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Short Communication

Impact of tetrodotoxin application and lidocaine supplementation on equine jejunal smooth muscle contractility and activity of the enteric nervous system in vitro

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ABSTRACT

By blocking the enteric nervous system (ENS) using tetrodotoxin (TTX), previous studies have documented the contractility-enhancing (CE) effects of lidocaine in equine intestinal smooth muscle (SM) at the level of SM cells and/or interstitial cells of Cajal (ICC). The present study examined the impact of ENS deactivation on CE lidocaine effects, and investigated the effects of lidocaine on ENS activity. TTX application did not affect the CE effects of lidocaine, indicating that these were not mediated by TTX-sensitive sodium channels. Application of TTX or ≥100 mg/L lidocaine reduced ENS activity. Although such concentrations of lidocaine exceed therapeutic blood concentrations, tissue concentrations may be higher with the potential to reduce ENS activity and impair intestinal motility in vivo. Improved understanding of underlying mechanisms is relevant for therapeutic use of lidocaine in horses with postoperative ileus.

Lidocaine is widely used for stimulating intestinal motility in horses suffering from postoperative ileus (POI) (Van Hoogmoed et al., 2004). However, cellular targets and mechanisms involved in its prokinetic effects are still unclear. Physiologically, intestinal motility depends on the intrinsic contractility of the circular (CSM) and longitudinal (LSM) smooth muscle (SM) layers and a superordinate coordination which is provided by the enteric nervous system (ENS). The ENS can be deactivated using tetrodotoxin (TTX) (Boddy et al., 2004). As reviewed by Lee and Ruben (2008), TTX is a potent neurotoxin which binds to voltage-gated sodium channels, inhibiting the initiation and propagation of action potentials.

By blocking the ENS using TTX, previous studies documented contractility-enhancing (CE) effects of lidocaine at the level of SM cells and/or ICC (Guschlbauer et al., 2010; Tappenbeck et al., 2013). The present study aimed to examine the impact of ENS deactivation on CE lidocaine effects in equine small intestine, and in turn to determine effects of lidocaine on ENS activity, assessed by measuring the contractile response of SM to electric field stimulation (EFS). It was hypothesised that the CE effects of lidocaine are not affected by TTX application, while lidocaine administration reduces ENS activity.

During standard median laparotomies, segments of distal jejunum were collected from healthy horses. After resection, the segments were transferred into modified Krebs–Henseleit buffer and CSM and LSM strips were prepared as described previously (Tappenbeck et al., 2013). The SM strips were placed into organ baths and connected to isometric force transducers (Hottinger Baldwin Messtechnik). At regular intervals of 15 min, the SM strips were stimulated by EFS (10 s, 10 Hz, 30 V). Five minutes after the first EFS, 1 µmol/L TTX (Biotrend Chemicals) was administered to half of the SM strips in order to block responses of the ENS (Boddy et al., 2004; Guschlbauer et al., 2010; Tappenbeck et al., 2013). Every 15 min lidocaine (lidocaine hydrochloride, Sigma-Aldrich), dissolved in distilled water, was added cumulatively to half of the organ baths (see Supplementary material in the online version at doi:10.1016/j.tvjl.2014.05.014) starting 30 min after administration of TTX, resulting in lidocaine concentrations of 5, 25, 50, 100 and 200 mg/L.

The isometric force development of the SM strips was defined as contractility and was described by frequency (F, peaks/min), amplitude (A, mN) and mean active force of contractions (MAF, mN) in accordance with our previous study (Tappenbeck et al., 2013). Changes in F, A and MAF throughout the trial were calculated by subtracting values before TTX application from data received from each further evaluation period and were defined as ΔF (Fig. 1), ΔA (Fig. 2) and ΔMAF (Fig. 3). The contractile response of SM strips to EFS (Fig. 4) was defined as impulse and was calculated by multiplying the MAF (mN) during the impulse with the duration of the impulse(s) (see Supplementary material in the online version at doi:10.1016/j.tvjl.2014.05.014).

Two-way repeated measures (RM) analyses of variance (ANOVA) (GraphPad Prism 4.0, GraphPad Software) were performed to assess

