

In vitro investigation of the effect of prostaglandins and nonsteroidal anti-inflammatory drugs on contractile activity of the equine smooth muscle of the dorsal colon, ventral colon, and pelvic flexure

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Objectives—To determine the in vitro effect of prostaglandin E₂ (PGE₂), PGF_{2α}, PGI₂; and nonsteroidal anti-inflammatory drugs (NSAID; ie, flunixin meglumine, ketoprofen, carprofen, and phenylbutazone) on contractile activity of the equine dorsal colon, ventral colon, and pelvic flexure circular and longitudinal smooth muscle.

Animals—26 healthy horses.

Procedure—Tissue collected from the ventral colon, dorsal colon, and pelvic flexure was cut into strips and mounted in a tissue bath system where contractile strength was determined. Incremental doses of PGE₂, PGF_{2α}, PGI₂, flunixin meglumine, carprofen, ketoprofen, and phenylbutazone were added to the baths, and the contractile activity was recorded for each location and orientation of smooth muscle.

Results—In substance P-stimulated tissues, PGE₂ and PGF_{2α} enhanced contractility in the longitudinal smooth muscle with a decrease or no effect on circular smooth muscle activity. Prostaglandin I₂ inhibited the circular smooth muscle response with no effect on the longitudinal muscle. The activity of NSAID was predominantly inhibitory regardless of location or muscle orientation.

Conclusions and Clinical Relevance—In the equine large intestine, exogenous prostaglandins had a variable effect on contractile activity, depending on the location in the colon and orientation of the smooth muscle. The administration of NSAID inhibited contractility, with flunixin meglumine generally inducing the most profound inhibition relative to the other NSAID evaluated in substance P-stimulated smooth muscle of the large intestine. The results of this study indicate that prolonged use of NSAID may potentially predispose horses to develop gastrointestinal tract stasis and subsequent impaction. (*Am J Vet Res* 2000;61:1259–1266)

Impactions of the large intestine can contribute to a substantial proportion of gastrointestinal tract lesions in horses.¹ Although the cause of impactions of the large intestine remains obscure, implicating factors include poor quality feed, advanced age, debilitation,

poor dentition, luminal parasites, restricted exercise or change in the amount of physical activity, and abnormal gastrointestinal tract motility patterns.¹ Regulation of gastrointestinal tract motility involves an intricate and delicate balance among various neural, humoral, and local components with input from the central, autonomic, and enteric nervous systems. Endogenous prostaglandins are locally released as products of arachidonic acid metabolism via the cyclooxygenase pathway.^{2,3} The predominant prostaglandins that regulate gastrointestinal tract function include prostaglandin E₂ (PGE₂), F_{2α} (PGF_{2α}), and I₂ (PGI₂), which exert substantial effects on water and electrolyte movement, mucus secretion, blood flow, and motility.^{2,4,5} Of these subtypes, PGE₂ is the major prostaglandin found in the gastrointestinal tract system.⁶ However, generalizations of prostaglandin activity in the intestine are difficult because of variations reported among species, routes of administration, and concentrations.

The effect of various prostaglandin subtypes on gastrointestinal tract motility depends on the region of the gastrointestinal tract evaluated and the orientation of the smooth muscle (circular vs longitudinal).⁷ For example, in humans and guinea pigs PGE₂ causes contraction of the longitudinal smooth muscle while relaxing the circular smooth muscle in in vitro investigations.⁷ Prostaglandin F_{2α} induces contractions in the circular and longitudinal muscles of the small and large intestine in guinea pigs, humans, cats, and dogs and increases the frequency of spike potentials and contractility of the canine small intestine in vivo.^{4,7-9} In horses, PGE₁ is associated with significant decreases in the electric activity in the stomach, colon, and small colon.¹⁰

The role of endogenous prostaglandins in motility regulation is suggested by the clinical impression that administration of nonsteroidal anti-inflammatory drugs (NSAID) is associated with an increased occurrence of impactions of the large intestine (cecal) in horses.¹¹ In the management of equine injury and disease, common NSAID include propionic acids (ketoprofen), pyrazolones (phenylbutazone), and aminonicotinic acid (flunixin meglumine), primarily for their antipyretic, anti-inflammatory, and analgesic effects. Although NSAID such as phenylbutazone and flunixin meglumine are commonly used for relief of signs of pain associated with musculoskeletal lesions, they are also associated with complications including mucosal

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ulceration and renal papillary necrosis caused by inhibition of cyclooxygenase activity.^{12,13} The effect of NSAID on cyclooxygenase activity in the horse has been established. In 1 study, ponies given phenylbutazone and PGE₂ remained clinically normal without developing low serum protein concentrations or mucosal injury of the gastrointestinal tract, whereas ponies receiving only phenylbutazone did develop low serum protein concentrations and mucosal injury.¹⁴

In a previous study,¹⁵ we evaluated the effect of NSAID and the various prostaglandin subtypes on contractile activity of the taenia of the equine ventral large intestine. In that study, PGE₂ and PGF_{2α} had an excitatory effect as observed by the increase in frequency and amplitude of contractions, whereas PGI₂ inhibited contractile activity. Further, all NSAID evaluated significantly reduced contractile activity. The purpose of the study reported here was to evaluate the effect of PGE₂, PGF_{2α}, and PGI₂ on contractile activity of the circular and longitudinal smooth muscle of the equine dorsal colon, ventral colon, and pelvic flexure.

