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Distribution of Neurokinin 2 and 3 Receptor mRNA In the Normal Equine Gastrointestinal Tract and Effect of Inflammation on Neurokinin 1, 2, And 3 Receptor mRNA In the Equine Jejunum

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1. Abstract

Objectives: To quantify neurokinin 2 and 3 receptor mRNA from nine regions throughout the equine intestinal tract, and to evaluate the effect of jejunal ischemia/reperfusion and intraluminal obstruction on neurokinin 1, 2, and 3 receptor mRNA.

Methods: Specimens were harvested from 5 adult horses euthanized for reasons unrelated to gastrointestinal disease for the study of normal distribution of neurokinin receptor mRNA. Jejunal segments from 6 healthy adult horses subjected to intraluminal distension or ischemia/reperfusion injury were harvested to study the influence of inflammation on neurokinin 1, 2, and 3 receptor mRNA expression. RNA was isolated from normal tissues and also from tissues that underwent either a sham operation (control), 60 minutes of ischemia followed by 60 minutes of reperfusion (ISO), or 120 minutes of intraluminal distension (ILD) as part of an inflammatory model. RNA was reverse transcribed into cDNA. NK2 and NK3 primers were designed and mRNA was quantified using real-time PCR for all experimental groups.

Results: Expression of NK2 receptor mRNA was highest for the duodenum and the body of the cecum. NK3 mRNA expression had high variability. In the inflammatory model, no statistically significant difference was noted between treatment groups for NK1 or NK3 receptor mRNA. NK2 receptor mRNA expression was significantly decreased for ILD when compared to control.

Conclusions: The description of neurokinin receptor mRNA distribution throughout the equine intestinal tract is an important initial step towards determining potential clinical applications of tachykinin agonists and antagonists, as well as their role in gastrointestinal ischemia/reperfusion and intraluminal obstruction injury.

2. Keywords:

Substance P; Neurokinin; Tachykinin; Neuropeptide; Equine; Gastrointestinal; Ischemia/Reperfusion, Inflammation

3. Abbreviations

GIT – Gastrointestinal tract **G-protein** – Guanine nucleotide-binding protein

SP – Substance P

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NK – Neurokinin

NP – Neuropeptide

HK – Hemokinin

PCR – Polymerase chain reaction

PF – Pelvic flexure

ILD – Intraluminal distension

ISO – Ischemia/reperfusion injury

4. Introduction

Tachykinins are a group of neuropeptides that includes Substance P (SP), Neurokinin A (NKA), Neurokinin B (NKB), Neuropeptide K (NPK), Neuropeptide γ (NPγ), Endokinin C, Endokinin D and Hemokinin 1 (HK1) [1]. NPK and NPγ are variants with NH_2 terminal extensions on NKA, which have similar physiologic actions as NKA [1]. Endokinin C and D have weak bioactivity and HK 1 has an unknown function [1]. Research to date, has thus shown SP, NKA and NKB to be the relevant mammalian tachykinins. Tachykinins act via binding to one of three G-protein-coupled neurokinin receptors: NK1, NK2 and NK3. All three tachykinins will bind to all three receptors to act centrally or peripherally. However, SP has the strongest affinity for NK1 and NKA has the highest affinity for NK2; NKB has the strongest affinity for the NK3 receptor [2].

SP, NKA and NKB all play a role in the development of intestinal inflammation [3]. A variety of experimental models of inflammation in rats and humans have been used to demonstrate the therapeutic benefit of neurokinin antagonists in the management of inflammatory bowel disease symptoms [4]. However, the potential for crucial species differences in NK receptor function exists. For example, NK3 antagonists, while ineffective in rats, significantly decreased intestinal permeability, myeloperoxidase activity (a measure of neutrophil infiltration) and histologic damage in guinea pigs [6].

Tachykinin antagonists have been shown to be protective against enteritis by decreasing intra-luminal fluid hypersecretion [30, 31] and decreasing TNFα release from macrophages [32]. Less tissue [33, 34] and histologic [31, 35] damage due to enteritis have also been documented in the presence of neurokinin antagonists. Both natural and synthetic NK1 [5] and NK2 [6] antagonists have been shown to decrease tissue damage in experimental gastrointestinal inflammation. It is also important to note that tachykinins seem to be involved in the early stages of inflammation, with antagonists being less protective when examined after seventy-two hours compared the first twenty-four [7]. This suggests that their therapeutic benefit may be the highest in acute inflammation.

