the role of extracellular calcium in the SCFA-induced contractile responses, experiments were performed in a solution that did not contain any extracellular calcium (zero extracellular calcium) or in physiologically normal calcium solution (1.5 mmol of CaCl₂/L) after the addition of nifedipine^p (1 μmol/L), a voltage-dependent calcium-channel antagonist, or various concentrations of ethylene glycol-bis (β-aminoethylether)-N,N,N',N'-tetra-acetate^q (EGTA; 0.1 to 0.5 mmol/L), a chelator of extracellular calcium. To determine the role of intracellular calcium in the SCFA-induced contractile response, tissue strips were incubated with 10 μmol of ryanodine,^r a plant alkaloid that inhibits calcium storage in the sarcoplasmic reticulum of smooth muscles.¹⁸

Each experimental protocol consisted of 4 longitudinal and 4 circular tissue strips, comprising 2 tissue strips prepared in circular and 2 strips prepared in longitudinal muscle orientations from each of the proximal and distal portions of the colon. Data obtained from identical muscle sites (eg, 2 longitudinal muscle strips from the proximal portion of the colon) were used to calculate a mean value. In the SCFA dose-response experiments, SCFA concentrations (1, 10, 50, or 100 mmol/L) and tissue baths were randomized. Each tissue strip served as its own control sample (ie, null procedure).

Each SCFA solution was prepared daily in distilled water. All compounds were added in 10- to 100-μl aliquots to achieve the desired molarity.

Data analysis—At the completion of each experiment,

the length and weight of each tissue strip were determined and used to calculate the cross-sectional area of the tissue. The cross-sectional area was calculated by using the following equation: area = mass/(density × length). Tissue density of 1.05 g/cm³ was assumed.¹⁹ Isometric forces were standardized on the basis of tissue cross-sectional area and reported as described elsewhere.^{12,14,15}

Multiple treatments were analyzed by repeated-measures ANOVA and paired *t*-tests.²⁰ Effects of tetrodotoxin, atropine, nifedipine, EGTA, ryanodine, or zero extracellular calcium solution on baseline responses to propionate or butyrate were analyzed by use of a 1-way ANOVA and *t*-test. It was not possible to make statistical comparisons between proximal and distal portions of the colon because of potential differences in muscle contributions at the 2 sites.

Data were expressed as mean ± SEM. Differences were considered significant at values of *P* < 0.05.

Results

Isometric stress response to sodium butyrate—Sodium butyrate (1 to 100 mmol/L) caused tonic contractions of longitudinal smooth muscle obtained from the proximal and distal portions of the canine colon with a maximal contractile response (P_{max}) at 50 mmol of butyrate/L (n = 7; Fig 1). Responses for 50 mmol of butyrate/L were 35-37% of the responses obtained with maximal concentrations of AMCh (10⁻⁴ mol/L), and these values differed significantly (Table 1). Butyrate at doses of 1 to 100 mmol/L did not have an effect on circular smooth muscle from the proximal and distal portions of the colon (data not shown).

Isometric stress responses to sodium propionate—Sodium propionate (1 to 100 mmol/L) caused tonic contractions of longitudinal smooth muscle obtained from the proximal and distal portions of the canine colon with a P_{max} at 50 mmol of propionate/L (n = 8; Fig 2). Responses to 50 mmol of propionate/L were 29-33% of the responses obtained with maximal concentrations of AMCh, and these values differed significantly (Table 1). Propionate at doses of 1 to 100 mmol/L did not have an effect on circular smooth muscle from the proximal and distal portions of the colon (data not shown).

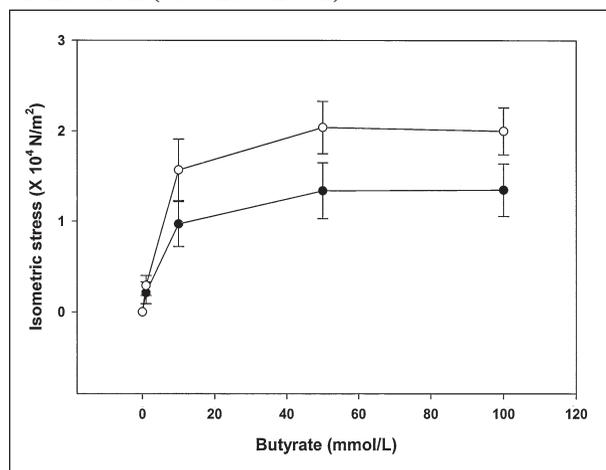


Figure 1—Isometric stress responses (mean ± SEM; n = 7) of longitudinal smooth muscle tissues obtained from the proximal (solid circle) and distal (open circle) portions of the canine colon to various concentrations of sodium butyrate.

