In vitro responses of equine colonic arterial and venous rings to adenosine triphosphate

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Objective—To evaluate the in vitro effects of adenosine triphosphate (ATP) on vasomotor tone of equine colonic vasculature.

Sample Population—Arteries and veins from the left ventral colon of 14 mixed-breed horses euthanized for reasons unrelated to cardiovascular or gastrointestinal tract disease.

Procedures—Endothelium-intact and -denuded arterial and venous rings were precontracted with 10−6 and 1.8 × 10−5 M endothelin-1, respectively. In 1 trial, endothelium-intact rings were also incubated with 10−4 M Nω-nitro-L-arginine methyl ester (L-NAME) to inhibit nitric oxide (NO) production. Adenosine triphosphate (10−6 to 10−4 M) was added in a noncumulative manner, and relaxation percentage versus time curves were generated. Areas under the curves (ie, percentage of relaxation time) were calculated.

Results—Relaxation response of arterial and venous rings to ATP was dose-dependent. Percentage of relaxation time in response to 10−6 M ATP was significantly greater, compared with that for rings not treated with ATP. Removal of endothelium attenuated but did not eliminate the relaxation response. Addition of L-NAME did not attenuate the relaxation response in arteries. At higher concentrations, the vascular response to ATP was biphasic.

Conclusions and Clinical Relevance—ATP applied to equine colonic arterial and venous rings with and without intact endothelium induced a biphasic response characterized by transient contraction followed by slow, substantial, and sustained relaxation. This ATP-induced response is possibly mediated by a mechanism other than NO. Adenosine triphosphate may be a useful treatment to modulate colonic vasomotor tone in horses with strangulating volvulus of the ascending colon. (Am J Vet Res 2001;62:1928–1933)

Strangulating volvulus of the ascending colon in horses is a disease characterized by colonic luminal obstruction and vascular occlusion that results in colonic ischemia, mucosal necrosis, and vascular thrombosis.1 Colonic blood flow remains significantly less than baseline values for at least 4 hours after surgery to correct complete experimentally induced arteriovenous occlusion of the ascending colon.2 This disease is associated with high mortality, which may be related to a sustained reduction of blood flow and hypoperfusion attributable to increased vascular smooth-muscle contraction and continued ischemic injury. Endothelial damage in the colonic vasculature develops subsequent to ischemia-reperfusion injury, and this damage can be exacerbated by endotoxin.3 Therefore, the sustained decrease in colonic blood flow in horses with strangulating volvulus may be associated with damage to the endothelium of colonic vessels. This damage, in turn, will lead to the release of vasoconstrictive agents and loss of endothelium-derived vasorelaxants, which subsequently may result in vasoconstriction.

Extracellular purines have important and diverse effects on many biological processes, including regulation of vascular tone.4 Adenosine triphosphate is principally an endothelium-dependent vasodilator that is rapidly metabolized and has a brief duration of action.5 The vasodilatory effects of ATP are mediated primarily through activation of purine receptors located on the endothelium and vascular smooth muscle.6 When activated by ATP purinergic 2Y (P2Y) receptors located on endothelial cells couple to G-proteins and activate phospholipase C, which leads to formation of inositol-3-phosphate (IP3) and mobilization of intracellular calcium.7 Vasodilatation occurs as a result of calcium-dependent activation of endothelial nitric oxide synthase (NOS) with subsequent generation of nitric oxide (NO) and endothelial-derived hyperpolarizing factor (EDHF).8 Generation of protein kinase C and subsequent phosphorylation of mitogen-activated protein kinase appears to be the pathway by which P2Y receptors on endothelial cells mediate prostacyclin synthesis and release to generate additional vascular relaxation.9 Purinergic 2Y receptors are also found on vascular smooth muscle and mediate vasodilatation.10 The mechanism underlying P2Y-mediated smooth-muscle relaxation is not known but may involve activation of potassium channels.11 When ATP is degraded to adenosine by ectonucleotidases, an adenosine purine receptor (predominantly A1) is activated, leading to vascular smooth-muscle relaxation.12

We have recently evaluated the local colonic and systemic hemodynamic alterations associated with IV infusion of a combination of ATP and magnesium chloride (ATP-MgCl2) in healthy anesthetized adult horses.13 Administration of ATP-MgCl2 at an infusion
rate of 0.3 mg of ATP/kg of body weight/min resulted in a significant decrease in colonic vascular resistance, principally via vasodilatation.11 These results suggest that ATP-MgCl₂ could have beneficial effects during low-flow conditions in the gastrointestinal tract by regulating vascular tone.