Materials and Methods

The University Animal Use and Care Committee approved this study. Tissue was collected from horses euthanized for reasons unrelated to the gastrointestinal tract. The horses had a mean age of 12 years (range, 2 to 25 years), with a mean weight of 470 kg (range, 375 to 560 kg). Immediately following euthanasia with an overdose of pentobarbital, the large intestine was exteriorized through an incision in the linea alba, and 10 × 10-cm full-thickness portions of the pelvic flexure, dorsal colon, and ventral colon at the level of the sternal and diaphragmatic flexures were removed. The tissue sections were processed as described.¹⁶ The tissue was immersed in a modified Krebs buffer solution (containing in mM: NaCl 110, KCl 4.6, CaCl₂ 2.5, NaHCO₃ 24.8, KH₂PO₄ 1.2, MgSO₄ 1.2, and dextrose 5.6; pH 7.3 to 7.4 when equilibrated with 95% O₂/5% CO₂). The tissue was pinned flat under slight tension in a dissecting dish containing sufficient Krebs buffer solution to keep the tissue completely immersed and cut into strips that were 2-mm wide by 10-mm long and parallel to the circular and longitudinal smooth muscle. The strips were mounted on glass hooks in a tissue bath system (20 ml capacity), which contained oxygenated Krebs buffer solution warmed to 37.5 ± 0.5 C as described.¹⁶ The other end of the muscle strip was connected to a polygraph chart recorder^a that measured tension using force transducers.^b After allowing the tissue to equilibrate in the baths for 45 minutes, the strips were stretched under a basal tension of 2 grams and allowed to equilibrate for an additional 60 minutes. During the equilibration period, the

Krebs buffer was changed at 20-minute intervals. Although spontaneous activity was generated by some of the muscle strips during this time, substance P^c (40 μl of 10⁻⁷M) was added to each bath to generate a consistent contractile pattern.¹⁶ Each run consisted of 4 circular and 4 longitudinal strips, with a minimum of 3 runs performed for each drug evaluated. Therefore, each drug was tested on a minimum of 12 muscle strips with only 1 drug tested per muscle strip during each run. The concentrations of prostaglandins and NSAID added to the muscle baths were 1 × 10⁻⁹, 3 × 10⁻⁹, 1 × 10⁻⁸, 3 × 10⁻⁸, 1 × 10⁻⁷, 3 × 10⁻⁷, 1 × 10⁻⁶, 3 × 10⁻⁶, and 1 × 10⁻⁵ M. Phasic contractions of the muscle strips were recorded as described.¹⁶ Contractile activity was recorded prior to the administration of any drug to establish baseline activity. Following drug addition to the muscle baths, contractile activity of the muscle strips was recorded for 180 seconds with a 10-minute interval between the addition of drugs. The inhibition or excitation generated was determined by comparing the strength of contraction (in Newtons [N]) generated during the 180-second interval to the contractile force obtained at baseline. In a previous study,¹⁵ we determined that contractility did not change as a function of time or tissue fatigue.

The PGI₂, PGE₂, and PGF_{2α}^c were kept as stock solutions of 10⁻²M in dimethyl sulfoxide (DMSO) solution stored at 4 C or prepared fresh the day of the experiment. The smallest volume of DMSO that would dissolve the NSAID to prepare a 10⁻²M stock solution was 400 μl. To determine that the contractile effects were not caused by the addition of DMSO to the muscle baths, the diluted DMSO in Krebs buffer was added to isolated muscle strips with no effect. On the day of the study, dilutions of the prostaglandins were freshly prepared in Krebs solution. The phenylbutazone, ketoprofen, and carprofen were purchased in crystalline form initially prepared as 10⁻²M stock solutions.^c The flunixin meglumine was purchased in solution from the manufacturer.^d

Statistical analysis—Statistical analysis was performed by an ANOVA to determine whether there was a significant difference in treated strips compared with baseline contractile activity and between treatments. Post hoc tests (Fishers least significant difference) were used if a significant difference was detected ($P \leq 0.05$).

Results

Exogenous prostaglandins had a variable effect on contractile strength of smooth muscle depending on prostaglandin concentration, location in the large intestine, and orientation of the smooth muscle (Table 1). Prostaglandin I₂ did not significantly affect contractile strength (frequency or amplitude of contractions) of circular or longitudinal smooth muscle of the dorsal colon, compared with baseline values (Fig 1). In the

Table 1—Mean in vitro effective concentration 50 (EC₅₀) of prostaglandins and nonsteroidal anti-inflammatory agents in the circular and longitudinal smooth muscle layers of equine large intestine

Agent	Ventral colon		Dorsal colon		Pelvic flexure	
	CM	LM	CM	LM	CM	LM
Phenylbutazone	2.6 × 10 ⁻⁶	1.5 × 10 ⁻⁵	4.0 × 10 ⁻⁶	1.5 × 10 ⁻⁵	2.9 × 10 ⁻⁶	5.9 × 10 ⁻⁷
Ketoprofen	5.9 × 10 ⁻⁷	4.7 × 10 ⁻⁶	6.7 × 10 ⁻⁷	4.7 × 10 ⁻⁶	8.2 × 10 ⁻⁷	7.7 × 10 ⁻⁷
Flunixin meglumine	7.2 × 10 ⁻⁷	6.5 × 10 ⁻⁷	3.3 × 10 ⁻⁷	6.5 × 10 ⁻⁷	2.6 × 10 ⁻⁷	2.3 × 10 ⁻⁶
Carprofen	1.6 × 10 ⁻⁶	3.3 × 10 ⁻⁶	6.7 × 10 ⁻⁷	3.3 × 10 ⁻⁶	4.8 × 10 ⁻⁷	5.6 × 10 ⁻⁷
PGI ₂	4.7 × 10 ⁻⁷	2.9 × 10 ⁻⁶	1.1 × 10 ⁻⁵	1.4 × 10 ⁻⁶	2.6 × 10 ⁻⁶	1.5 × 10 ⁻⁶
PGF _{2α}	4.9 × 10 ⁻⁶	1.2 × 10 ⁻⁵	2.1 × 10 ⁻⁶	1.4 × 10 ⁻⁵	2.6 × 10 ⁻⁶	5.0 × 10 ⁻⁷
PGE ₂	4.3 × 10 ⁻⁷	3.9 × 10 ⁻⁶	6.6 × 10 ⁻⁶	2.3 × 10 ⁻⁶	4.7 × 10 ⁻⁶	2.5 × 10 ⁻⁶

CM = Circular smooth muscle layer. LM = Longitudinal smooth muscle layer. PGI₂ = Prostaglandin I₂. PGF_{2α} = Prostaglandin F_{2α}. PGE₂ = Prostaglandin E₂.