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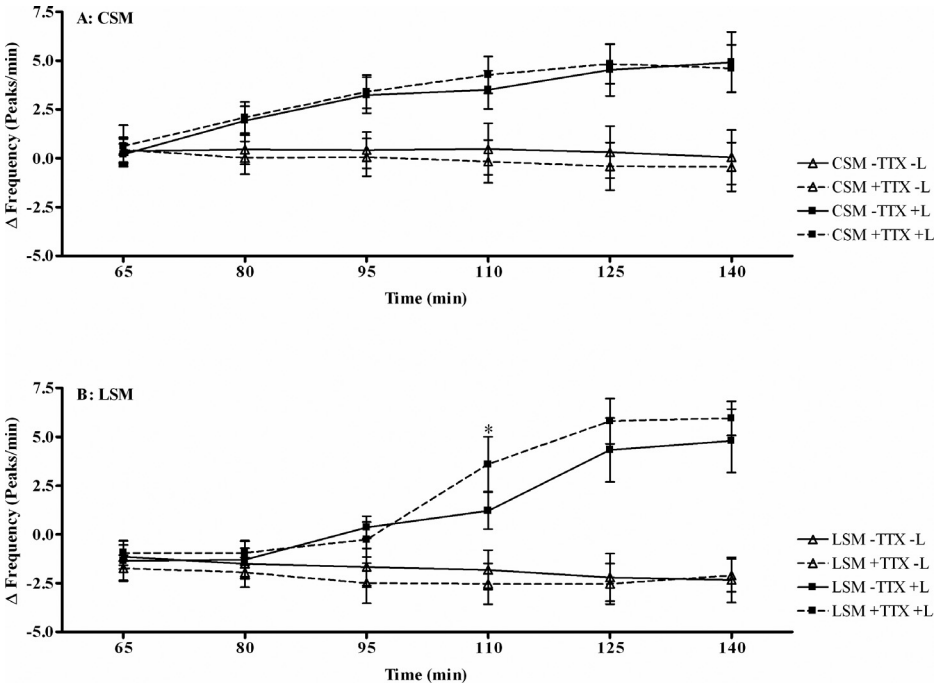

Fig. 1. Frequency (ΔF) of contractions in circular smooth muscle (CSM; A) and longitudinal smooth muscle (LSM; B) with or without application of tetrodotoxin (+TTX; –TTX) in the presence (+L) or absence (–L) of increasing lidocaine concentrations. As lidocaine was applied to the organ baths at regular intervals, each lidocaine concentration resembled a distinct time point during experimental procedures, with concentrations of 5, 25, 50, 100 and 200 mg/L lidocaine at 80, 95, 110, 125 and 140 min after start of the experiment. Data are given as means ± SE of $n = 10$ horses. Paired Student's t tests comparing ΔF in the absence and presence of TTX were performed for each time point, with $^{*}P < 0.05$ indicating significant differences.

the impact of TTX application and lidocaine treatment on ΔF, ΔA, ΔMAF and impulses. Paired Student's t tests (GraphPad Prism 4.0) compared ΔF, ΔA, ΔMAF and impulses in the absence and presence of TTX for each time point. Values were presented as means ± SE of $n = 10$ horses with each mean consisting of $n = 2$ values/horse. P values < 0.05 were regarded as statistically significant.

Lidocaine administration affected the contractility in SM with or without TTX application (Table 1). Application of TTX did not alter

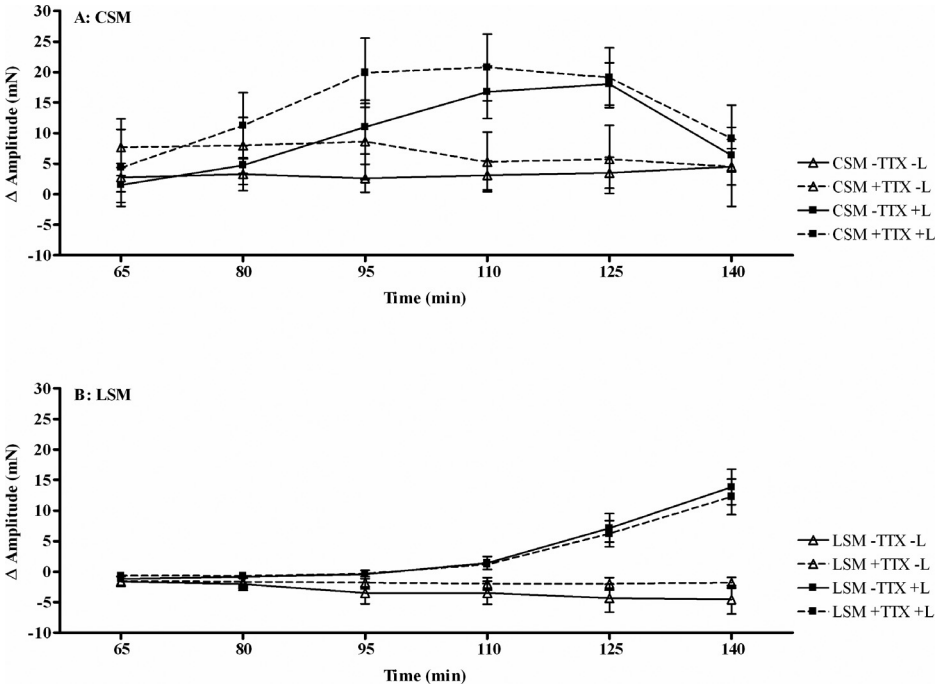

Fig. 2. Amplitude (ΔA) of contractions in circular smooth muscle (CSM; A) and longitudinal smooth muscle (LSM; B) with or without application of tetrodotoxin (+TTX; –TTX) in the presence (+L) or absence (–L) of increasing lidocaine concentrations. As lidocaine was applied to the organ baths at regular intervals, each lidocaine concentration resembled a distinct time point during experimental procedures, with concentrations of 5, 25, 50, 100 and 200 mg/L lidocaine at 80, 95, 110, 125 and 140 min after start of the experiment. Data are given as means ± SE of $n = 10$ horses. Paired Student's t tests comparing ΔA in the absence and presence of TTX at each time point did not detect any significant differences.