Colic events in horses often have major influences on gastrointestinal tract motility. Ischemia and reperfusion injury is a serious problem in the colic process, especially after strangulating obstructions, which often lead to a decrease in intestinal propulsive motility. A period of ischemia may lead to cell injury caused by an increase in ROS; reperfusion injury damages intestinal tissues when oxygenated blood returns to the affected area and subsequently there is an increase in cell membrane permeability. Horses with ischemia and reperfusion injury are prone to development of postoperative ileus. Lidocaine is the most commonly used prokinetic agent for postoperative management of horses with colic.

In an in vitro study, investigators detected an ameliorating effect of lidocaine on jejunal motility after creation of an in vivo ischemia and reperfusion injury. Impaired smooth muscle contractility recovered and was similar to the contractility of undamaged control tissues. During in vitro incubation of smooth muscle preparations, lidocaine decreases cell membrane permeability, which results in diminished release of the marker enzyme CK. These results indicate that lidocaine can improve smooth muscle contractility and ba-

**Effects of in vivo lidocaine administration at the time of ischemia and reperfusion on in vitro contractility of equine jejunal smooth muscle**

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**Objective**—To determine whether administration of lidocaine during ischemia and reperfusion in horses results in concentrations in smooth muscle sufficient to protect against the negative consequences of ischemia-reperfusion injury on smooth muscle motility.

**Animals**—12 horses.

**Procedures**—Artificial ischemia and reperfusion injury of jejunal segments was induced in vivo in conjunction with lidocaine treatment during ischemia (IRL) or without lidocaine treatment (IR). Isometric force performance was measured in vitro in IRL and IR smooth muscle preparations with and without additional in vitro application of lidocaine. Lidocaine concentrations in smooth muscle were determined by means of high-performance liquid chromatography. To assess the influence of lidocaine on membrane permeability, activity of creatine kinase and lactate dehydrogenase released by in vitro incubated tissues was determined biochemically.

**Results**—In vivo administration of lidocaine allowed maintenance of contractile performance after an ischemia and reperfusion injury. Basic contractility and frequency of contractions were significantly increased in IRL smooth muscle tissues in vitro. Additionally, in vitro application of lidocaine achieved further improvement of contractility of IR and IRL preparations. Only in vitro application of lidocaine was able to ameliorate membrane permeability in smooth muscle of IR and IRL preparations. Lidocaine accumulation could be measured in in vivo treated samples and serum.

**Conclusions and Clinical Relevance**—In vivo lidocaine administration during ischemia and reperfusion had beneficial effects on smooth muscle motility. Initiating lidocaine treatment during surgery to treat colic in horses may improve lidocaine’s prokinetic features by protecting smooth muscle from effects of ischemia and reperfusion injury. (Am J Vet Res 2011;72:1449–1455)
sic cell functions through cellular repair mechanisms. The exact pathways for the repair mechanisms on a cellular level are unknown. However, improving contractility of small intestinal smooth muscle after ischemia and reperfusion injury is essential for the recovery of coordinated propulsive intestinal motility.

In the study reported here, it was hypothesized that repair of IR injured cells could be supported by lidocaine and that the injury could also be ameliorated by simultaneous administration of lidocaine during ischemia and reperfusion. To test this hypothesis, a study was conducted in which lidocaine was infused IV during a period of in vivo artificially induced ischemia and reperfusion. We proposed that the immediate effects of lidocaine in smooth muscle during reperfusion after ischemia would attenuate the negative consequences of ischemia and reperfusion on contractility and thus protect smooth muscle tissues. After the measurement of basic contractile performance of jejunal smooth muscle samples to assess the effect of in vivo administration of lidocaine, we also added lidocaine to the buffer in organ baths to assess further improvement of smooth muscle motility. Accumulation of lidocaine in tissues was considered essential for the lidocaine effects. Therefore, to assess lidocaine distribution and accumulation in body tissues, lidocaine concentrations were measured in blood samples and jejunal smooth muscle samples.