Table 1—Effects of various calcium solutions or ryanodine on contractile responses in longitudinal smooth muscle tissues obtained from the proximal and distal portions of the canine colon for various short-chain fatty acids and acetylmethylcholine (AMCh)

Solution	AMCh (0.1 mmol/L)		Acetate (50 mmol/L)		Butyrate (50 mmol/L)		Propionate (50 mmol/L)	
	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
CaCl ₂ (1.5 mmol/L)	5.54 ± 0.67	3.86 ± 0.59	0.66 ± 0.22	0.72 ± 0.24	2.05 ± 0.43	1.35 ± 0.31	1.65 ± 0.51	1.27 ± 0.29
EGTA (0.5 mmol/L)	—	—	0.14 ± 0.06	0.21 ± 0.04*	0.45 ± 0.11*	0.23 ± 0.10*	0.34 ± 0.11*	0.19 ± 0.09*
Ca ²⁺ (0 mmol/L)	—	—	0*	0*	0.10 ± 0.05*	0.10 ± 0.04*	0*	0*
Ryanodine (10 μmol/L)	—	—	—	—	2.16 ± 0.31	1.28 ± 0.28	1.72 ± 0.26	1.33 ± 0.29

Values are reported as (mean ± SEM) × 10⁴ N/m².
 *Within a column, value differs significantly (*P* < 0.05) from response for CaCl₂ (1.5 mmol/L).
 — Not measured. EGTA = Ethylene glycol-bis (β-aminoethylether)-N,N,N',N'-tetra-acetate.

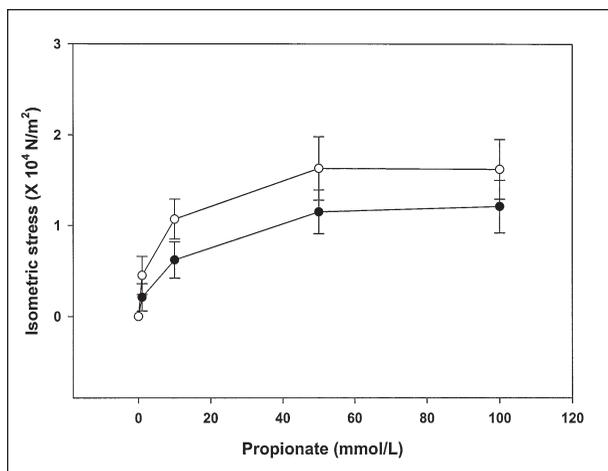


Figure 2—Isometric stress responses (mean ± SEM; n = 8) of longitudinal smooth muscle tissues obtained from the proximal (solid circle) and distal (open circle) portions of the canine colon to various concentrations of sodium propionate.

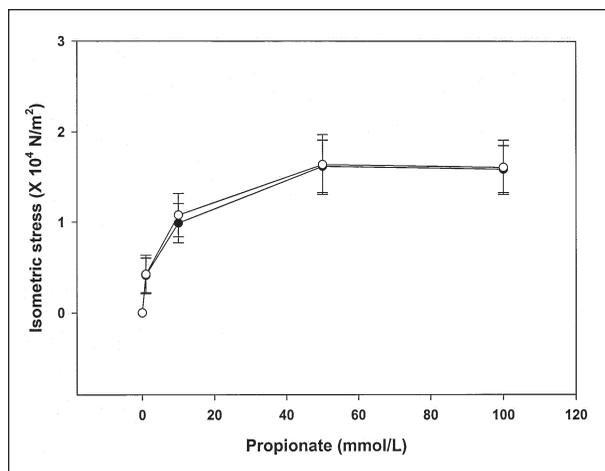


Figure 4—Isometric stress responses (mean ± SEM; n = 8) of longitudinal smooth muscle tissues obtained from the proximal portion of the canine colon to various concentrations of sodium propionate after prior incubation with (solid circle) and without (open circle) atropine (1 μmol/L).

