Journal of Feline Medicine and Surgery (2003) **5**, 167–173
doi:10.1016/S1098-612X(03)00002-0

jfms

Short chain fatty acids stimulate feline colonic smooth muscle contraction

Mark P Rondeau*, Karen Meltzer, Kathryn E Michel, Catherine M McManus, Robert J Washabau

Gastrointestinal Physiology Laboratory, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104-6010, USA

The effect of short chain fatty acids (SCFA) on feline colonic smooth muscle contraction was evaluated in vitro. Colonic tissue was obtained from seven healthy male and female adult cats and seven healthy male and female kittens. Longitudinal and circular colonic smooth muscle strips from proximal and distal colon were incubated with SCFA (acetate, butyrate and propionate; 1–100 mM). SCFA-induced contractions were compared to responses obtained using maximal concentrations (10^{-4} M) of acetylcholine (ACh). The calcium dependence of the SCFA response was investigated by incubating with nifedipine (1 μM) or verapamil (1 μM).

Acetate, butyrate and propionate elicited isometric stress responses (0.25–1.98× 10^4 N/m^2) in longitudinal, but not circular, smooth muscle from both the proximal and distal colon of adult cats. Maximal responses were attained at 50 and 100 mM SCFA. Maximal butyrate and propionate responses were 29 and 19% of the maximal ACh response (10^{-4} M), respectively. Acetate was least effective in stimulating contractile responses. Nifedipine and verapamil abolished all responses. Contractile responses in kittens were similar to those observed in adult cats, but were smaller in amplitude.

Results of these studies have shown that SCFA stimulate longitudinal colonic smooth muscle contractions in kittens and adult cats in vitro. These SCFA-induced contractions involve activation of calcium influx. These in vitro findings may account for some of the effects of dietary fiber on feline colonic motility in vivo.

Date accepted: 12 December 2002

Introduction

Short chain fatty acids (SCFA) are the end products of bacterial fermentation of dietary fiber in the hindgut of nonruminant mammals. Fiber is defined as plant components that are resistant to degradation by mammalian digestive enzymes, especially amylase. Substrates for bacterial fermentation include structural polysaccharides (cellulose, hemicellulose, some pectins), nonstructural polysaccharides (pectins, gums, mucilages) and structural nonpolysaccharides (lignin) (Hickman 1998). Acetate, propionate and butyrate account for the majority of SCFA produced in the feline colon, and they are found in individual concentrations as high as 150 mM (Brosey et al 2000). SCFA are readily absorbed and rapidly metabolized by colonic epithelial cells (Buguat 1987, Bergman 1990). They have several physiologic effects including promotion of colonocyte differentiation and proliferation (LeDuc et al 1994), stimulation of sodium and water reabsorption (Ruppin et al 1980, Herschel et al 1981), inhibition of pathogenic bacterial growth (Izat et al 1990), production of metabolic energy, and enhancement of colonic blood flow (Bergman 1990). Recent work in our laboratory has shown that SCFA also stimulate canine colonic longitudinal smooth muscle contraction (McManus et al 2002).

Colonic motility disorders, such as megacolon, are more common in feline than in canine patients (Washabau and Hasler 1996, Jergens and Willard 2000). Recurring episodes of constipation or obstipation may culminate in the development of

An abstract of these data was presented at the 2002 ACVIM Forum in Dallas, TX.

*Corresponding author. Tel.: +1-215-573-8608; fax: +1-215-573-6050. E-mail: rondeau@vet.upenn.edu

1098-612X/03/030167+07 $30.00/0

megacolon. Idiopathic dilated megacolon is the end stage of colonic dysfunction, where affected cats have permanent loss of colonic structure and function (Washabau and Holt 1999). Traditional medical therapy has consisted of removal of impacted feces, followed by treatment with laxatives and/or colonic prokinetic agents. With the withdrawal of cisapride (Propulsid; Janssen) from the North American and western European markets, there are no readily available effective colonic prokinetic drugs for use in the cat. Dietary fiber has been advocated as an effective natural laxative because of its effects of increasing fecal water content, decreasing intestinal transit time, and increasing frequency of defecation (Washabau and Holt 1999).