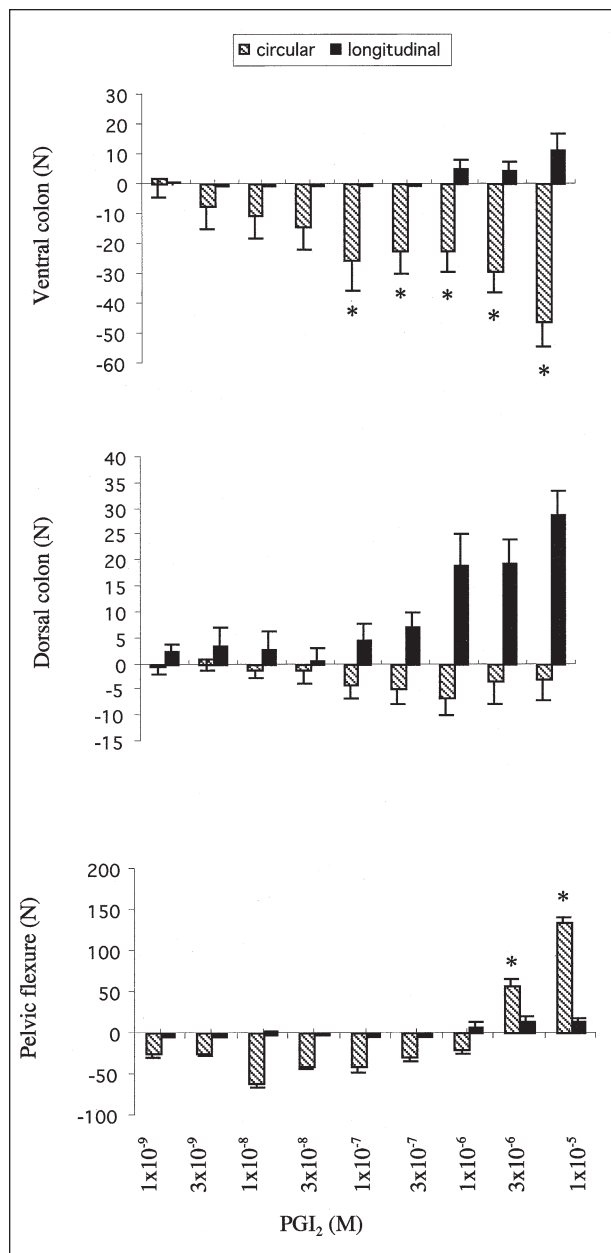


Figure 1—Mean contractile response following the administration of prostaglandin I₂ (PGI₂, in M concentration) to the ventral colon, dorsal colon, and pelvic flexure of the large intestine depicted with concentration (M) on the X axis and newtons (N) on the y axis. *Significant difference from baseline at $P < 0.05$.

ventral colon, a significant decrease in contractile strength of circular smooth muscle was detected at PGI₂ concentrations $\geq 1 \times 10^{-7}$ M, compared with baseline values, whereas a significant effect on contractile strength of longitudinal smooth muscle occurred only at the highest concentration tested (1×10^{-5} M PGI₂). Contractile strength of longitudinal smooth muscle of the ventral colon at PGI₂ concentrations $> 3 \times 10^{-7}$ M were significantly different from lower concentrations but not from each other. Contractile strength of circular smooth muscle of the ventral colon at the highest concentration of PGI₂ tested (1×10^{-5} M) was significantly different from other PGI₂ concentrations, except