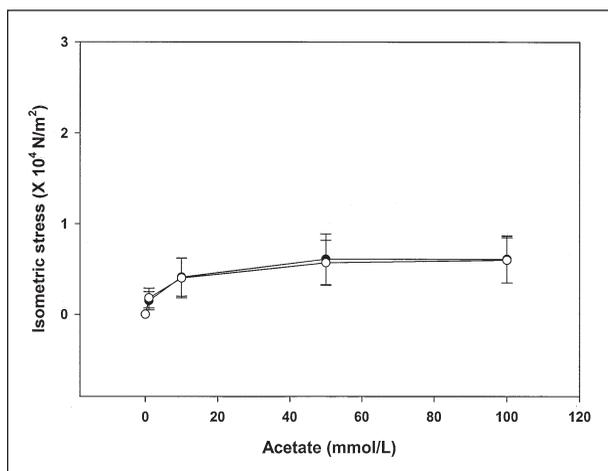


Figure 3—Isometric stress responses (mean ± SEM; n = 7) of longitudinal smooth muscle tissues obtained from the proximal (solid circle) and distal (open circle) portions of the canine colon to various concentrations of sodium acetate.

Isometric stress responses to sodium acetate—Sodium acetate (1 to 100 mmol/L) caused tonic contractions of longitudinal smooth muscle obtained from the proximal and distal portions of the canine colon with a P_{max} at 50 mmol/L (n = 7; Fig 3). Responses to 50 mmol of acetate/L were 11-18% of the responses obtained with maximal concentrations of AMCh, and these values differed significantly (Table 1). Acetate at

doses of 1 to 100 mmol/L did not have an effect on circular smooth muscle from the proximal and distal portions of the colon (data not shown).

Effect of prior incubation of atropine on isometric stress response to sodium propionate—Responses to propionate (1 to 100 mmol/L) in tissues obtained from the proximal portion of the colon were not affected by prior incubation of tissues with 10⁻⁶M atropine (n = 8; Fig 4). Atropine (10⁻⁶M) abolished responses to 100 mmol of AMCh/L.

Effect of tetrodotoxin, nifedipine, zero extracellular calcium solution, or EGTA on isometric stress responses to sodium propionate—Responses to propionate (1 to 100 mmol/L) in tissues obtained from the proximal portion of the colon were not affected by tetrodotoxin (alone or in combination with atropine), but nifedipine and zero extracellular calcium solution significantly affected the response and abolished the isometric stress response to propionate (n = 6; Fig 5). The longitudinal smooth muscle response to propionate was significantly attenuated (> 60%) but not completely abolished by the addition of EGTA (Table 1).

Effect of tetrodotoxin, nifedipine, zero extracellular calcium solution, or EGTA on isometric stress responses to sodium butyrate—Responses to butyrate (1 to 100 mmol/L) in tissues obtained from the proxi-

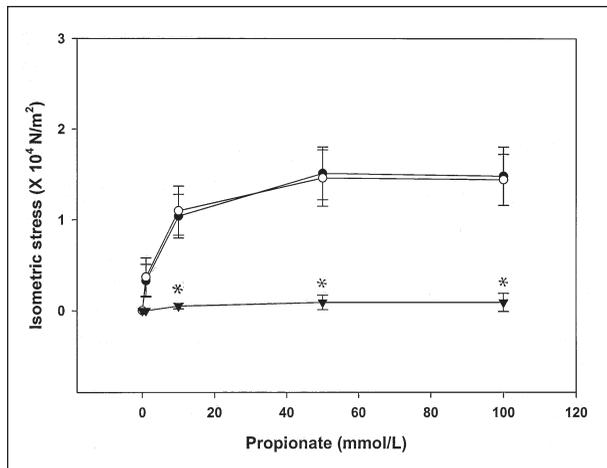


Figure 5—Isometric stress responses (mean \pm SEM; $n = 6$) of longitudinal smooth muscle tissues obtained from the proximal portion of the canine colon to various concentrations of sodium propionate alone (solid circle) or to various concentrations of sodium propionate after prior incubation with tetrodotoxin ($1 \mu\text{mol/L}$; open circle) or nifedipine ($1 \mu\text{mol/L}$; solid inverted triangle). *Nifedipine significantly ($P < 0.05$) inhibited smooth muscle responses at this concentration of sodium propionate.