Materials and Methods

Animals—Twelve adult warmblood horses (6 mares and 6 geldings) were used in the study. Horses were 3 to 22 years old and weighed 450 to 535 kg. Horses were healthy and had no gastrointestinal tract disorders. Two weeks before surgery, horses were moved to individual stalls and received an anthelmintic. Horses had unlimited access to hay and water while in the stalls. Procedures were approved by the State Office for Consumer Protection and Food Safety in accordance with the German Animal Welfare Law.

Surgical procedures for induction of ischemia-reperfusion injury—A modified jejunal ischemia and reperfusion injury technique was used as described elsewhere. Food was withheld from all horses for 6 hours before surgery. Horses received xylazine hydrochloride (0.8 to 1.1 mg/kg, IV) as a preanesthetic medication. Anesthesia was induced by IV administration of diazepam (0.05 mg/kg) and ketamine hydrochloride (2.2 mg/kg). Intratracheal intubation was performed, and anesthesia was maintained by simultaneous administration of isoflurane in 100% oxygen and a CRI of ketamine (1 mg/kg/h). Dobutamine, lactated Ringer's solution, and hydroxyethyl starch were administered to maintain a mean arterial blood pressure > 60 mm Hg during anesthesia.

After the induction of anesthesia, horses were positioned in dorsal recumbency. The abdominal region was aseptically prepared, and routine laparotomy was performed. To create an ischemia and reperfusion injury, mesenteric vessels of a 25-cm segment located in the distal portion of the jejunum approximately 1.5 m oral to the end of the ileocaecal fold were ligated with Penrose drains; ligatures were applied in a manner intended to maintain integrity of the vessels. Thus, a hemorrhagic strangulating obstruction was simulated. The lumen of the 25-cm segment was obstructed by the application of Penrose drains circumferentially around the jejunum at each end of the segment. The intestinal segment was distented by infusion of warm (37°C) Ringer's solution into the lumen to achieve an intraluminal pressure of 20 mm Hg. Ischemia was maintained for 15 minutes. During the ischemic period, the jejunum was replaced into the abdominal cavity. Afterward, ligatures on the mesenteric vessels and the jejunum were removed, and the intraluminal fluid was manually emptied 1 time into the cecum. The jejunal segment was replaced into the abdominal cavity for another 15 minutes (reperfusion). After the reperfusion period, the jejunal segment (ie, IR tissue) was resected.

In vivo infusion of lidocaine—Immediately after resection of the first segment, a second jejunal segment was created in the same horse in the exact same manner as for the IR segment. After the ischemic period was initiated by ligation of mesenteric vessels and obstruction of the lumen, a bolus of a 2% solution of lidocaine was infused (1.3 mg/kg, IV); the infusion lasted 10 minutes. This was followed by CRI of lidocaine (0.05 mg/kg/min) for the remaining 5 minutes of the ischemic period. For reperfusion, ligatures were removed from the vessels and jejunum, and the jejunal segment was replaced into the abdominal cavity for 15 minutes. After the reperfusion period, this jejunal segment (ie, IRL tissue) was resected (approx 35 minutes after resection of the first segment).

Tissue preparation—Immediately after resection, jejunal tissues were placed in a modified Krebs-Henseleit buffer (NaCl, 117.0 mmol/L; KCl, 4.7 mmol/L; CaCl₂, 2.5 mmol/L; MgCl₂, 1.2 mmol/L; NaH₂PO₄, 1.2 mmol/L; NaHCO₃, 25.0 mmol/L; and glucose, 11.0 mmol/L) aerated with 95% O₂ and 5% CO₂ (pH, 7.4; 38°C), prepared as described elsewhere. Briefly, strips of circular smooth muscle of equal size and weight (+ strips/sample) were prepared and mounted into a force measurement apparatus with 8 isometric force transducers. To simulate the effect of CRI, lidocaine (25 mg/L) was added to the transport buffer and organ bath buffer of IRL tissues. Furthermore, preliminary experiments revealed that the basal contractility was equivalent in IRL tissues incubated with and without supplemental lidocaine in the transport buffer and organ bath buffer. However, a rapid washout effect was observed in tissues incubated without lidocaine supplementation. To avoid this washout effect, supplementation of the transport buffer and organ bath buffer was necessary. To evaluate the effects of lidocaine on smooth muscle cells and ICCs, IR and IRL tissues were pretreated with tetrodotoxin (1 μmol/L) to deactivate neurons of the enteric nervous system. Successful inhibition was verified by a lack of response to electric field stimulation.