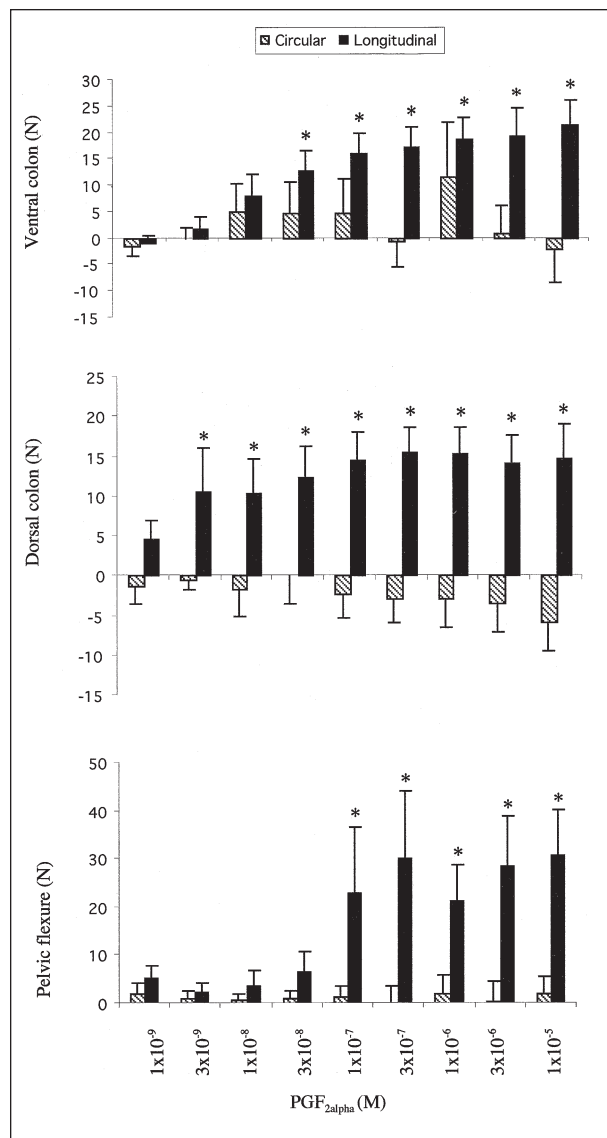


Figure 2—Mean contractile response following the administration of PGF_{2α} to equine smooth muscle of the large intestine. See Figure 1 for key.

1×10^{-7} and 3×10^{-6} M. No significant effect of PGI₂ was observed on contractile strength of longitudinal smooth muscle of the pelvic flexure. Prostaglandin I₂ concentrations $> 3 \times 10^{-6}$ and 1×10^{-5} M ($P = 0.01$ and 0.01 , respectively) resulted in a significant increase in contractile strength of circular smooth muscle of the pelvic flexure compared with baseline values.

Prostaglandin F_{2α} consistently increased the contractile strength of longitudinal smooth muscle of the dorsal colon, ventral colon, and pelvic flexure (Fig 2). In contrast, no significant effect of PGF_{2α} was observed on contractile strength of circular smooth muscle of the ventral colon, dorsal colon, and pelvic flexure. Prostaglandin F_{2α} caused an increase in contractile strength of circular smooth muscle of the ventral colon and pelvic flexure, whereas PGF_{2α} caused an initial increase in contractile strength of circular smooth muscle of the dorsal colon that was followed by a persistent decline at higher concentrations.

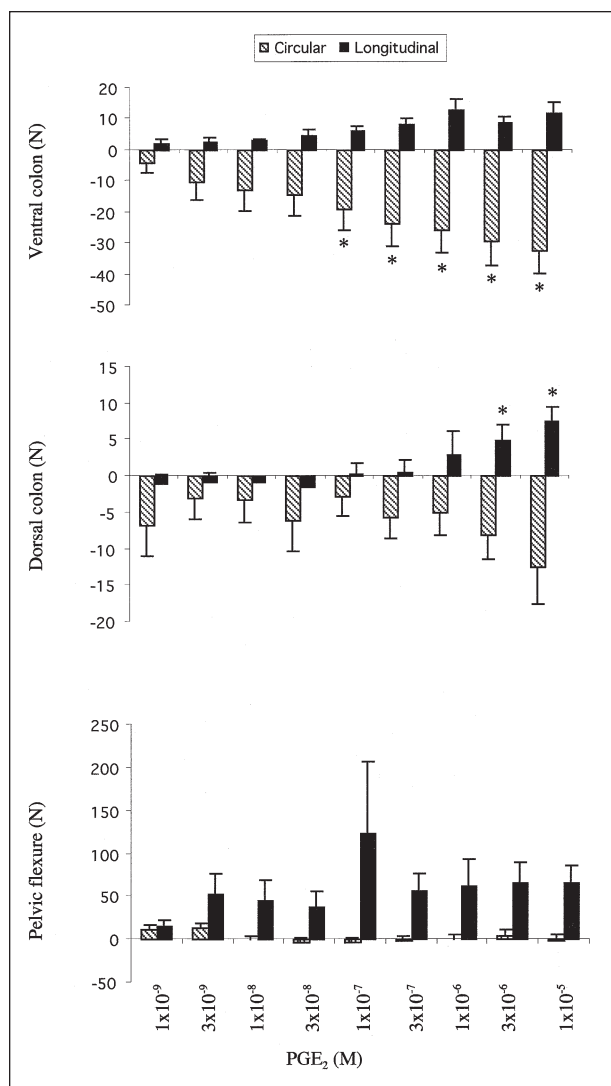


Figure 3—Mean contractile response following the administration of PGE₂ to equine smooth muscle of the large intestine. See Figure 1 for key.

Prostaglandin E₂ increased the amplitude and frequency of contractions in the longitudinal smooth muscle of the dorsal and ventral colon at concentrations > 3 × 10⁻⁶ M and 1 × 10⁻⁷ M; (Fig 3). Mean contractile strength of longitudinal smooth muscle of the dorsal colon (relative to baseline) increased from 4.9 N at PGE₂ concentrations of 3 × 10⁻⁶ M to 7.5 N at PGE₂ concentrations of 1 × 10⁻⁵ M. Contractile strength of the longitudinal smooth muscle of the ventral colon increased from 6.1 N at a PGE₂ concentration of 1 × 10⁻⁷ M to 11.7 N at a PGE₂ concentration of 1 × 10⁻⁵ M. However, no significant effect was seen on the circular and longitudinal smooth muscle of the pelvic flexure and the circular smooth muscle of the dorsal colon. In contrast to the effect seen on the longitudinal smooth muscle, PGE₂ consistently and significantly decreased contractile strength of circular smooth muscle of the ventral colon. The contractile strength decreased from -19.2 N at a PGE₂ concentration of 1 × 10⁻⁷ M to -32.5 N at a PGE₂ concentration of 1 × 10⁻⁵ M.