The goal of the study described here was to evaluate the in vitro effects of SCFA on colonic smooth muscle from feline proximal and distal colon. We hypothesized that SCFA may have a prokinetic effect on feline colonic smooth muscle, similar to effects previously reported in canine colonic smooth muscle (McManus et al 2002). An SCFA-mediated prokinetic effect could provide another mechanism by which dietary fiber would be beneficial in the treatment of feline colonic motility disorders.

Materials and methods

Tissues

Colonic tissue derived from euthanized adult cats and kittens was harvested for use in this study. The tissue donors included seven healthy, sexually intact male and female adult cats (1–2 years of age) and seven healthy, sexually intact male and female kittens (3–5 months of age). Study animals were euthanized (using an intravenous overdose of pentobarbital) as part of an ongoing, but unrelated study[1] at the university. Cats 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 anthelminthics. All cats were fed the same commercially available diet, and food was withheld for 8 h prior to euthanasia. An institutional animal care and use committee approved all procedures.

[1]The authors gratefully acknowledge the provision of animal tissue by Dr Mark Haskins of the University of Pennsylvania (NIH grant #s DK54481 and DK25759).

Preparation of colonic muscle tissue

Immediately following euthanasia, the entire colon was removed from each cat through a midline incision and placed in hydroxyethylpiperazine ethanesulfonic acid (HEPES)[2] buffer solution for the subsequent determination of mechanical properties. Colonic segments were placed in silicone elastomer[3] coated dissection dishes containing HEPES buffer solution (137.3 mmol of NaCl/l, 5 mmol of KCl/l, 1 mmol of $MgCl_2$/l, 1.5 mmol of $CaCl_2$/l, 10 mmol of glucose/l, and 5 mmol of HEPES/l) at a pH of 7.4 at room temperature (20–22°C). HEPES buffer was used because it is a zwitterionic buffering system that has excellent buffering capacity (McManus et al 2002); incubation of acetate, propionate, or butyrate at concentrations ranging from 1 to 100 mM does not change the pH of the buffering system. The colon was opened longitudinally along the mesenteric surface, and luminal contents were gently removed, using repetitive HEPES buffer washes. To approximate in vivo conditions as closely as possible, tissue strips with intact mucosa and submucosa were prepared from the proximal (1–5 cm distal to the ileocecal sphincter) and distal (1–5 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 were then transferred to continuously aerated (100% O_2) and warmed (37° C) 10 ml tissue baths[4] 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[5], as described elsewhere (Fleischmann et al 1993, Kume et al 1994). After a 45-min 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

[2]*N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid], Sigma Chemical Co, St Louis, MO.
[3]Sylgard 184 silicone elastomer, Dow Corning, Midland, MI.
[4]50-1569, 10-ml jacketed organ baths, Harvard Apparatus, South Natick, MA.
[5]50-7905, isometric force transducers, Harvard Apparatus, South Natick, MA.

10^{-4} M acetylcholine (ACh)[6] was achieved. Active isometric forces were recorded at each tissue length. Optimal muscle length was subsequently maintained for the duration of each experiment. Acquisition and analysis of data for active isometric forces were performed by use of transducer amplifiers[7], computer software[8], and a computer[9], as described elsewhere (Washabau et al 1994, Washabau and Stalis 1996).

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 ACh (10^{-4} M) or with noncumulative (single bolus) concentrations of sodium acetate[10], sodium butyrate[11], or sodium propionate[12] (1–100 mM). To evaluate the role of extracellular calcium in the SCFA-induced contractile responses, experiments were performed following incubation with nifedipine[13] (1 μM) or verapamil[14] (1 μM).