NK receptors 1, 2 and 3 play a role in modulation of gastrointestinal motility, with a suggested involvement in the generation of Migrating Motor Complexes [8]. Initially, SP was found to have a positive contractile effect on intestinal smooth muscle [9]. Further study revealed NKA as the most potent tachykinin agonist to affect contractility [10]. The complex mode of action that tachykinins exhibit, however, includes both excitatory myogenic and inhibitory neurogenic effects [11-14]. The distribution of receptors in both muscle and nerves therefore is expected to have a significant impact on the expected contractile response.

Little information is available on tachykinins and their receptors in horses. Most studies have evaluated SP immunoreactivity [15-17]. One study examined the distribution of NK receptors in the pelvic flexure [18] and another quantified NK1 receptors but not NK2 or 3 receptors in all regions of the intestinal tract [19]. A positive contractile effect to NKA and NKB was observed in the equine duodenum and the ileum, with circular muscle being more responsive than longitudinal [20]. It was also concluded that SP acts as a neurotransmitter in the equine jejunum, based on the presence of SP immunoreactivity in the myenteric plexus [16] and the release of SP from myenteric neurons [21]. It is surprising, however, that tachykinins and their neurokinin receptors have not

been more thoroughly examined in the horse, when considering that Substance P was first extracted from equine intestinal tissues in 1931 [9].

Ileus, or the cessation of aboral motility, has been reported to have a mortality rate as high as 80 percent when it occurs postoperatively in horses [22]. The release of inflammatory mediators is one of the proposed causative factors for the development of ileus [23, 24], however, its etiology is complex and not completely understood. Improvement in prokinetic drug therapy for this condition is needed since post-operative ileus remains an important cause of complications and mortality in equine medicine.

The purpose of this study was to evaluate the distribution of neurokinin receptors 2 and 3 in smooth muscle of various anatomic regions in the equine intestinal tract by quantifying mRNA expression using real-time PCR. Further, we sought to evaluate the effect of ischemia/reperfusion and intraluminal obstruction on the distribution of mRNA expression of neurokinin receptors 1, 2 and 3 in the equine jejunum.

5. Materials and Methods

5.1 Sample collection

a. Distribution of NK2 and NK3 receptors

Experimental protocols were approved by the University of Guelph Animal Care Committee. Intestinal samples were collected from 5 mature horses (3 Standardbred and 2 Thoroughbred) euthanized for reasons unrelated to gastrointestinal disease less than 1 hour after euthanasia, as previously described [19]. Euthanasia was performed by intravenous administration of barbiturate overdose. Full thickness samples from the antimesenteric side of the intestine were harvested from the following regions: duodenum (D), jejunum (J), ileum (I), body of the cecum (C), right ventral colon (RV), left ventral colon (LV), pelvic flexure (PF), right dorsal colon (RD) and left dorsal colon (LD). Samples were rinsed with ambient temperature saline, and tissue freezing compound[a] was added. These samples were placed in liquid nitrogen, then stored at -80°C for further processing. Small pieces of smooth muscle were later dissected on dry ice from each sample and stored in 100 mg aliquots at -80°C until RNA was extracted.

b. Inflammatory model

Six mature (mean age 5.5 years), healthy horses were used for the study of changes in NK1, NK2 and NK3 receptor distribution in the jejunum after ischemia and subsequent reperfusion (ISO) or intraluminal distension (ILD). None of the horses used for the study had evidence of gastrointestinal disease or systemic disease, and none had been treated with motility-modifying or anti-inflammatory agents. Anesthetic and surgical procedures have been previously described[25]. Briefly, all horses were sedated with xylazine[b] (0.2 to 0.5 mg/kg, IV) and anaesthetized with guaifenesin[c] (100 mg/kg IV) and ketamine[d] (2.2 mg/kg, IV). General anaesthesia was maintained with halothane in oxygen with intermittent positive pressure ventilation. All horses were positioned in dorsal recumbency for the procedure. A routine ventral midline laparotomy incision was made, and six jejunal segments were identified, beginning 100 cm distal to the duodenocolic ligament. These segments were each approximately 25 cm in length, supplied by a separate jejunal arcade artery and vein, and separated by a dividing 25 cm jejunal segment. Two segments were randomly designated ILD, two as ISO and two as control segments. ILD was created by occluding the lumen at each end of the segment with penrose drains, and infusing a sterile crystalloid[e]