The NSAID caused a predominantly inhibitory

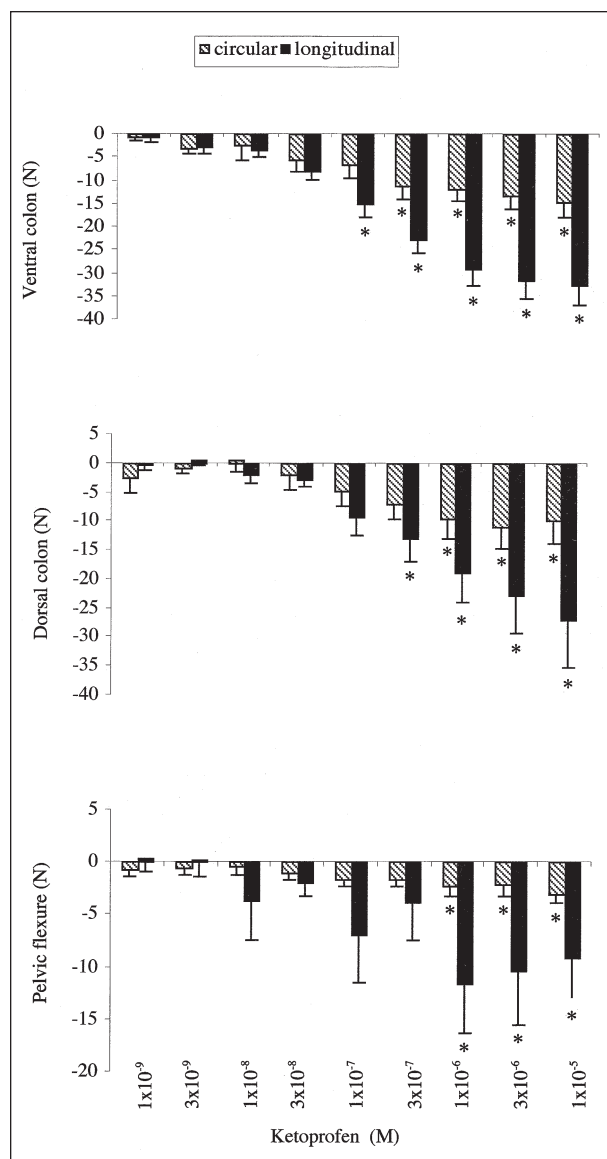


Figure 4—Mean contractile response following the administration of ketoprofen to equine smooth muscle of the large intestine. See Figure 1 for key.

effect in all regions of the colon and in the circular and longitudinal smooth muscle. In the ventral colon, contractile strength of circular smooth muscle was significantly decreased at concentrations of ketoprofen > 1 × 10⁻⁷ M (*P* = 0.03) and at ketoprofen concentrations > 3 × 10⁻⁸ M (*P* = 0.03 M) for the longitudinal smooth muscle. In the dorsal colon, higher concentrations were required in the circular and longitudinal smooth muscle to reduce contractile strength, compared with the ventral colon. In the circular smooth muscle, contractile strength was decreased at a ketoprofen concentration of 1 × 10⁻⁶ M (*P* = 0.01), whereas contractile strength of the longitudinal smooth muscle was decreased at ketoprofen concentrations > 3 × 10⁻⁷ M (*P* = 0.02). Although low concentrations of ketoprofen (1 × 10⁻⁹ M and 3 × 10⁻⁹ M) were associated with increased contractile strength of the longitudinal smooth muscle of the pelvic flexure, compared with baseline values,

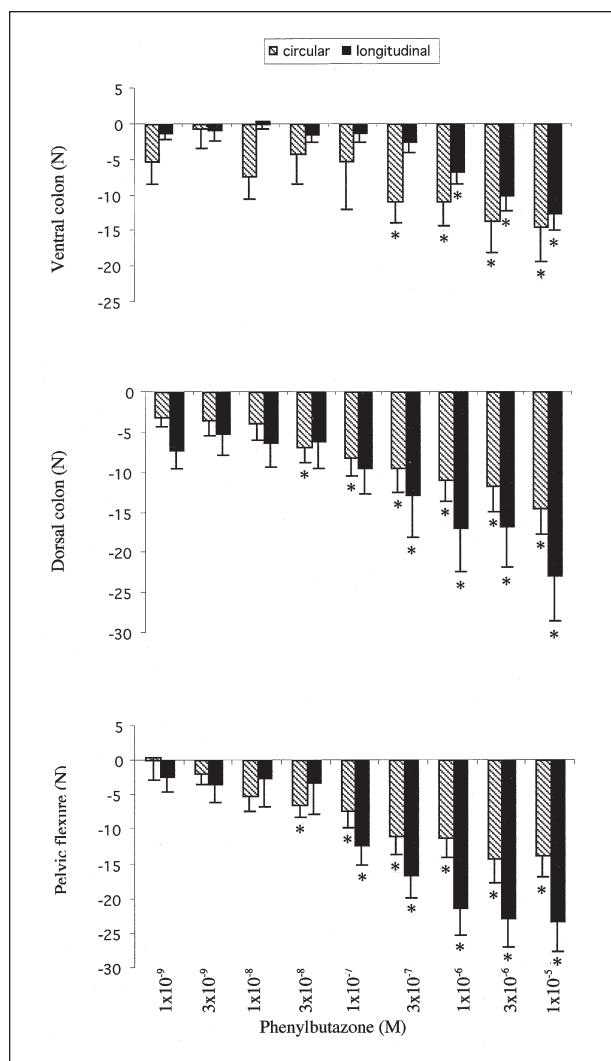


Figure 5—Mean contractile response following the administration of phenylbutazone to equine smooth muscle of the large intestine. See Figure 1 for key.

subsequent additions of higher concentrations of ketoprofen were progressively inhibitory. In the pelvic flexure, ketoprofen concentrations $> 1 \times 10^{-6}$ M were significantly different from baseline for the circular and longitudinal smooth muscle (Fig 4).