Each experimental protocol consisted of four longitudinal and four circular tissue strips, comprising two tissue strips prepared in circular and two strips prepared in longitudinal muscle orientations from each of the proximal and distal portions of the colon. Data obtained from identical muscle sites (eg, two longitudinal muscle strips from the proximal portion of the colon) were used to calculate a mean value. In SCFA dose–response experiments, SCFA concentrations (1, 10, 50, or 100 mM) 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 10–100 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/cm^3 was assumed (Herlihy and Murphy 1973). Isometric forces were standardized on the basis of tissue cross-sectional area and reported as described elsewhere (Washabau et al 1991a,b).

Multiple treatments were analyzed by repeated-measures ANOVA and paired t-tests (Tallarida and Murray 1987). Effects of nifedipine and verapamil on baseline responses were analyzed by use of a one-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 two sites.

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

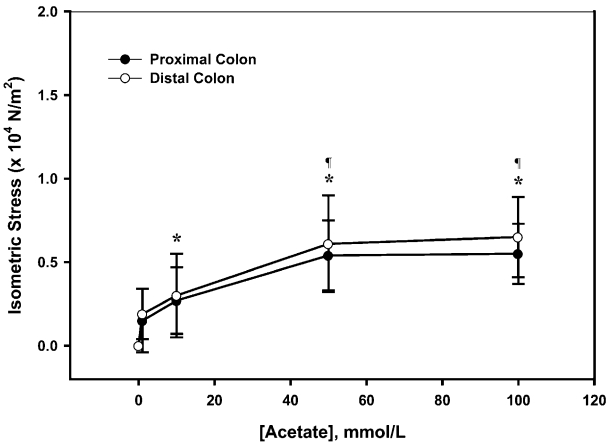

Fig 1. Dose–response curves for acetate (1–100 mM) in adult feline longitudinal colonic (proximal and distal) smooth muscle (n=7). *Significantly greater ($P<0.05$) than responses seen at 1 mM acetate. ¶Significantly greater ($P<0.05$) than responses seen at 1 or 10 mM acetate.

Results

Isometric stress responses to sodium acetate

Sodium acetate (1–100 mM) caused tonic contractions of longitudinal smooth muscle from the proximal and distal colon of adult cats (n=7) with a maximal contractile response (P_{max}) at 50 and 100 mM acetate (Fig 1). The 100 mM acetate responses were less than 5% of the responses obtained with maximal concentrations (10^{-4}) of ACh ($P<0.05$). Contractile responses at 10, 50, and 100 mM acetate concentrations were all significantly greater ($P<0.05$) than responses at 1 mM acetate. Acetate at doses of 1–100 mM was without effect on circular smooth muscle from proximal and distal colon (data not shown). Responses

[6]Acetyl-β-methylcholine bromide, Sigma Chemical Co, St Louis, MO.
[7]HW-Model 800, Lakeshore Technologies, Chicago, IL.
[8]SW-STP-8, Lakeshore Technologies, Chicago, IL.
[9]Dell Optiplex GXMT 5166, Dell Computers, Austin, TX.
[10]Sodium acetate, Aldrich Chemical, Milwaukee, WI.
[11]Sodium butyrate, Aldrich Chemical, Milwaukee, WI.
[12]Sodium propionate, Aldrich Chemical, Milwaukee, WI.
[13]Nifedipine, Sigma Chemical Co, St Louis, MO.
[14]Verapamil, Sigma Chemical Co, St Louis, MO.

Table 1. Maximal responses of colonic smooth muscle from adult feline and kitten proximal and distal colon

	Butyrate (P_{max}; $\times 10^4$ N/m^2)	Propionate (P_{max}; $\times 10^4$ N/m^2)
Adult feline		
Distal longitudinal	1.46±0.21	0.82±0.18
Distal circular	0	0
Proximal longitudinal	1.31±0.16	0.95±0.22
Proximal circular	0	0
Kitten		
Distal longitudinal	0.99±0.19	0.66±0.18
Distal circular	0	0
Proximal longitudinal	0.86±0.21	0.63±0.16
Proximal circular	0	0

P_{max}=peak active isometric stress resulting from SCFA stimulation. Values are mean±SEM for seven observations.

in longitudinal colonic smooth muscle in kittens (n=7) were similar to those in adult cats, but they were of smaller amplitude (data not shown).