Because the endothelium is damaged during periods of colonic ischemia and reperfusion, the effects of ATP on regulation of vascular tone may be diminished or abolished as a result of loss of endothelial-derived vasorelaxants, specifically NO. The purpose of the study reported here was to determine the effects of ATP on vasomotor tone (specifically the vasodilatory response) of isolated equine colonic arterial and venous rings with and without intact endothelium and after in vitro exposure to the NOS inhibitor No-nitro-L-arginine methyl ester (L-NAME). We hypothesized that ATP would cause concentration-dependent vasodilatation in arterial and venous rings. Moreover, any vasodilatory response in arterial rings would be significantly attenuated as a result of removal of the endothelium or exposure to L-NAME. Finally, because veins typically respond less than arteries to endothelium-dependent relaxation12 and the predominant site of NO action is arterial resistance vessels,13 we predicted that in vitro responses of venous rings to ATP would be minimal affected by lack of intact endothelium or exposure to L-NAME.

Materials and Methods

Horses and collection of mesenteric vessels—This study was approved by the Institutional Animal Care and Use Committee of Louisiana State University. Segments of mesenteric vessels were collected from the left ventral colon of 14 adult horses destined for euthanasia for reasons unrelated to gastrointestinal tract and vascular disease and euthanatized with sodium pentobarbital (100 mg/kg, IV). Vessels were collected and placed in chilled oxygenated (95% O₂ and 5% CO₂) Tyrode solution (136.87 mM NaCl; 2.68 mM KCl; 11.90 mM NaH₂CO₃; 5.55 mM dextrose; 1.81 mM CaCl₂; 1.07 mM MgCl₂; 3.06 mM NaH₂PO₄) until rings were prepared.

Preparation of vascular rings—The colonic artery and vein were cannulated with an 18-gauge catheter and placed in an organ bath containing oxygenated Tyrode solution at 37 °C. One side of the vessel ring was fixed to the floor of the organ bath, and the other side was attached to a force-displacement transducer interfaced with a polygraph.23,24 An initial tension of 2 g was applied to the rings, which were then allowed to equilibrate for 45 minutes; tension at the end of this period was termed resting tension. Results of published studies23,24 from our laboratory indicate that 2 g of tension applied to colonic vessels results in optimum vessel responsiveness. Bath solutions were replaced and tension readjusted to 2 g at 15-minute intervals except following the final wash.

Experimental protocol—Two experiments were performed. The first (trial 1) was designed to determine the influence of endothelium on response of vessels to ATP. Arterial and venous rings from 7 horses were prepared with intact endothelium (endo+; n = 8 [arterial] and 8 [venous]) or with endothelium removed by use of gentle mechanical debridement (endo–; 8 [arterial] and 8 [venous]).11,15,16 Sixteen tissue baths were used during trial 1, and 2 runs were performed for specimens from each horse to accommodate the 4 ring types (ie, arterial endo+, arterial endo–, venous endo+, and venous endo–). A run was defined as 2 ring types/16 tissue baths; therefore, to accommodate all 4 ring types, 2 runs were performed. Ring types for each horse were randomly assigned to each run. After equilibration for 45 minutes in Tyrode solution, arterial and venous rings were precontracted with 10⁻⁴ M and 1.8 × 10⁻⁵ M endothelin-1 (ET-1), respectively (Table 1). Stock solutions of 10⁻⁴ M ET-1 were prepared in distilled water and stored in 70-ml aliquots at −70 °C until used. On the day of the experiment, the ET-1 stock solution was thawed, and appropriate dilutions were made in distilled water. Endothelin-1 was added to 8 equilibrated rings of each type, and tension was measured continuously until the contractile response plateaued. A single concentration of ATP (10⁻⁴ to 10⁻⁵ M in distilled water) was then added to 1 ring of each type. Tension was determined continuously for 30 minutes, and these data were used to generate an ATP response curve (ie, relaxation percentage vs time) for each ring type and each ATP concentration. Two rings of each type were exposed only to ET-1; these rings served as time controls.