solution into the lumen via an intraluminal 18 gauge catheter. Pressures, measured by a pressure transducer, were maintained at 25 cmH_2O for 120 minutes. ISO was created by occluding major mesenteric arteries and veins with rubber-shod haemostats. Collateral vessels were also occluded with rubber-shod clamps. Ischemia was maintained for 60 minutes, followed by 60 minutes of reperfusion. Control segments were marked with non-absorbable suture. Full thickness 10 x 10 cm samples were harvested from the antimesenteric region in the centre of each segment. Samples were rinsed with saline, and smooth muscle was dissected free from surrounding tissue. The smooth muscle samples were then rinsed a second time, wrapped in tinfoil, placed in liquid nitrogen, and stored at -70°C until RNA was extracted.

5.2 RNA Isolation

a. Distribution of receptors

RNA isolation of samples has been previously described [19]. Briefly, smooth muscle samples were minced, then homogenized in 1 mL of isolation reagent[f] and 0.5mL of 2.4 mm zirconia beads using a cell disrupter[g]. Samples were centrifuged (4800 rpm for 1 minute) and the supernatant was placed in a new tube. RNA was isolated according to the manufacturer's instructions[f] (routine guanidine-chloroform-phenol extraction).

b. Inflammatory model

RNA isolation of samples has been previously described [25]. Briefly, smooth muscle samples were minced and homogenized in 10 volumes of buffer (250mM sucrose and 50mM tris-HCl; pH 7.4) by a homogenizer[h]. Samples were then centrifuged (1,000 X g for 15 minutes) and washed 4 times, before being resuspended in 5 volumes of 50mM tris-HCl buffer [25]. RNA was isolated according to the manufacturer's instructions[f].

5.3 Removal of Contaminating DNA

A DNase inactive reagent[i] was used to remove trace genomic DNA contamination for samples used in the determination of receptor distribution. DNA-free[j] was used on samples for the inflammatory model studies. Spectrophotometry was used to determine purity and concentrations of RNA samples (OD 260/280 ratio), and gel electrophoresis was used to determine whether RNA was intact.

5.4 First-strand cDNA synthesis

The method of first-stand cDNA synthesis has been previously described [19]. Briefly, 6µg RNA from each sample was reverse transcribed in a reaction volume of 75µL (including M-MLV reverse transcriptase 5x buffer, KCl, dNTPs, random primers, RNasin and M-MLV reverse transcriptase). A thermal cycle[k] was then used (to incubate at 70°C, 37°C, heat at 99°C and cool the samples at 5°C). Repeating this process for each sample, without reverse transcriptase, confirmed appropriate removal of contaminating DNA. For the inflammatory model, a synthesis system[l] was used generate cDNA from 3µg of RNA from each sample. All samples were then stored at -80°C until analysis.

5.5 Primer design

NK1 receptor primers had previously been designed [19]. NK2 and NK3 primers were designed using a sequence database and primer[m] software, and were based on the bovine NK2 and NK3 sequences. Conditions for real-time PCR were optimized using a real-time PCR apparatus[n], following preliminary experiments with a thermal cycler[o].

5.6 Standard Curve Construction

Standard curves were prepared from cDNA pooled from each sample. For each curve, four dilutions (1, 1/10, 1/100 and 1/1000) were used and each was run 6 times. Standard curves for β-actin, NK1, NK2 and NK3 receptors were then used to generate coefficient files, which were used in the quantification of mRNA expression. Pooled cDNA samples were used as a calibrator.

5.7 Real-time Quantitative PCR

Primers were used to quantify equine cDNA in samples using the real-time PCR apparatus[p]. Conditions were: activation at 95ºC for 2 min, followed by 50 amplification cycles (95ºC/15 s; 55ºC/25 s; 72ºC/35 s), acquisition of fluorescence (75ºC/1 s), melting (55–95ºC with a temperature transition rate of 0.1ºC per second and continuous fluorescence measurement) and cooling to 40ºC. A reaction volume of 20µL was used and nucleic acid stain[q] identified product. Samples were run in triplicate for β-actin (previously determined to be a suitable house-keeping gene[19], and target genes NK1, NK2 and NK3. A positive control (calibrator cDNA) and negative control (water) were included in each run. Analysis was performed using relative quantification[r] computer software. The amount of target cDNA (NK1, NK2 or NK3 receptor) in a sample was compared to the amount of reference cDNA (β-actin) and a ratio was generated using the coefficient files.