Phenylbutazone decreased the contractile strength of circular smooth muscle of the pelvic flexure and dorsal colon at concentrations $> 3 \times 10^{-8}$ M. Phenylbutazone decreased the contractile strength of circular smooth muscle of the ventral colon at a concentration of 1×10^{-7} M (Fig 5). In the ventral colon, dorsal colon, and pelvic flexure, phenylbutazone decreased the contractile strength of longitudinal smooth muscle at concentrations $> 1 \times 10^{-6}$ M, 3×10^{-7} M, and 1×10^{-7} M, respectively. At lower concentrations of phenylbutazone, the contractile activity of circular smooth muscle of the ventral colon fluctuated by increasing and decreasing in strength, although phenylbutazone concentrations $> 3 \times 10^{-8}$ M consistently decreased contractile strength. In the longitudinal smooth muscle of the ventral colon, a similar pattern was observed in that contractile strength increased

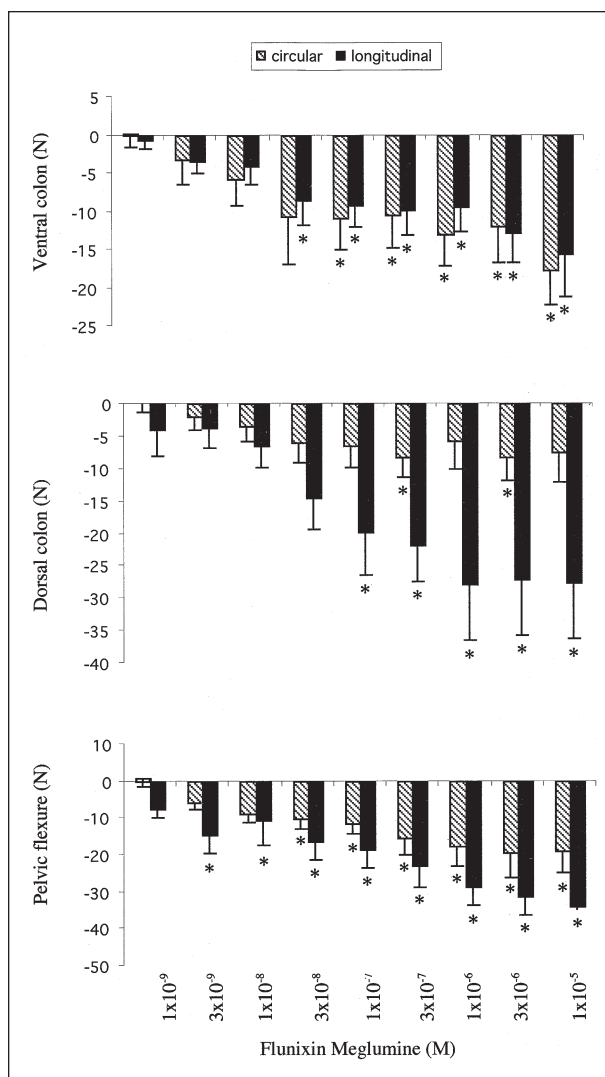


Figure 6—Mean contractile response following the administration of flunixin meglumine to equine smooth muscle of the large intestine. See Figure 1 for key.

above baseline at phenylbutazone concentrations $\leq 3 \times 10^{-8}$ M. In the dorsal colon and pelvic flexure, the addition of phenylbutazone caused a steady decline in contractile strength of longitudinal smooth muscle and frequency and amplitude of contractile activity.

The addition of flunixin meglumine to smooth muscle of the ventral colon decreased contractile strength relative to baseline values, although the magnitude of the decrease in contractile strength was greater for the circular than the longitudinal smooth muscle (Fig 6). Thus, a significant decrease in contractile strength of circular smooth muscle occurred at a flunixin concentration of 1×10^{-7} M with a mean difference from baseline of 10.98 N, compared with a flunixin concentration of 3×10^{-8} M with a mean difference of 8.7 N for longitudinal smooth muscle. In the dorsal colon, the response to flunixin was similar in longitudinal smooth muscle to that observed in the ventral colon. However, the circular smooth muscle of the dorsal colon appeared less sensitive to flunixin, because a significant decrease in contractile strength occurred at the higher flunixin

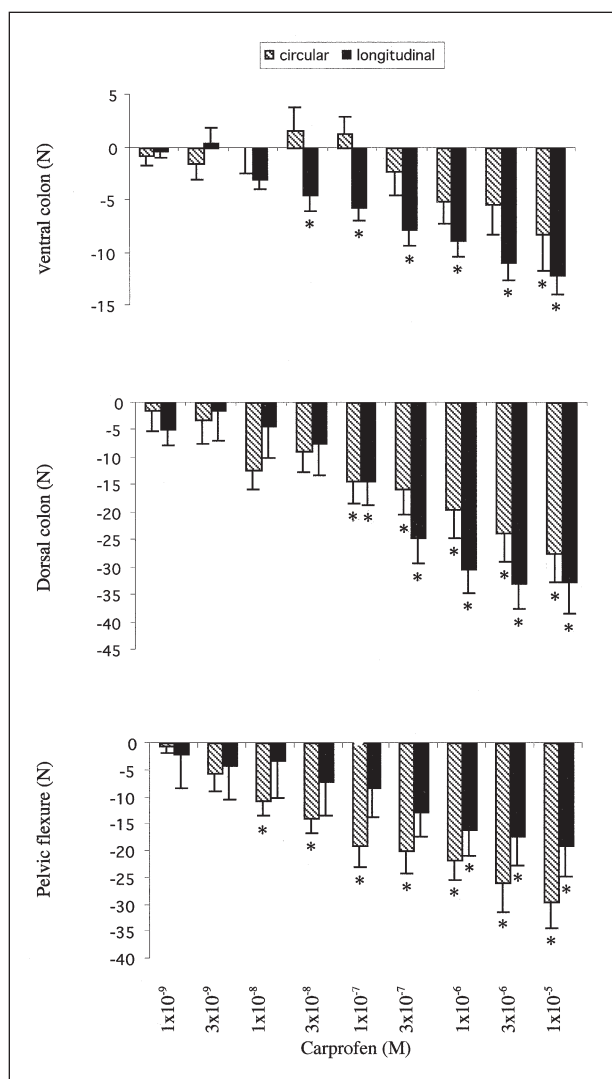


Figure 7—Mean contractile response following the administration of carprofen to equine smooth muscle of the large intestine. See Figure 1 for key.