The second experiment (trial 2) was designed to determine the influence of NO in response of vessels to ATP. Arterial and venous rings from 7 horses different from those used in trial 1 were prepared with intact endothelium. Eight tissue baths were used during this trial. Two runs were performed for each ring type (arterial and venous), with the order randomized for all specimens from any given horse. Rings were precontracted with ET-1 as described for trial 1. Vessel rings (arterial, n = 8; venous, 8) were then incubated with freshly prepared 10⁻⁴ M L-NAME for 30 minutes before adding ATP, as described for trial 1. Fresh solutions of ATP and L-NAME were prepared daily. One ring of each type was exposed only to ET-1; this ring served as the time control. In addition, 1 ring of each type was treated with 10⁻⁴ M ATP in the absence of L-NAME; this ring served as the positive control to determine whether there were significant differences in relaxation response to 10⁻⁴ M ATP between vessels used in trial 1 and 2.

Histologic examination—Sets of arterial and venous rings from each horse were prepared during each trial and placed in neutral-buffered 10% formalin. Vessel rings were routinely processed and stained with H&E. Cross-sectional segments were examined histologically to evaluate the integrity of the endothelial and smooth muscle layers.

Determination of relaxation percentage—Tension was measured 1, 3, 5, 7, 9, 11, 13, and 15 minutes after addition of ATP. Tension of the time-control rings (no ATP added) was also measured at these times. Fifteen minutes was selected as the end-point, because contraction induced by ET-1 in the time-control rings was stable during this period, indicating that any relaxation (ie, decrease in tension) was attributable to ATP and not secondary to loss of contraction induced by

<table>
<thead>
<tr>
<th>Ring type</th>
<th>L-NAME</th>
<th>Tension (mg)</th>
<th>Tension/dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial+</td>
<td>No</td>
<td>1,322 ± 105.2</td>
<td>156.0 ± 22.1</td>
</tr>
<tr>
<td>Arterial+</td>
<td>Yes</td>
<td>1,319 ± 102.1</td>
<td>167.1 ± 15.9</td>
</tr>
<tr>
<td>Arterial–</td>
<td>No</td>
<td>1,936 ± 136.7</td>
<td>155.1 ± 16.6</td>
</tr>
<tr>
<td>Arterial–</td>
<td>Yes</td>
<td>1,494 ± 105.9</td>
<td>142.5 ± 13.0</td>
</tr>
<tr>
<td>Venous+</td>
<td>No</td>
<td>2,624 ± 232.4</td>
<td>285.5 ± 33.5</td>
</tr>
<tr>
<td>Venous+</td>
<td>Yes</td>
<td>2,624 ± 232.4</td>
<td>285.5 ± 33.5</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SEM.
ET-1. Relaxation percentage at each time point was determined according to the following formula:

\[
\text{Relaxation \%} = \left( \frac{\text{tension}_{ET-1} - \text{tension}_{ATP}}{\text{tension}_{ET-1}} \right) \times 100
\]

where tension_{ET-1} is the stable tension induced by preconstriction with ET-1, and tension_{ATP} is the tension recorded in treated or time-control rings after addition of ATP to the treated rings. Relaxation percentage versus time curves were generated from these data points, and the area under the curve (AUC) was estimated by use of the trapezoid method. The AUC represented the integrated percentage of relaxation over time and was reported as the percentage of relaxation time (% minutes). Only the area above the x-axis (relaxation) was included in the calculation. Moreover, AUC was considered continuous and followed a normal distribution on the basis of results of the Shapiro-Wilk test with failure to reject the null hypothesis of normality at \( P \leq 0.05 \).

**Statistical analyses**—Percentage of relaxation time (ie, AUC) in response to each concentration of ATP was compared among ring types by use of a fixed-effect linear model assuming a nested factorial design. To account for the fact that different ring types were prepared from different horses, the model included a fixed effect of trial. Trial 1 included 4 ring types (arterial endo+, arterial endo–, venous endo+, and venous endo–), whereas trial 2 included 2 types (arterial t-NAME+ and venous t-NAME+). Thus, ring type was nested within trial, with ATP concentration (the repeated effect) factored over ring type. The interaction term of ring type and concentration was used as the error term to test for significant main effects. When there were significant \(( P \leq 0.05 \) ) effects of concentration and interaction of ring type and concentration, selected ad hoc comparisons were made by use of least square means. Within ring type, percentage of relaxation time in response to each concentration of ATP was compared with percentage of relaxation time for time-control rings. Percentage of relaxation time in response to 10–3M ATP was selected ad hoc comparisons were made by use of least square means. Within ring type, percentage of relaxation time in response to 10–3M ATP was compared with percentage of relaxation time for time-control rings after addition of ATP to the treated rings. Percentage of relaxation time was also compared between rings treated with 10–4M and 1.8 × 10–4M endothelin-1 (ET-1), respectively.