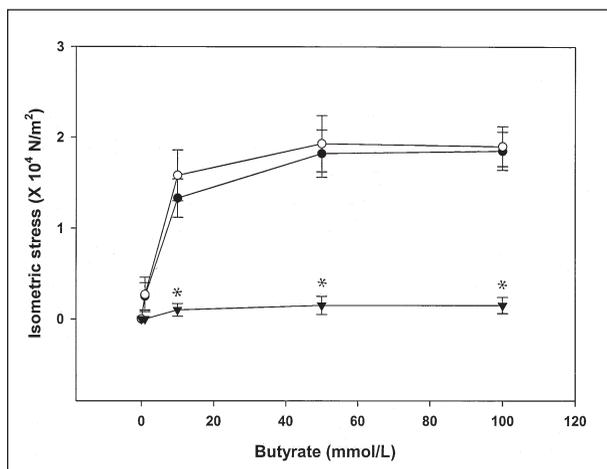


Figure 6—Isometric stress responses (mean \pm SEM; $n = 8$) of longitudinal smooth muscle tissues obtained from the proximal portion of the canine colon to various concentrations of sodium butyrate alone (solid circle) or to various concentrations of sodium butyrate after prior incubation with tetrodotoxin ($1 \mu\text{mol/L}$; open circle) or nifedipine ($1 \mu\text{mol/L}$; solid inverted triangle). *Nifedipine significantly ($P < 0.05$) inhibited smooth muscle responses at this concentration of sodium butyrate.

mal portion of the colon were not affected by tetrodotoxin (alone or in combination with atropine), but nifedipine and zero extracellular calcium solution significantly affected the response and abolished the isometric response to butyrate ($n = 8$; Fig 6). The longitudinal smooth muscle response to butyrate was attenuated ($> 71\%$) but not completely abolished by the addition of EGTA ($n = 7$; Table 1).

Effect of ryanodine on isometric stress responses to sodium butyrate or sodium propionate—Contractions following noncumulative additions of butyrate (50 mmol/L) or propionate (50 mmol/L) were not affected by prior incubation of tissues with $10 \mu\text{mol}$ of ryanodine ($n = 8$; Table 1).

Discussion

Analysis of results of the study reported here revealed that SCFA (ie, acetate, butyrate, and propionate) stimulate contraction of canine colonic longitudinal, but not circular, smooth muscle *in vitro*. Similar results have been reported for the distal portion of the gastrointestinal tract in rats.^{21,22} Short-chain fatty acids at concentrations similar to those used in the experiments reported here induced contractions of the terminal ileum²¹ and middle and distal, but not proximal, portion of the colon in rats.²² To our knowledge, similar *in vitro* studies have not been reported in other animal species.

The SCFA-induced longitudinal smooth muscle contractions may be physiologically relevant, because they were observed for concentrations of SCFA that are found in the gastrointestinal tract of dogs. Several studies^{2-4,23} have documented that total SCFA concentrations of the proximal and distal portions of the colon range from 150 to 200 mmol/L in dogs fed meat- or cereal-based diets; thus, SCFA concentrations used in the experiments reported here are similar to those reported *in vivo*.

The amplitude of the SCFA-induced responses suggests that they may contribute to *in vivo* colonic motility patterns such as propulsion, retropulsion, or maintenance of muscular tone. Contractions induced by butyrate and propionate (50 mmol/L) were 37 and 23%, respectively, of the responses to maximal concentrations of AMCh for tissues obtained from the same site. These SCFA-induced responses are similar to responses to acetylcholine and substance P in the canine colon reported elsewhere.²⁴ Short-chain fatty acids at concentrations similar to those used in the experiments reported here can stimulate *in vivo* propulsive motility in the ileum of dogs⁸⁻¹⁰ but apparently not the colon of dogs.¹¹ Analysis of our data would suggest that endogenous SCFA may contribute more to the overall muscular tone of the colon instead of propulsive motor activity. Tonic activity would be consistent with the sustained concentrations of SCFA resulting from ongoing fermentation of fiber in the colon of dogs.

Sodium butyrate was the most effective SCFA, whereas sodium acetate elicited the least substantial response in the proximal and distal portions of the colon. Sodium propionate elicited contractions intermediate in magnitude between sodium butyrate and acetate. Similar results were reported²² for the colon of rats, but all SCFA appear to be equally effective in the ileum of rats.²¹ The maximum isometric stress response for each SCFA appeared to be approximately 50 mmol/L , although the extracellular concentration of SCFA in the vicinity of smooth muscle cells may only be a fraction of that concentration because of metabolism by colonocytes.^{3,4} Contractile responses observed in the canine colon are consistent with maximal responses reported in the ileum of rats.²¹