6. Statistical Analysis

A generalized linear mixed-model (ANOVA) accounting for the random effect of horse was employed. NK1, NK2 and NK3 receptors were analyzed for significant differences between bowel segments and in the second study between control, ISO and ILD. Factors included in the model were bowel section or ISL/ILD treatment group. The assumptions of the ANOVA were assessed by comprehensive residual analyses. A Shapiro-Wilk test, a Kolmogorov-Smirnov test, a Cramer-von Mises test, and an Anderson-Darling test were conducted to assess overall normality. Residuals were plotted against predicted values and explanatory variables to determine patterns in the data that suggest outliers, unequal variance or other problems. If the overall F- test was significant, post hoc Tukey-Kramer tests were applied. Significance was set at $p \leq 0.05$[s].

7. Results

7.1 Generation of PCR product with NK2 and NK3 primers

In order to determine the quality of primers designed, gel electrophoresis was used to confirm the generation of a single product of predicted length (not shown). The absence of primer-dimer compounds was also verified in this way. The NK2 and NK3 primers designed for this study, which were based on bovine NK2 and NK3 sequences, as well as the housekeeping gene β-actin and NK1 receptor sequences previously determined [19] are presented in Table 1.

Table 1: Primers used to quantify receptor gene expression in equine intestinal tissue using Real Time PCR.

Gene	Forward Primer Nucleotide Sequence	Reverse Primer Nucleotide Sequence
β-actin	5' CTTCCAGCCCTC CTTCC 3'	5' GTCCCACCCGAC AGCAC 3'
NK1	5' ACGGGTCACGC AGATGT 3'	5' GGGCTACTACTC CACCACAGA 3'
NK2	5' CTTGAGAGCAA CACCACAGG 3'	5' TGGCTGGCATAG ACGAAGTT 3'
NK3	5' ATTTCGCTGGTG TCCCTTC 3'	5' CTCTTCTTCCGAC TGGATGTG 3'

7.2 NK2 Receptor mRNA Expression

Mean ratios of NK2 mRNA gene expression to β-actin gene expression, normalized to a calibrator sample, are reported in Figure 1.