Isometric stress responses to sodium butyrate

Sodium butyrate (1–100 mM) caused tonic contractions of longitudinal smooth muscle from the proximal and distal colon of adult cats (n=7) with a maximal contractile response (P_{max}) at 50 and 100 mM (Table 1, Fig 2). The 100 mM butyrate responses were 29% of the maximal ACh (10^{-4} M) response. Contractile responses at 10, 50, and 100 mM butyrate concentrations were all significantly greater (P<0.05) than responses at 1 mM butyrate. Butyrate at doses of 1–100 mM was without effect on circular smooth muscle from proximal and distal colon (Table 1). Responses in longitudinal colonic smooth muscle in kittens (n=7) were similar to those in adult cats, but they were of smaller amplitude (Table 1).

Isometric stress responses to sodium propionate

Sodium propionate (1–100 mM) caused tonic contractions of longitudinal smooth muscle from the proximal and distal colon of adult cats (n=7) with a maximal contractile response (P_{max}) at 50 and 100 mM (Table 1, Fig 3). The 100 mM propionate responses were 19% of the maximal ACh (10^{-4} M) responses. Contractile responses at 10, 50, and 100 mM propionate concentrations were all significantly greater (P<0.05) than responses at 1 mM propionate. Propionate at doses of 1–100 mM was without effect on circular smooth muscle from proximal and distal colon (Table 1). Responses in longitudinal colonic smooth muscle

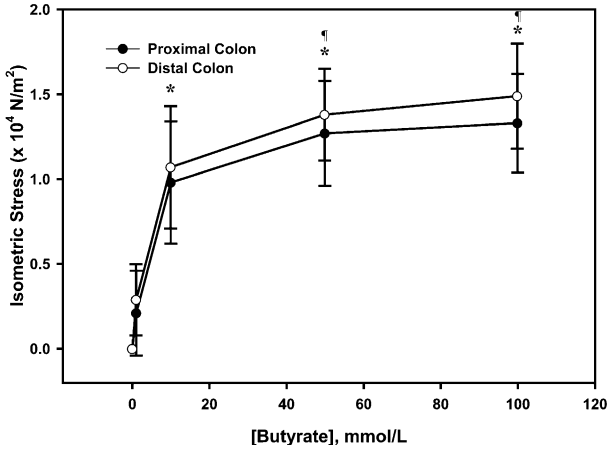

Fig 2. Dose–response curves for butyrate (1–100 mM) in adult feline longitudinal colonic (proximal and distal) smooth muscle (n=7). *Significantly greater (P<0.05) than responses seen at 1 mM butyrate. ¶Significantly greater (P<0.05) than responses seen at 1 or 10 mM butyrate.

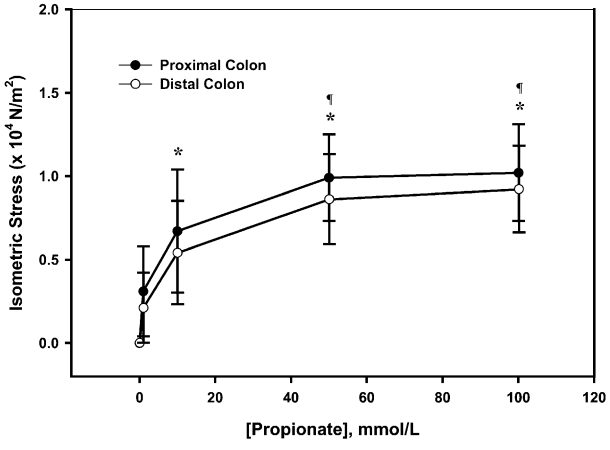

Fig 3. Dose–response curves for propionate (1–100 mM) in adult feline longitudinal colonic (proximal and distal) smooth muscle (n=7). *Significantly greater (P<0.05) than responses seen at 1 mM propionate. ¶Significantly greater (P<0.05) than responses seen at 1 or 10 mM propionate.