**Results**

Histologic evaluation of each representative ring type indicated that intact endothelium and smooth muscle was present in endo+ rings. In endo– rings, endothelium was completely removed without damage to the smooth muscle layer. Colonic arterial and venous rings precontracted with ET-1 relaxed in response to treatment with ATP regardless of whether

![Image](347x232 to 551x399)

![Image](348x414 to 550x581)

**Table 2**—Adenosine triphosphate-induced relaxation of precontracted* equine colonic arterial and venous rings with intact (+) or denuded (–) endothelium.

<table>
<thead>
<tr>
<th>Ring type (n)</th>
<th>t-NAME†</th>
<th>Max relaxation‡ (%)</th>
<th>Max attenuation§ (%)</th>
<th>Max relaxation‡ (%)</th>
<th>Max attenuation§ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial (8)</td>
<td>No 36.71 ± 4.39</td>
<td>NA</td>
<td>56.90 ± 5.94</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Arterial (8)</td>
<td>No 24.42 ± 9.16</td>
<td>–30.06 ± 22.19</td>
<td>33.99 ± 12.52</td>
<td>–45.17 ± 18.45</td>
<td></td>
</tr>
<tr>
<td>Arterial (8)</td>
<td>Yes 36.98 ± 9.29</td>
<td>1.425 ± 18.54</td>
<td>75.09 ± 14.76</td>
<td>33.80 ± 25.62</td>
<td></td>
</tr>
<tr>
<td>Venous (8)</td>
<td>No 56.94 ± 8.47</td>
<td>NA</td>
<td>59.46 ± 3.55</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Venous (8)</td>
<td>No 36.65 ± 5.98</td>
<td>–29.16 ± 12.58</td>
<td>41.28 ± 4.15</td>
<td>–30.31 ± 6.47</td>
<td></td>
</tr>
<tr>
<td>Venous (8)</td>
<td>Yes 34.30 ± 7.60</td>
<td>–29.52 ± 17.98</td>
<td>62.47 ± 5.85</td>
<td>6.35 ± 10.72</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as mean ± SEM.

*Vascular rings were precontracted with ET-1. t-NAME was added to inhibit nitric oxide synthesis. †Relaxation was determined by use of the equation: tension_{ET-1} – tension_{ATP} / tension_{ET-1}. ‡Attenuation was determined by subtracting the maximum relaxation of rings without endothelium or exposed to t-NAME from that of rings with intact endothelium divided by maximum relaxation of rings without endothelium or exposed to t-NAME.§Max = Maximum. NA = Not applicable.

![Figure 1](348x414 to 550x581)
endothelium was intact or rings were treated with l-NAME (Table 2). Concentrations of ATP ranging from 10^{-8} to 10^{-3}M induced an initial rapid and transient contraction followed by relaxation in all ring types examined (Fig 1). Concentration of ATP and ring type, as well as the interaction of these 2 terms, significantly affected percentage of relaxation time (Fig 2). However, contraction of the time-control rings did not significantly change over the 15-minute evaluation period in either trial.

In trial 1, percentage of relaxation time (ie, AUC) was significantly less 15 minutes after addition of 10^{-4} or 10^{-3}M ATP to arterial and venous endo+ and venous endo- rings, compared with time-control rings. Response of arterial and venous endo- rings to either 10^{-4} or 10^{-3}M ATP did not differ significantly.

Percentage of relaxation time (ie, AUC) for rings precontracted with ET-1 and treated with 10^{-4}M ATP alone was not significantly different between trials 1 and 2. In trial 2, percentage of relaxation time for arterial and venous rings exposed to l-NAME in response to 15 minutes of treatment with 10^{-4} or 10^{-3}M ATP were significantly different from those determined in response to treatment with ET-1 alone (Fig 2). In addition, percentage of relaxation time in response to 10^{-4}M ATP differed significantly between arterial and venous endo- rings in trial 1 and l-NAME–treated arterial and venous rings in trial 2, respectively. Percentage of relaxation time of venous endo+ rings in response to 10^{-3}M ATP in trial 1 also differed significantly from that for l-NAME–treated venous rings in trial 2. Percentage of relaxation time did not differ between l-NAME–treated arterial and venous rings in response to either 10^{-3} or 10^{-4}M ATP.