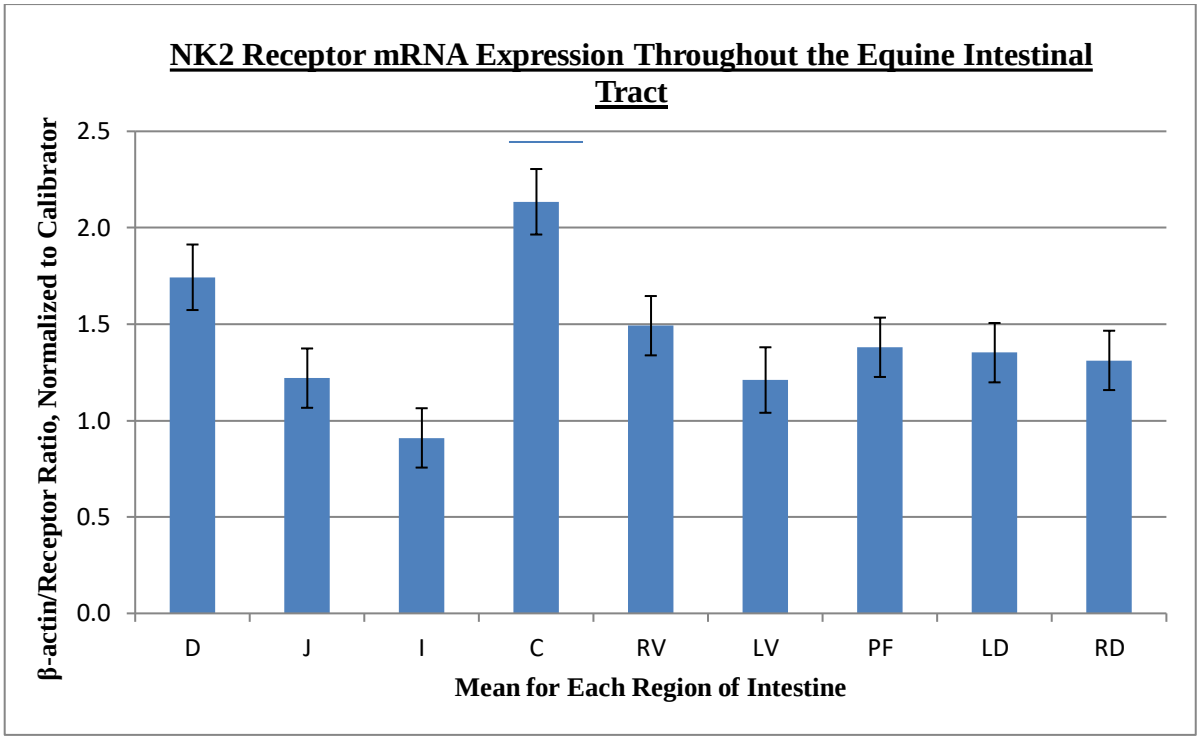

Figure 1: Mean NK2 receptor mRNA receptor expression from 5 horses, as a ratio to β-actin expression and normalized to a calibrator sample, for 9 regions of the intestinal: duodenum (D), jejunum (J), ileum (I), body of the cecum (C), right ventral colon (RV), left ventral colon (LV), pelvic flexure (PF), right dorsal colon (RD) and left dorsal colon (LD). * – indicates findings that were significantly different from each other ($p = 0.0107$). † - indicates intestinal segments that showed significant differences from the cecum (jejunum ($p = 0.0039$), ileum ($p \leq 0.0001$), left ventral colon ($p = 0.0066$), pelvic flexure ($p = 0.0269$), left dorsal colon ($p = 0.0194$), and right dorsal colon ($p = 0.0121$)).

NK2 receptors were not evenly distributed throughout the equine intestinal tract. A significant difference (as determined by 2-way ANOVA) was evident between intestinal segments ($p \leq 0.0003$). In the small intestine, the duodenum showed the highest level of NK2 receptors compared to other segments. Tukey-Kramer adjustment for comparison of means showed that duodenal NK2 mRNA concentration was significantly higher than that in the ileum ($p = 0.0107$). Four of the five horses in the study showed higher levels of NK2 receptors in the duodenum than other regions of the small intestine. NK2 receptor levels were next highest in the jejunum, followed by the ileum. Four of five horses showed their lowest small intestinal concentrations of NK2 receptors in the ileum.

In the large intestine, the cecum showed the highest mean level of NK2 mRNA expression compared to other segments. Tukey-Kramer adjustment showed that levels of NK2 receptors in the cecum were significantly higher than the jejunum ($p = 0.0039$), ileum ($p \leq 0.0001$), left ventral colon ($p = 0.0066$), pelvic flexure ($p = 0.0269$), left dorsal colon ($p = 0.0194$), and right dorsal colon ($p = 0.0121$).

7.3 NK3 Receptor mRNA Expression

Mean ratios of NK3 mRNA gene expression to β-actin gene expression, normalized to a calibrator sample, are reported in (Figure 2). NK3 receptor mRNA was detected at relatively low levels in all areas of the intestinal tract in 3 of 5 horses, but there were no significant differences between segments. Two of five horses showed very low, to undetectable concentrations of NK3 mRNA throughout the intestinal tract. One horse showed