The reason for differences in the amplitude of SCFA-induced contractions was not readily apparent but may relate to differences in the activation of calcium influx. Stimulatory effects of specific SCFA could be mediated by receptor occupancy or by ion-channel

activation. The mechanism of the SCFA-induced response was investigated by incubating muscle strips with tetrodotoxin, an inhibitor of the fast sodium channel of enteric neurons, and atropine, a nonselective muscarinic cholinergic antagonist, prior to the addition of each SCFA. Tetrodotoxin and atropine did not have a significant effect on the responses of longitudinal smooth muscle to butyrate or propionate, suggesting that the SCFA effects are not mediated by enteric cholinergic neurons. Responses to butyrate and propionate were abolished or greatly attenuated by prior incubation of tissues with nifedipine (inhibitor of the L-type calcium channel), a zero extracellular calcium solution, or EGTA. Responses to SCFA were not affected by prior incubation with ryanodine, a plant alkaloid that inhibits calcium storage in the sarcoplasmic reticulum of smooth muscles.¹⁸ Analysis of these data suggests that ryanodine-sensitive intracellular calcium stores do not contribute to SCFA-induced contractions, although other ryanodine-insensitive stores could have contributed to these contractions. Even so, the response of canine colonic longitudinal smooth muscle to SCFA appears to be largely dependent on the influx of extracellular calcium through membrane calcium channels. Results obtained with nifedipine further suggest involvement of the L-type calcium channel. Our findings are consistent with results²¹ reported for ileal longitudinal smooth muscle of rats. Short-chain fatty acids induce contractions in ileal longitudinal smooth muscle cells of rats that are accompanied by increases in the concentration of cytosolic free calcium, both of which are abolished by prior treatment with verapamil, a voltage-dependent calcium-channel antagonist.²¹ The dependence of the SCFA response on extracellular calcium in canine colonic longitudinal smooth muscle is consistent with the dependence of longitudinal smooth muscles on extracellular calcium with other agonists and in other species.²⁵⁻²⁷ For example, studies of longitudinal muscle strips from the intestines of guinea pigs and humans support the notion that in calcium-free medium or in calcium-containing medium after the addition of calcium-channel blockers, contraction induced by muscarinic agonists is abolished or is replaced by a transient contraction when high concentrations of an agonist are used. In muscle cells isolated from the longitudinal muscle layer, contraction and the increase in intracellular calcium concentration induced by agonists are abolished by withdrawal of calcium from the medium or addition of calcium-channel blockers, implying that the source of calcium responsible for contraction in these cells is extracellular.^{25,26}

Short-chain fatty acids could have other effects on smooth muscle contraction. Rapid permeation of smooth muscle cells may decrease the intracellular pH of muscle cells, resulting in decreased potassium conductance and depolarization of the cell membrane.²⁸ Intracellular pH was not measured in this set of experiments, but SCFA at concentrations of 1 to 100 mmol/L did not alter the pH of the HEPES buffering system.

Short-chain fatty acids did not have an effect on canine colonic circular smooth muscle. These findings are consistent with those of another study,²⁶ indicating

that agonist-induced contractions of circular smooth muscle are not dependent on extracellular calcium. Instead, circular smooth muscle cells are dependent on the mobilization of intracellular calcium. Removal of calcium from the medium or addition of a calcium-channel blocker does not inhibit the contractile response of circular muscle cells nor the corresponding increase in intracellular calcium concentrations.

^aProvided by Dr. Mark Haskins, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa.

^bEukanuba, Iams Pet Foods, Dayton, Ohio.

^c(N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid], Sigma Chemical Co, St Louis, Mo.

^dSylgard 184 silicone elastomer, Dow Corning, Midland, Mich.

^e50-1569, 10-ml jacketed organ baths, Harvard Apparatus, South Natick, Mass.

^f50-7905, isometric force transducers, Harvard Apparatus, South Natick, Mass.

^gAcetyl-β-methylcholine bromide, Sigma Chemical Co, St Louis, Mo.

^hHW-Model 800, LakeShore Technologies, Chicago, Ill.

ⁱSW-STP-8 Lakeshore Technologies, Chicago, Ill.

^jDell Optiplex GXMT 5166, Dell Computers, Austin, Tex.

^kSodium acetate, Aldrich Chemical, Milwaukee, Wis.

^lSodium butyrate, Aldrich Chemical, Milwaukee, Wis.

^mSodium propionate, Sigma Chemical Co, St Louis, Mo.

ⁿTetrodotoxin, Sigma Chemical Co, St Louis, Mo.

^oAtropine sulfate, Sigma Chemical Co, St Louis, Mo.

^pNifedipine, Sigma Chemical Co, St Louis, Mo.

^qEthylene glycol-bis (β-aminoethylether)-N,N,N',N'-tetra-acetate, Sigma Chemical Co, St Louis, Mo.

^rRyanodine, Sigma Chemical Co, St Louis, Mo.

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