**Discussion**

Results of the present study indicate that ATP can overcome ET-1-induced vascular contraction. In addition, the relaxation response of equine colonic arterial and venous rings to ATP was dose-dependent and, at high concentrations, biphasic (ie, an initial transient contraction followed by slow relaxation). Removal of endothelium attenuated but did not eliminate the relaxation response in either arterial or venous rings. Moreover, the contribution of NO to the relaxation response was minimal and more appreciable in venous rings.

In contrast to results of numerous studies indicating that the mechanism of ATP-induced vasodilatation is principally mediated by NO, our results suggest that NO does not appreciably contribute to the endothelium-dependent component of the relaxation response to ATP in normal equine colonic vessels. In a study by Simonsen et al that evaluated the effect of ATP on vasomotor tone of isolated coronary small arteries from lambs, mechanical removal of the endothelium but not inhibition of NOS partially reduced relaxation elicited by exogenous ATP. These results indicate that ATP relaxed ovine coronary small arteries through receptors located on smooth muscle and endothelial cells. Furthermore, NO was excluded as a mediator of the endothelial component of the relaxation response to ATP. Results of our present study correlate with results from the study by Simonsen et al and results of an in vivo study performed in our laboratory in which we failed to identify a significant increase in colonic arterial and venous plasma NO concentrations in horses treated with ATP-MgCl2 despite the development of profound colonic vasodilatation.

There are several possible explanations for the observations noted in the present study. Although we did not evaluate its role, prostacyclin may play an important role in endothelium-dependent ATP relaxation in equine colonic arterial and venous rings.
Further studies using indomethacin to block prostacyclin synthesis are necessary to elucidate the role of prostacyclin in ATP-induced vasodilation. Additionally, activation of P2Y receptors with subsequent IP₃ formation and calcium mobilization can lead to the synthesis of EDHF, which may also contribute to the vascular relaxation response.⁴

Another possible explanation for the apparent lack of involvement of NO in the vasodilatory response to ATP is that NOS was not completely inhibited in trial 2. The concentration of L-NAME that we used (10⁻⁴ M) is comparable to that used in other studies¹⁵,¹⁷,²⁰ that evaluated the role of NO in the vascular response in horses. Concentrations of L-NAME ranging from 10⁻⁶ M to 10⁻⁴ M have been shown to be effective in blocking NO production. In our initial pilot studies, we compared colonic vascular responsiveness to acetylcholine in the presence of 10⁻⁶ and 10⁻⁴ M L-NAME. Our results indicated that 10⁻⁴ M L-NAME yielded better blockade of NOS than 10⁻⁶ M. Furthermore, results from an in vivo pharmacokinetic study⁶ of L-NAME in horses indicated that systemic and pulmonary hypertension rapidly develop following IV administration of a bolus injection of L-NAME. Therefore, incomplete blockade of NOS is an unlikely explanation of our results.

One observation that was not expected was the blockade of the endothelium-dependent component of the relaxation response to 10⁻⁴ M ATP when colonic venous rings were treated with L-NAME. The same blockade was not observed in response to 10⁻⁴ M ATP or in arterial rings. This finding is in contrast to results of a study²¹ indicating that the predominant site of action of NO is arterial resistance vessels. An explanation for these findings is that at physiologic concentrations of ATP (10⁻⁴ M), endothelium-dependent relaxation in colonic veins is NO mediated. Although speculative, at superphysiologic concentrations of ATP (10⁻³ M), other endothelium-dependent vasodilators (eg, prostacyclin and EDHF) may become more important than NO. Why L-NAME blocked the endothelium-dependent component of the relaxation response to 10⁻⁴ M ATP in colonic veins but not arteries is not known and warrants further investigation.

Endothelin-1 was selected as our precontractile agent for several reasons. First, because ATP is a slowly relaxing agent, we wanted to use a precontractile agent that would sustain the contraction for a minimum of 15 minutes. This would allow us to more fully evaluate the in vivo effects of ATP on vasomotor tone. Secondly, because ET-1 is the most potent vasoconstricting agent presently identified and because it has been implicated as a cause of decreased blood flow that commonly occurs during ischemia,²² we wanted to determine whether ATP could overcome ET-1–induced vasoconstriction. Concentrations of ET-1 used in the present study were selected on the basis of results of a study by Venugopal et al²³ indicating that ET-1 concentrations required to induce 50% maximum contraction are 2.3 × 10⁻⁴ M and 6.7 × 10⁻⁴ M in colonic arteries and veins, respectively. In pilot studies in our laboratory, these concentrations induced a minimum of 500 mg of contraction, which was the minimum response that was stable and consistent among all ring types examined. Vessel rings that did not attain 500 mg of tension were rejected from analysis in the present study.