concentration of 1×10^{-6} M. In the pelvic flexure, contractile strength of circular and longitudinal smooth muscle was significantly inhibited, compared with baseline values at a flunixin concentration of 3×10^{-8} M, although the magnitude of the decrease in contractile strength was greater for the longitudinal smooth muscle relative to the circular smooth muscle.

Carprofen, similarly to the other NSAID, consistently decreased contractile strength of smooth muscle in all regions of the large intestine (Fig 7). In the dorsal colon and pelvic flexure, a significant decrease in contractile strength of the circular and longitudinal smooth muscle occurred at similar carprofen concentrations. For example, the smooth muscle of the dorsal colon was significantly affected at carprofen concentrations $> 1 \times 10^{-7}$ M, whereas carprofen concentrations $> 1 \times 10^{-8}$ and 1×10^{-6} M significantly affected the circular and longitudinal smooth muscle of the pelvic flexure, respectively. The circular smooth muscle of the ventral colon, however, was not significantly affected by carprofen, although the contractile strength appeared to be

decreased. Further, of the areas of the large intestine investigated, carprofen had a smaller effect on the smooth muscle of the ventral colon relative to the dorsal colon and pelvic flexure. Thus, at the highest concentration of carprofen (1×10^{-5} M), the contractile strength of longitudinal smooth muscle of the ventral colon was decreased to 12.1 N, compared with 32.8 N for the longitudinal smooth muscle of the pelvic flexure.

Discussion

Prostaglandins perform important regulatory functions in the gastrointestinal tract, including maintenance of blood flow, immunoregulation, promotion of inflammatory mediator synthesis and motility effects and are thought to exert a substantial effect on gastrointestinal tract fluid and electrolyte (eg, chloride) transport mechanisms.¹⁷⁻²⁰ Therefore, it is reasonable to speculate that inhibition of prostaglandin production via NSAID is likely to have an effect on gastrointestinal tract function. There appears to be a high occurrence of impactions of the large intestine associated with NSAID use, which may be the result of NSAID-mediated inhibition of chloride secretion or reduction in prostaglandins that stimulate contractile activity.^{15,21} Further support for the effect of NSAID on cyclooxygenase function is evident from effects of aspirin, phenylbutazone, and flunixin on serum thromboxane concentrations in horses.²² Results of other studies indicate that the effect of NSAID can persist for longer periods than would be anticipated by their calculated half-life, and a single intravenous injection can inhibit cyclooxygenase activity for at least 24 hours in inflammatory models.^{23,24} This effect may account for the high occurrence of lesions in the large intestine of horses that are on protracted courses of NSAID. Potentially, gastrointestinal tract inflammation secondary to ischemic or distension injury, for example, may prolong cyclooxygenase inhibition from NSAID.

In our study, although the concentrations of NSAID required to significantly inhibit contractile activity varied depending on location (dorsal colon versus ventral colon), they consistently inhibited contractility throughout the large intestine including the circular and longitudinal smooth muscle. The effects of the NSAID were most likely the result of inhibition of cyclooxygenase or some other mechanism and not a toxic pharmacologic effect. This was determined from work in which the application of PGE₂ restored contractile activity in tissue inhibited by NSAID.¹⁵ It is likely that other factors are involved in the effect of NSAID on contractile activity. This is apparent in the circular smooth muscle, for example, where inhibition of inhibitory prostaglandins (ie, PGE₂) would have been expected to increase contractile activity. Although the NSAID generally did not inhibit the circular smooth muscle as substantially as the longitudinal smooth muscle, it is possible that there is a regional distribution of prostaglandin species. It is also possible the NSAID-induced inhibition of the cyclooxygenase pathway may allow for the preferential diversion of arachidonic acid metabolism toward the lipoxygenase pathway. The effect of ketoprofen may be related to its potential ability to inhibit the lipoxygenase pathway and subsequent

production of leukotrienes.²⁵ The products of the lipoxygenase include various leukotrienes that may also induce gastrointestinal tract injury.^{26,27}

In our study, variations in contractile strength occurred in the circular and longitudinal smooth muscle by the same prostaglandin subtype. Prostaglandin E₂, for example, was associated with a decrease in contractile strength in the circular smooth muscle, whereas an excitatory effect occurred in the longitudinal smooth muscle. The inhibitory response of the circular smooth muscle by PGE₂ was also consistent with the results of a study²⁸ evaluating the effect of endotoxin on colonic motility. In that study, prostaglandins E and I were found to decrease contractile activity of the circular smooth muscle, whereas PGF_{2α} stimulated colonic activity; however, effects on the longitudinal smooth muscle of the colon were not evaluated. Results of studies in other species also detected a differential response in the circular and longitudinal smooth muscle by the same prostaglandin species. In guinea pigs, for example, PGE₂ and PGF_{2α} increased contractile activity in the longitudinal smooth muscle, whereas the circular smooth muscle was inhibited.²⁹ The variable effect of the same prostaglandin species in the same region of the gastrointestinal tract on the circular and longitudinal smooth muscle is likely related to differences in receptor type, density, and other local neuropeptides. However, the exact mechanism of activity on the cellular level remains speculative. For example, PGE₂ is thought to increase contractile activity potentially by increasing intracellular cytosolic calcium concentrations, reducing the synthesis of cAMP, or inducing the release of acetylcholine from the myenteric plexus.⁶