Basal contractile activity and lidocaine responses—Smooth muscles have spontaneous contractions that can be defined on the basis of their amplitude (isometric force of contractions) and frequency, determined by the activity of ICCs. By use of these 2 variables, the area under the curve for all contractions within 1 minute (contractility) was calculated. Subsequently, all 3
variables were used to define the isometric force performance of equine intestinal smooth muscle. Each strip was mounted into an organ bath filled with 10 mL of Krebs-Henseleit buffer and fitted with an isometric force transducer. The initial tension of muscle strips was adjusted to 2 g. Another study and published data have indicated that 2 g of tension results in optimal muscle length for isometric force development in equine jejunum. Isometric contractile forces of smooth muscle tissues were measured with a chart recorder (4.8 kHz/direct current), and data were collected with data acquisition software.

After equilibration of IR and IRL smooth muscle strips, basal contractile activity was recorded for 30 minutes. Isometric force performance was determined on the basis of amplitude, frequency of contractions, and contractility. Thereafter, responses for additional supplementation with lidocaine were measured in IR and IRL tissues in buffer supplemented with 12.3 μL of a lidocaine stock solution (20 mg/mL). The lidocaine solution was pipetted directly into the organ baths, thereby adjusting the buffer to a final lidocaine concentration of 25 and 50 mg/L for IR+L and IRL+L tissues, respectively. The most effective in vitro lidocaine concentration was identified in other studies conducted to determine the dose-dependent effects for lidocaine. Dose-dependent effects of dilutions of 2% lidocaine solution containing the preservatives methylparaben, propylparaben, and sodium EDTA were tested in a previous study, however, no effects of the preservatives or time were observed as assessed by the use of isometric force measurements.

Membrane permeability—To evaluate cell membrane permeability, the release of specific markers (CK and LDH) was measured after in vitro incubation of jejunal IR and IRL smooth muscle tissues. Two segments (1 × 1 cm) of each muscle sample were prepared and incubated for 5 minutes in 5 mL of Krebs-Henseleit buffer aerated with 95% O2 and 5% CO2 (pH, 7.4; 38°C). One segment of each sample was incubated in buffer supplemented with lidocaine (25 mg/L). After incubation, the buffer was divided into aliquots, which were stored at –20°C until analysis. Activities of LDH and CK were measured after in vitro incubation of jejunal tissues, compared with results for IR tissues in buffer supplemented with 12.3 μL of a lidocaine stock solution (20 mg/mL). The lidocaine solution was pipetted directly into the organ baths, thereby adjusting the buffer to a final lidocaine concentration of 25 and 50 mg/L for IR+L and IRL+L tissues, respectively. The most effective in vitro lidocaine concentration was identified in other studies conducted to determine the dose-dependent effects for lidocaine. Dose-dependent effects of dilutions of 2% lidocaine solution containing the preservatives methylparaben, propylparaben, and sodium EDTA were tested in a previous study, however, no effects of the preservatives or time were observed as assessed by the use of isometric force measurements.

Lipid extraction—Samples were extracted with methanol-chloroform used in accordance with a commonly performed extraction procedure. Then, samples (500 μL of serum and 300 μL of ultrapure water or 500 μg of jejunal tissue and 800 μL of ultrapure water) were homogenized with 2 mL of methanol and 1 mL of chloroform. After 30 minutes of mixing by rotation, tissue samples were centrifuged (2,000 × g for 3 minutes at 7°C); supernatant was collected and transferred into a new reaction tube. The residual pellet was stored at –20°C until analyzed via the Bradford method to quantify the amount of protein. After additional centrifugation of the supernatant (2,000 × g for 10 minutes at 7°C), the upper phase was collected and centrifuged again. The entire lower phase was collected and concentrated by evaporation to dryness in an analytic high-speed rotary vacuum evaporator. After removing all traces of solvent and water, the extract was mixed (vortexed for 120 seconds) with 2 mL of methanol.