To evaluate relaxation responses in vascular ring preparations, the rings must first be contracted to establish tension so a relaxation response can be detected. One limitation of in vitro relaxation studies is the inability to control the amount of stable contraction induced by a vasoconstricting agent or by use of electrical field stimulation. The magnitude of the response to a vasodilating agent may vary depending on the amount of initial tension generated. The effect of various tensions on the relaxation response to ATP was not evaluated in the present study. Therefore, results of other studies using the same ring preparations that we examined may vary if the relaxation response is influenced by the initial tension generated.

We used an unstable form of ATP rather than a stable analog to induce vasodilation. The ATP used in the present study was the same as that used in our in vivo studies, although in our in vivo studies,¹¹,²² magnesium chloride was added. Because significant vasodilatation occurs in vivo after IV administration of ATP-MgCl₂, it was important to determine the mechanism of this vasodilatory response. In particular, it is vital to future studies evaluating the efficacy of ATP for treatment of horses with colonic ischemia to determine whether the form of ATP used clinically could cause vasodilatation in vessels without endothelium.

Unlike our in vivo studies¹¹,²² in which the effects of ATP and magnesium chloride were evaluated, we only evaluated the in vitro effects of ATP in the present study. Magnesium has been reported to be a potent vasodilating agent and can potentiate in vivo vasodilatation when combined with ATP.²³ However, we do not believe that magnesium was a major contributor to the in vivo vasodilatory response that we detected previously. In an in vitro pilot study, we found that compared with the ATP-induced relaxation response, addition of magnesium chloride (10⁻³ M to 1⁻¹ M) did not enhance the relaxation response of equine colonic arterial and venous rings with intact or denuded endothelium precontracted with ET-1. Additionally, magnesium chloride alone did not cause appreciable relaxation of colonic vascular rings with intact or denuded endothelium precontracted with ET-1.

Because the form of ATP used in the present study is subjected to degradation to adenosine by tissue ectonucleotidases, it was not possible to determine whether ATP or adenosine mediated the smooth-muscle component of the vascular relaxation response. Both P₂Y receptors, which respond to ATP, and A₂ receptors, which respond to adenosine, have been identified on vascular smooth muscle.⁴ Additional studies incorporating specific adenosine blocking agents are required to determine what percentage of the smooth-muscle relaxation response is attributable to ATP versus adenosine.

Results of previous studies¹⁰ indicate that high concentrations of ATP cause transient vascular smooth-muscle contraction followed by slow sustained relaxation. Similar results were detected in the present study. Contraction induced by ATP is mediated via activation of P₂X receptors located on smooth muscle.
cells. Ligand binding to P2X receptors results in the rapid and nonselective passage of cations (Na⁺, K⁺, Ca²⁺) across the cell membrane, which results in an increase in intracellular concentrations of Ca²⁺, membrane depolarization, and smooth muscle relaxation. Whether the same mechanism is responsible for ATP-induced vasoconstriction in horses is not known. The concentration-dependent initial transient contraction induced by high concentrations of ATP combined with the lack of specific antagonists to block this response complicates interpretation of the relaxation effects of ATP.

The vascular rings used in the present study were collected from horses without gastrointestinal tract or vascular abnormalities. We attempted to mimic the effect of diseases of the colon that lead to endothelial dysfunction by mechanically removing the endothelium from some of these rings. However, disease could alter in vivo and in vitro vascular responses to ATP. Additionally, the responses and mechanisms induced by ATP may vary along the colonic vascular bed according to region. Thus, results of the vascular segments used in this study may not be predictive of microvasculature responses, which are important to the various components of the intestinal wall.

In the present study, exogenous ATP applied to equine colonic arterial and venous rings precontracted with ET-1 in vitro caused a biphasic response characterized by an initial and transient contraction followed by a slow, substantial, and sustained relaxation. The relaxation response was attenuated by removal of the endothelium but was not inhibited by blocking NO production, suggesting that relaxation is not mediated via NO. Further studies evaluating the role of prostacyclin and adenosine in the relaxation response are required to further define the mechanism of action of ATP-induced vasodilatation. In addition, because vasodilatation does occur in the absence of endothelium, further studies evaluating the in vivo efficacy ATP-MgCl₂ to modulate vasomotor tone in horses with colonic ischemia are also warranted.

### References