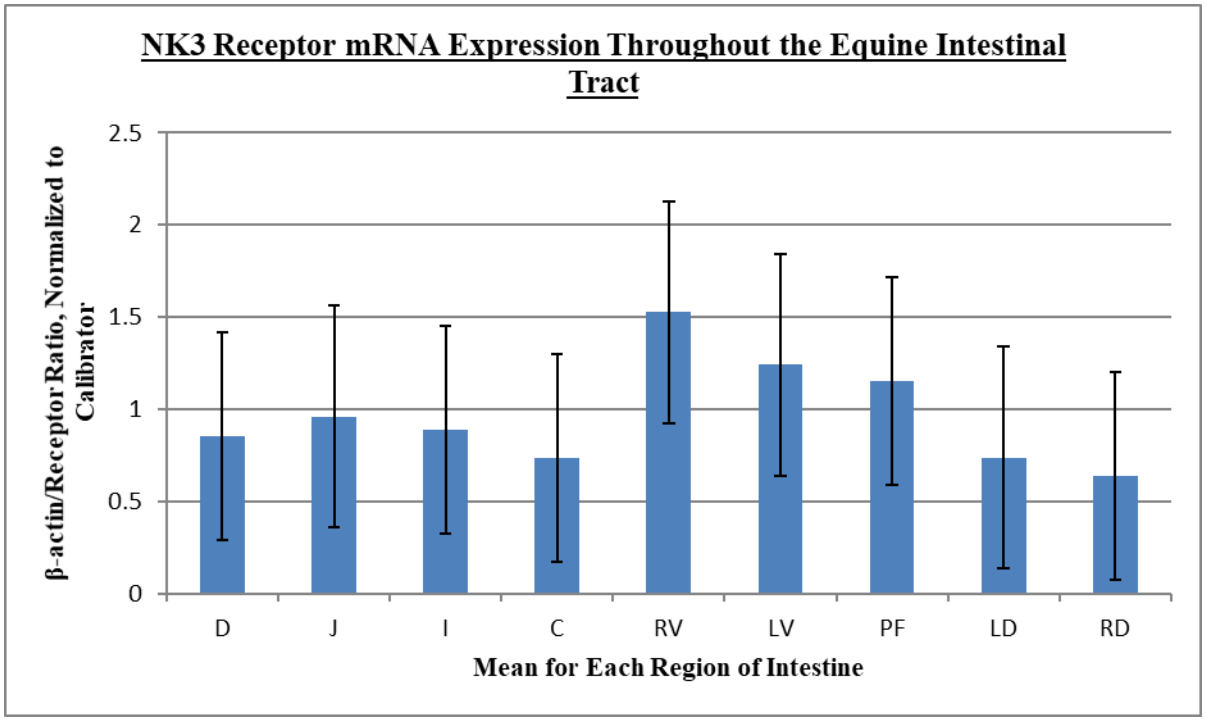

Figure 2: Mean NK3 receptor mRNA receptor expression from 5 horses, as a ratio to β-actin expression and normalized to a calibrator sample, for 9 regions of the intestinal tract: duodenum (D), jejunum (J), ileum (I), body of the cecum (C), right ventral colon (RV), left ventral colon (LV), pelvic flexure (PF), right dorsal colon (RD) and left dorsal colon (LD).

higher overall values in the large intestine than the other four horses.

7.4 Inflammatory Model

Mean ratios of change in NK1, NK2 and NK3 receptor mRNA gene expression to β-actin gene expression, normalized to a calibrator sample, in response to ILD or ISO are reported in Figure 3.