HPLC measurement—Prepared samples (50 μL) were injected onto the column of a chromatograph. Untreated samples were used as negative control samples in HPLC measurements. A flow rate of 0.5 μL/min was used; column temperature was maintained at 25°C. Absorbance of the effluent was measured at 205 nm. The mobile phase for elution of lidocaine consisted of 300 mL of methanol and 200 mL of NH4HCO3 (10 mmol/L; pH, 10.5). All samples were extracted, and lidocaine contents were quantified in duplicate. Quantitative assessment of lidocaine concentrations in tissue and serum samples was validated during recovery experiments.

Statistical analysis—Data were reported as mean ± SEM for 12 horses, with 4 IR and 4 IRL tissues from each horse. Significant differences in basal contractility, frequency of contractions, and amplitude were detected with a paired Student t test. Significant differences for basal and lidocaine-supplemented contractile responses were calculated by use of a 2-way ANOVA for matched observations for in vivo lidocaine infusions (IR and IRL) and in vitro lidocaine supplementation (IR+L and IRL+L), with Bonferroni post hoc tests used to assess significant differences between IR and IRL versus IR+L and IRL+L. Values of P < 0.05 were considered significant. All statistical analyses were performed by use of statistical software.

Results

Basal contractile activity and lidocaine responses—All circular smooth muscle strips of the distal portion of the jejunum of each horse had spontaneous contractile activity. There were significant increases in basal contractility and basic mean frequency of contractions in IRL tissues, compared with results for IR tissues (Figure 1). Basal force of contractions (amplitude) did not differ significantly between IR and IRL tissues.

Lidocaine response—The addition of 25 mg of lidocaine/L significantly (P < 0.001; 2-way ANOVA) increased in vitro contractility of IR+L and IRL+L tis-
sues. This effect was more pronounced in IR+L tissues, compared with IR tissues (P = 0.01; Bonferroni post hoc test; Figure 1). In IR+L tissues, this increase was based on a significant (P < 0.001; Bonferroni post hoc test) increase in the frequency of contractions, compared with the frequency of contractions for the IR tissues. However, only a slight change of frequency was detected between IRL and IRL+L tissues. Because the extent of the effect of in vitro lidocaine supplementation depended on the in vivo lidocaine infusion status, a significant interaction was detected. The force of contractions (amplitude) was significantly increased in IR+L and IRL+L tissues (P < 0.001; 2-way ANOVA), compared with results for the IR and IRL tissues. The effect was more pronounced in IR+L tissues, compared with IR tissues (P < 0.001; Bonferroni post hoc test).

Membrane permeability—No significant difference of CK and LDH release in untreated IR and IRL tissues was detected (Figure 2). After incubation in buffer supplemented with lidocaine (25 mg/L), CK and LDH activities for IR+L and IRL+L tissues were significantly (P < 0.001; 2-way ANOVA) decreased, compared with activities for the IR and IRL tissues. The decrease in CK release was significantly (P = 0.01; Bonferroni post hoc test) more pronounced in IRL+L tissues, compared with release in IRL tissues. In contrast, release of LDH was significantly (P < 0.001; Bonferroni post hoc test) more diminished in IR+L tissues, compared with release in IR tissues.

Lidocaine concentrations in serum and jejunal tissues—The HPLC method was validated for use in equine serum and jejunal tissues. The linear range of the assay for serum and jejunal smooth muscle in horses was between 25 and 500 ng/mL. Recovery of lidocaine was established at 95% for serum and 92.5% for jejunal smooth muscle. Limit of detection for lipid extracts of serum and smooth muscle samples was 5 ng/mL, and limit of quantification was 25 ng/mL.