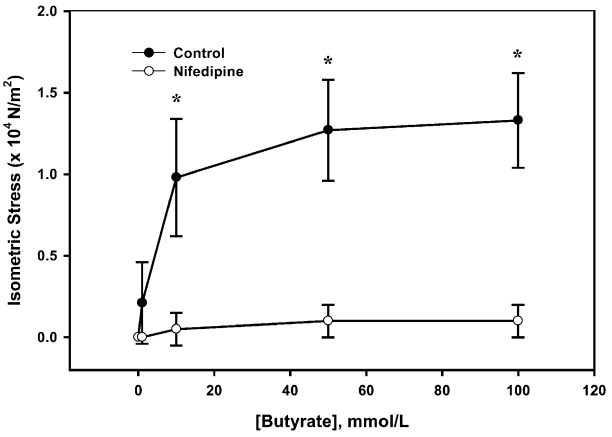

Fig 4. Dose–response curves for butyrate (1–100 mM) in adult feline longitudinal distal colonic smooth muscle in the presence or absence of nifedipine (1 μM) (n=7). ¶Significantly greater ($P<0.05$) than responses seen in the presence of 1 μM nifedipine.

of kittens (n=7) were similar to those in adult cats, but they were of smaller amplitude (Table 1).

Effect of nifedipine or verapamil on isometric stress responses

Isometric stress responses to butyrate (1–100 mM) in longitudinal smooth muscle from proximal and distal colon of adult cats were abolished by prior incubation with nifedipine (1 μM; Fig 4) or verapamil (1 μM; data not shown). Nifedipine and verapamil similarly abolished contractile responses to acetate and propionate (data not shown).

Discussion

Experiments conducted in this study have shown that SCFA induce contraction of longitudinal, but not circular, smooth muscle from the proximal and distal portions of the feline colon in vitro. These results are similar to those reported previously in the canine colon (McManus et al 2002). The stimulatory effect of SCFA on feline colonic longitudinal smooth muscle may provide another mechanism by which dietary fiber is clinically useful in the treatment of feline colonic motility disorders.

Previous experiments have shown that fermentation of fibrous substrates by feline colonic microflora results in colonic luminal SCFA concentrations in vivo similar to those used in this study (Brosey et al 2000). Total SCFA concentrations ranged from 46 to 190 mM in the proximal colon, and from 49 to 264 mM in the distal colon. Acetate, propionate, and butyrate are the most abundant SCFA, and they are found in the colonic lumen of cats in ratios of approximately 60:25:15. Acetate concentrations range from 18 to 146 mM, propionate concentrations range from 7 to 74 mM, and butyrate concentrations range from 4 to 27 mM. These are luminal concentrations that may underestimate concentrations in the vicinity of colonic smooth muscle cells, as SCFA are rapidly absorbed from the lumen by colonocytes (Macfarlane et al 1994). In this set of experiments, we tried to closely approximate concentrations of SCFA that have been reported in vivo, but we chose 100 mM as the maximal concentration because this appeared to be the concentration at which maximal effect was achieved in classic dose–response experiments. We cannot exclude the possibility that higher concentrations of SCFA could evoke higher contractile responses. We also cannot exclude synergistic or other effects of SCFA in the in vivo colon. In our experiments, we evaluated individual SCFA effects rather than combinations of two or three SCFA. Synergistic and/or other effects of SCFA combinations may occur in vivo, and warrant further investigation.