Location (ie, dorsal colon vs ventral colon and pelvic flexure) also appeared to be a factor in the method of response to the different prostaglandins. For example, in the dorsal colon, PGI₂ and PGF_{2α} did not have a significant effect on contractile strength of the circular smooth muscle, although there appeared to be a reduction in motility, whereas PGF_{2α} significantly increased contractile activity. Regional differences are also evident in humans where contractile strength in the proximal and distal aspect of the colon is increased by PGE₂, whereas no effect was seen in the midcolon.³⁰ In rabbits, prostaglandin E had no effect on the proximal portion of the colon, whereas the mid- and distal portions were sensitive to PGF_{2α}.³¹

In general, lower concentrations of PGE₂ and PGF_{2α} induced a significant effect on contractile activity for the circular and longitudinal smooth muscle, respectively. These smooth muscle layers significantly affect the digestion and transport of luminal contents. Contractions of the longitudinal smooth muscle reduce the distance that peristaltic waves must move luminal contents, and the alternating contraction and relaxation assists in mixing.³² Segmental and peristaltic contractions of the circular smooth muscle assist in mixing and propagating the food bolus, respectively. The propagation of feed material is the product of the combined influence of the activity of the circular and longitudinal smooth muscle.³³ In humans, the longitudinal smooth muscle is approximately one fifth to one third the thickness of the circular smooth muscle, with

the greatest accumulation of longitudinal muscles in the taenia of the colon.³³ In horses, it is possible that PGE₂ and PGF_{2α} are important regulatory prostaglandins for contractile activity, especially in the ventral colon where large aggregates of longitudinal muscle comprise the taenia. Potentially, long-term NSAID use may cause sustained inhibition of these prostaglandins and promote gastrointestinal tract stasis. A study¹⁵ conducted in our laboratory evaluated the effect of exogenous prostaglandins and NSAID on contractile activity of the lateral free band (taenia) of the ventral colon. We found that exogenous PGE₂ and PGF_{2α} significantly increased contractile activity, whereas PGI₂ caused an inhibitory response that was similar to the effect seen on the longitudinal smooth muscle in this study. Although PGE₂ caused a predominantly inhibitory effect in the circular smooth muscle, it is possible that this prostaglandin may not be found in substantial quantities or frequency. Further studies are indicated to evaluate the quantity and type of prostaglandin synthesized and released in various portions of the colon and how these factors change during and following gastrointestinal tract insult.

The variation observed in the activity of the prostaglandin subtypes at different concentrations may result from the interaction of high concentrations of these agents with other mediators. In rabbits, although low doses of PGF_{2α} significantly increased spike potential frequency, higher concentrations do not have a significant effect.⁷ In that study, possible explanations for this effect included that PGF_{2α} was acting as a partial smooth muscle antagonist such that additional concentrations would not yield more contractile activity. We also speculated that perhaps the higher concentrations induced a catecholamine release, which then negatively affected contractility. This may explain why some of the prostaglandins in our study such as PGI₂ induced contractile activity at the lower concentrations yet inhibited activity at the higher ranges.

In our study, substance P was added to the muscle strips to initiate contractile activity in all muscle strips, because some strips did not have spontaneous activity. However, we did not feel that the addition of this substance influenced our results, because the quantity was minimal, and the use of this agent in other motility investigations has been described.¹⁶ Following the administration of substance P, there was an increase in contractile activity characterized by increases in the amplitude and frequency of contractions. However, after approximately 10 minutes, contractions became regular. Baseline contractile patterns were not recorded until the contractile activity assumed a regular pattern.

The concentrations of prostaglandins and NSAID selected in our study were on the basis of preliminary work, which found that they were low enough not to cause any adverse effect on the tissue. Increasing the concentration from this initial amount allowed us to assess the minimal concentration necessary to detect a response. In horses, extrapolation of in vitro concentrations of NSAID and prostaglandins to in vivo concentrations is difficult. In a study²¹ using indomethacin and flunixin meglumine, concentrations of flunixin meglumine at 4 and 8 μg/ml were used in incubation solu-

tions, which are similar to the concentrations of NSAID evaluated in our study. Although the tissue was bathed in buffer containing specific concentrations of NSAID and prostaglandins, it is not possible to make direct correlations to the values reported in in vivo studies. However, concentrations of prostaglandins used were similar to those in previous studies to evaluate motility effects in the gastrointestinal tract and in a study evaluating the effect of phenylbutazone on gastrointestinal tract mucosa.^{10,34} In contrast to the results of our study, in vivo investigations did not find a significant effect of NSAID on motility of the large intestine.³⁵⁻³⁸ Although a specific reason for the discrepancy is unknown, it is interesting that our in vitro results support the clinical impression that NSAID use can precipitate impactions of the large intestine. Potential reasons for the variation in study outcomes are likely related to differences in study design, route of administration (intravascular versus direct exposure to the smooth muscle), duration of tissue exposure to the NSAID, or an unknown effect of cyclooxygenase inhibitors on local cellular mechanisms. Further investigation is warranted to determine how NSAID specifically interact with the gastrointestinal tract smooth muscle.

^aModel 70 polygraph, Grass Astromed, West Warwick, RI.

^bFTO3, Grass Astromed, West Warwick, RI.

^cSigma Chemical Co, St Louis, Mo.

^dSchering-Plough Animal Health Corp, Kenilworth, NJ.

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