All samples collected during in vivo lidocaine infusion had measurable lidocaine concentrations. Mean ± SEM serum lidocaine concentration was 97.17 ± 17.74 ng/mL.
Dose-dependent improvement of the force of contraction tile function, lidocaine was added to the transport buffer. To maintain a consistent plane of smooth muscle contrac-

membrane permeability as assessed by in vitro was only observed after supplementation of lidocaine for

The ICCs are susceptible to hypoxia, as induced by ischemia-reperfusion injury, and subsequently de-
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duce cellular damage that could cause dysfunction of ICCs and thereby decrease frequency of contractions. Additionally, the function of smooth muscle cells was disturbed, which resulted in a lack of adequate response to ICC signals. The force of contractions was also di-

Discussion

The objective of the study reported here was to elu-
cidate the influence of lidocaine on smooth muscle con-
tractility and to characterize possible mechanisms of its effects. In vivo administration of lidocaine via IV in-
fusion protected smooth muscle function against isch-
emia-reperfusion injury by maintaining the frequency of contractions. It appears possible that the effects de-
tected for the IRL samples were a combined effect of in
vivo infusion of lidocaine and in vitro incubation in the lidocaine-containing transport buffer. However, prelimi-
ary experiments revealed that transport of IRL tissue in transport buffer that contained lidocaine, compared with transport of IRL tissues in transport buffer with-
out lidocaine, did not result in alterations of amplitude, frequency of contractions, and contractility within the first 30 minutes of isometric force measurement. A rapid washout effect during incubation of IRL tissues in the organ bath was evident after that initial measurement. To maintain a consistent plane of smooth muscle contrac-
tile function, lidocaine was added to the transport buffer.

Significant increases in frequency of contractions after early incubation with lidocaine-containing trans-
port buffer indicates a beneficial effect on ICCs, which are the pacemaker cells of the gastrointestinal tract. In contrast, involvement of lidocaine-stimulated signals from the enteric nervous system could be excluded be-
cause tetrodotoxin, a blocker of the enteric nervous sys-
tem, was present throughout the in vitro incubations. The ICCs propagate variations in membrane potentials, slow waves, spontaneous action potentials, and spikes, which are transmitted to the intestinal smooth muscle cells. This coordinated cell-cell signal transmission re-
sults in smooth muscle contractions and is fundamen-
tally important for motility of the gastrointestinal tract. Spikes trigger the influx of extracellular calcium into the smooth muscle cells, and the extent of calcium in-
fluctes the force of contractions. Additionally, ade-
quate energy production is an essential prerequisite for development of maximal force of contraction. The harmonic concert of these physiologic processes at the smooth muscle cell level is an essential prerequisite for coordinated propulsive motility.

In vivo infusion of lidocaine significantly improved the frequency of contractions. This indicated that lido-
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Ischemia and reperfusion injury causes general cellular damage by excessive calcium influx and ROS production after reoxygenation of ischemic tissues. The ROS interact with cell membranes, damage proteins, and cause lipid peroxidation, thus resulting in per-
turbations in membrane permeability that lead to cell death. Increased intracellular calcium in ischemic tissue can lead to dissolution of lipid membranes. Both pathophysiologic pathways result in an increase in membrane permeability that causes cellular dysfunc-
tion. Lidocaine was able to decrease membrane perme-
ability of smooth muscle cells. However, this was only detected in vitro after incubation of IR and IRL samples in buffer supplemented with lidocaine. That decrease of membrane permeability was considered to subse-
sequently improve results for smooth muscle force as detected in vitro.