SCFA concentrations found in the feline colon are dependent on the type of fiber found in the diet. Fiber classifications have often been based on physicochemical properties (eg, solubility characteristics), but a more physiologically relevant method of classifying fiber is based on fermentability. Fibers with increased fermentability will result in increased SCFA production. In vitro fermentation techniques using feline colonic microflora have shown that the greatest total SCFA production occurs when fibers such as locust bean gum, guar gum, and citrus pectin are fed, while fibers such as cellulose, gum karaya, and xanthan gum result in production of lower concentrations of SCFA. Intermediate total SCFA concentrations are produced with beet pulp diets (Sunvold et al 1995). Although locust bean gum, guar gum, and citrus pectin resulted in higher total colonic SCFA concentrations, cats fed a diet rich in these fibers had unwanted side effects, such as increased fecal output and diarrhea (Sunvold et al 1995). When comparing colonic absorption of SCFA, absorption was greatest when cats were fed beet pulp compared with nonfiber, cellulose, and pectin/gum arabic (Bueno et al 2000). Beet pulp is a fiber source containing a mixture of soluble and insoluble fibers, as are soybean hulls, pea fiber, and oat fiber. It has been suggested a mixed fiber source may be the dietary

fiber source of choice for cats to optimize both SCFA production and fecal consistency (Bueno et al 2000), but more research will be needed to determine ideal fiber composition in feline diets.

Sodium butyrate produced the greatest contractile response, while sodium acetate produced the least response. These results are similar to those reported previously in the canine colon (McManus et al 2002). Further evaluation of specific dietary fiber sources and combinations of fiber may elucidate a method by which individual SCFA concentrations can be altered in vivo. Whether increased butyrate production in the in vivo colon would contribute significantly to improved colonic motility remains to be seen.

The contractile response to SCFA in feline colonic longitudinal smooth muscle appears to be dependent on influx of extracellular calcium through plasma membrane calcium channels. Contractile responses to all SCFA (acetate, butyrate, propionate) were abolished when the tissue was pre-incubated with nifedipine (1 μM) or verapamil (1 μM), which are inhibitors of the L-type calcium channel. This is consistent with results reported previously in canine colonic longitudinal smooth muscle (McManus et al 2002) and rat ileal longitudinal smooth muscle (Cherbut et al 1996). Based on this investigation, we cannot exclude the contribution of other sources (eg, sarcoplasmic reticulum) of calcium to the SCFA-induced responses, but the magnitude of the nifedipine and verapamil effects suggest that most of the SCFA-induced responses derive from extracellular calcium. Differences in the ability to activate calcium influx may explain the different amplitudes of response seen with individual SCFA.

Responses observed in the kitten colon were similar to those observed in adult cats for all SCFA, but they were smaller in amplitude. Possible explanations for these findings include differences in SCFA absorption, calcium mobilization, development of colonic smooth muscle, and cholinergic or other neurotransmitter responsiveness. Previous experiments have shown reduced contractile responses to ACh and substance P in colonic smooth muscle of kittens compared with those in adult cats, supporting the latter mechanism (McManus et al 2000).

SCFA stimulate contraction of feline longitudinal colonic smooth muscle in vitro. If these effects occur in vivo, it may provide another mechanism by which dietary fiber could be beneficial in cats with colonic motility disorders. It may also indicate a role for dietary fiber in the maintenance of normal colonic tone in health.

Acknowledgement

The authors graciously acknowledge the financial support of the National Institutes of Health (T35 RR07065-001).

References

Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* **70**, 567–590.

Brosey BP, Hill RC, Scott KC (2000) Gastrointestinal volatile fatty acid concentrations and pH in cats. *American Journal of Veterinary Research* **61**, 359–361.

Bueno AR, Cappel TG, Sunvold GD, Moxley RA, Reinhart GA, Clemens ET (2000) Feline colonic microbes and fatty acid transport: effects of feeding cellulose, beet pulp and pectin/gum arabic fibers. *Nutrition Research* **20**, 1319–1328.

Buguat M (1987) Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comparative Biochemistry and Physiology* **86B**, 439–472.

Cherbut C, Aube AC, Blottiere HM, Pacaud P, Scarpignato C, Galmiche JP (1996) In vitro contractile effects of short chain fatty acids in the rat terminal ileum. *Gut* **38**, 53–58.

Fleischmann BK, Washabau RJ, Kotlikoff MI (1993) Control of resting membrane potential by delayed rectifier potassium currents in airway smooth muscle cells. *Journal of Physiology* **469**, 625–638.