The underlying mechanism for the decrease in membrane permeability in smooth muscle cells and probably ICCs attributable to lidocaine is unclear. Hypothetically, it could be an effective mechanism to protect function of smooth muscle cells and ICCs by inhibiting the intracellular accumulation of calcium. Such inhibition helps to prevent the formation of ROS and therefore to decrease peroxidation of membrane lipids and subsequent cell injury. In vivo infusion of an initial bolus of lidocaine maintained only the frequency

Figure 3—Lidocaine concentrations in samples of serum (ng/mL [gray bars]) and jejunal smooth muscle (ng/mg of tissue [black bars]) obtained from 12 horses.

ng/mL (range, 10 to 155 ng/mL). Mean lidocaine con-
centration in jejunal smooth muscle tissues was 133.9 ± 24.49 ng/mg (range, 37 to 309 ng/mg). The accumu-
lation of lidocaine in serum and tissue samples after short-term IV infusion was highly variable among hors-
es. Comparing the means of lidocaine concentrations in serum and tissue samples, no differences were detected as assessed by use of the paired Student t test. However, evaluation of data for each horse revealed that the inter-individual variation was extremely high and did not appear to reflect an association between serum and tissue lidocaine concentrations (Figure 3).
of contractions, which indicated a dose-dependent effect on ICCs.

Both IR and IRL samples had comparable high membrane permeability, which could only be decreased by additional supplementation with lidocaine in the in vitro incubation. Improvement of membrane stability could prevent losses of macromolecules (eg, ATP) for maintaining energy metabolism of smooth muscle cells and could stabilize membrane potentials and subsequently cellular functions. In equine intestinal smooth muscle cells, contractile performance was increased. Originally, analogous studies in hypoxic and reperfused isolated rabbit hearts appeared to confirm this hypothesis. Investigators in 1 study found that lidocaine was able to decrease the release of adenosine, other ATP metabolites, and CK from heart muscle when administered during the hypoxic period, thereby increasing the contractile performance of cardiac muscle. Higher membrane stability of blood vessel endothelial cells was also assessed in a study of morphological changes in IR-injured intestinal wall influenced by lidocaine. In vivo treatment of the distal portion of the jejenum with lidocaine resulted in less edema in all tissue layers, which supported the concept that alterations in membrane permeability could contribute to lidocaine effects.

An essential prerequisite for the lidocaine effect reportedly is its presence in jejunal smooth muscle after a 15-minute bolus infusion. For CRI conditions as used in the treatment of postoperative ileus, serum lidocaine concentrations increase up to approximately 1,000 ng/mL after 3 hours and remain stable at approximately 950 ng/mL over a 96-hour infusion period. Investigators in 1 study detected a mean serum concentration of lidocaine of 891.1 ng/mL 3 hours after lidocaine infusion. Hence, for the experimental conditions in the study reported here, approximately one-tenth of the serum concentrations of the CRI were achieved after short-term infusion of lidocaine. Measurements on tissue samples were performed by use of HPLC to assess lidocaine concentrations in muscle samples. Detectable lidocaine concentrations could be confirmed in smooth muscle after short-term infusion. However, short-term infusion of lidocaine during ischemia and reperfusion resulted in lidocaine concentrations in serum and tissue samples that were highly variable among horses.

Smooth muscle samples were incubated with lidocaine (25 mg/L) in vitro. High doses of lidocaine used for in vitro studies were considered nontoxic for isolated smooth muscle tissues. It is known that in vivo plasma lidocaine concentrations are much lower than the in vitro effective concentrations. However, to our knowledge, there is no information currently available about lidocaine pharmacokinetics. Detailed knowledge of the pharmacokinetics of lidocaine is needed to extrapolate results of the present study to in vivo therapeutic conditions.

For the study reported here, we concluded that lidocaine was able to affect cell membrane permeability and concomitantly was able to improve smooth muscle function. However, in vitro concentrations of lidocaine used in this study exceeded the maximum therapeutic concentration in vivo. The extent of improvement is dependent on the dose of lidocaine. Basically, in vivo infusion of lidocaine prevented the decrease in frequency (IRL tissues) and the force of contractions was low. This indicated a protective effect on ICC function. Enhancing lidocaine concentrations in vitro resulted in an increase in the force of contractions in IR and IRL tissues, thereby restoring equivalent contractility. Decreased membrane permeability in IR and IRL samples may have contributed to the improvement in function. Hence, protective and repair mechanisms were induced by lidocaine and were both effective in maintaining the frequency of contractions and restoring the contractile performance of intestinal smooth muscle.

**References**