Herlihy JT, Murphy RA (1973) Length–tension relationship of smooth muscle of the hog carotid artery. *Circulation Research* **33**, 275–283.

Herschel DA, Argenzio RA, Southworth M, Stevens CE (1981) Absorption of volatile fatty acid, sodium, and water by the colon of the dog. *American Journal of Veterinary Research* **42**, 1118–1124.

Hickman MA (1998) Interventional nutrition for gastrointestinal disease. *Clinical Techniques in Small Animal Practice* **13**, 211–216.

Izat AL, Tidwell NM, Thomas RA, Reiber MA, Adams MH, Colberg M, Waldroup PW (1990) Effects of a buffered propionic acid in diets on the performance of broiler chicks and on microflora of the intestine and carcass. *Poultry Science* **69**, 818–826.

Jergens AE, Willard MD (2000) Diseases of the large intestine. In: Ettinger SJ, Feldman EC (eds), *Textbook of Veterinary Internal Medicine* (5th edn). Philadelphia, PA: WB Saunders, pp. 1238–1256.

Kume H, Hall IP, Washabau RJ, Takagi K, Kotlikoff MI (1994) β-Adrenergic agonists regulate K_{Ca} channels in airway smooth muscle by cAMP dependent and independent mechanisms. *Journal of Clinical Investigation* **93**, 371–379.

LeDuc LE, McRoberts JA, Vidrich A (1994) Eicosanoid production by a differentiated canine colonic epithelial cell line. *Gastroenterology* **106**, 297–305.

Macfarlane GT, Gibson GR, Macfarlane S (1994) Short chain fatty acid and lactate production by human intestinal bacteria grown in batch and continuous culture. In: Binder

HJ, Cummings J, Soergel KH (eds), *Short Chain Fatty Acids* (21st edn). Dordrecht, The Netherlands: Kluwer Academic, pp. 44–60.

McManus CM, Michel K, Simon DA, Washabau RJ (2000) Post-natal development of cholinergic and substance P responses in feline colonic smooth muscle. *Gastroenterology* **118**, A839.

McManus CM, Michel KE, Simon DM, Washabau RJ (2002) Effect of short-chain fatty acids on contraction of smooth muscle in the canine colon. *American Journal of Veterinary Research* **63**, 295–300.

Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG Jr (1980) Absorption of short-chain fatty acids by the colon. *Gastroenterology* **78**, 1500–1507.

Sunvold GD, Fahey GC, Merchen NR, Bourquin LD, Titgemeyer EC, Bauer LL, Reinhart GA (1995) Dietary fiber for cats: In vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. *Journal of Animal Science* **73**, 2329–2339.

Tallarida RJ, Murray RM (1987) *Manual of Pharmacologic Calculations with Computer Programs* (2nd edn). New York: Springer.

Washabau RJ, Hasler AH (1996) Constipation, obstipation and megacolon. In: August JR (ed), *Consultations in Feline Internal Medicine* (3rd edn). Philadelphia, PA: WB Saunders, pp. 104–113.

Washabau RJ, Holt D (1999) Pathogenesis, diagnosis, and therapy of feline idiopathic megacolon. *Veterinary Clinics of North America—Small Animal Practice* **29**, 589–603.

Washabau RJ, Stalis H (1996) Alterations in colonic smooth muscle function in cats with idiopathic megacolon. *American Journal of Veterinary Research* **57**, 580–587.

Washabau RJ, Wang MB, Ryan JP (1991a) Effect of muscle length on isometric stress and calcium-dependent myosin phosphorylation in smooth muscle. *American Journal of Physiology* **260**, G920–G924.

Washabau RJ, Wang MB, Ryan JP (1991b) Myosin light chain phosphorylation and contraction of smooth muscle. *American Journal of Physiology* **261**, G952–G957.

Washabau RJ, Wang MB, Ryan JP (1994) Myosin light chain phosphorylation and contraction of guinea pig gallbladder smooth muscle. *American Journal of Physiology* **266**, G469–G474.