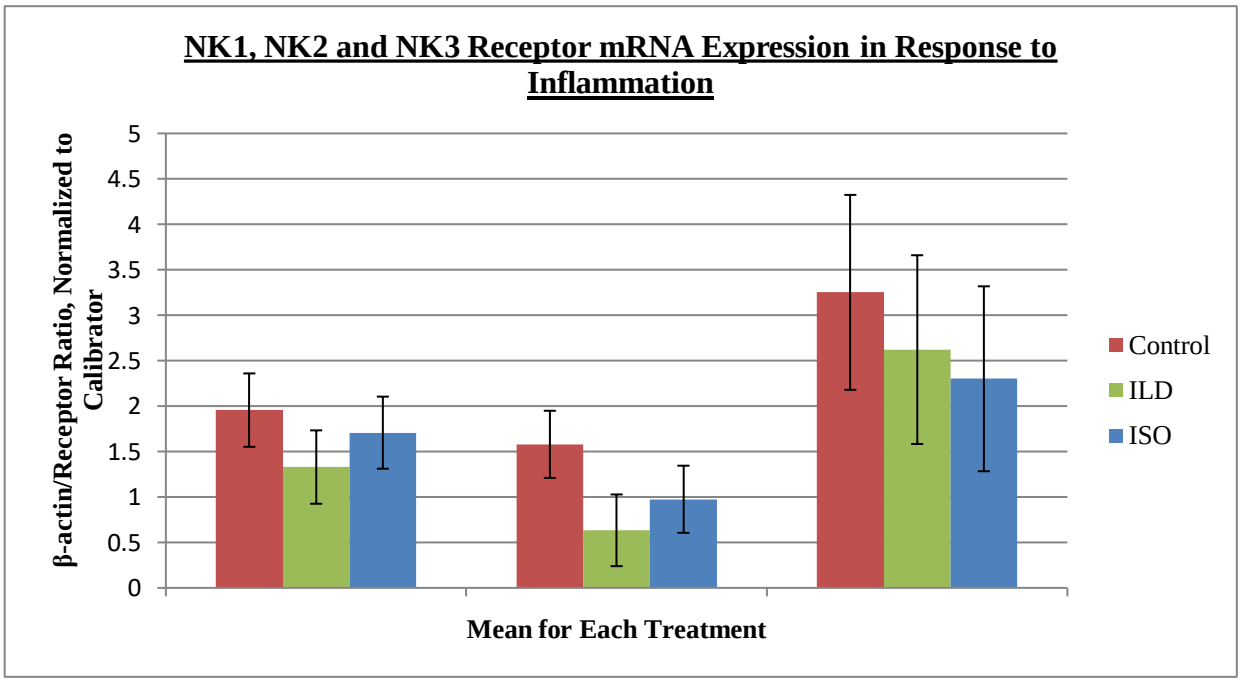

Figure 3: Mean NK1, NK2 and NK3 receptor mRNA expression from the jejunum of 6 horses, as a ratio to β-actin expression and normalized to a calibrator sample for Control (C), Ischemia reperfusion (ISO) and Intraluminal distention (ILD) segments. * - indicates segments that were significantly different from each other ($p = 0.021$).

For NK1 receptors, overall ANOVA analysis did not reveal any statistically significant differences between

treatment groups ($p = 0.1947$). However, pair-wise comparison revealed a trend for ILD NK1 mRNA expression to be lower than that of control ($p = 0.075$). For NK2 receptor analysis 1 outlier was removed. An overall ANOVA for NK2 receptor expression showed a significant difference in the inflammatory model ($p = 0.0216$). Tukey-Kramer analysis showed that NK2 receptors were higher in control samples than ILD ($p = 0.021$). A trend was also noted, for control to be higher than ISO ($p = 0.1200$). No significant differences were observed in NK3 mRNA expression between control, ischemic or distension treatments ($p \leq 0.6244$)

8. Discussion

The present study is the first to report distribution patterns for NK2 and NK3 tachykinin receptor mRNA throughout the equine intestinal tract. This is an initial step in the evaluation of the roles that neurokinins play in equine enteric physiology and pathology. Previously, the distribution of NK1 receptor mRNA in 9 regions of the equine intestinal tract was described [19]. NK receptors have also been quantified for the equine PF [18]. While tachykinin agonists and antagonists have been extensively studied in laboratory animals and in humans for the treatment of inflammatory bowel diseases and abnormal motility patterns, exploration of this topic in the equine species is still in its infancy.

Abnormal motility and inflammatory changes in the gastrointestinal tract are common problems in equine medicine [28]. Acute inflammatory changes characterize most cases of colic, due both to ischemia/reperfusion and intraluminal distension that occur secondary to strangulating obstruction injuries [29]. Acute inflammation during colic is also thought to contribute to post-operative ileus [23, 24]. Research to date in other species supports the benefit of NK antagonists in acute inflammation, with the most protective effects seen as pre-treatments or within the first 24 hours [7].

In addition to protective properties, NK therapy has shown therapeutic benefit for abnormal motility. For example, in rats, SP and NK2 receptor antagonists have been useful in restoring regular motility after postoperative intestinal atony [36, 37]. NK2 agonists seem to have the strongest contractile effect on smooth muscle of the small and large intestine [13]. Previous reports have shown a positive contractile response of isolated smooth muscle strips harvested from the equine intestinal tract to NKA, NKB [20] and SP [20, 21]. Circular muscle has also proven to be more responsive than longitudinal smooth muscle sections [18, 20, 21].

In the current study, the duodenum showed consistently high levels of NK2 receptor mRNA compared to other segments of the small intestine. In a similar study, NK1 receptor mRNA was also found to have high levels in the duodenum [19]. Other equine enteric receptors have also shown high levels in the duodenum [26]. While not statistically different from each other, levels of NK2 mRNA were next highest in the jejunum, followed by the ileum. This suggests that tachykinin agonists and antagonists would have their greatest effect in the proximal small intestine, as receptor concentration appears to decrease abnormally. Often, in the horse, inflammatory and motility abnormalities affect the distal small intestine most severely [46]. Tachykinins have been implicated in both phase II (the mixing phase) and the overall generation of MMCs (migrating motor complex) of the GIT [38, 39]. For example, in rats a SP antagonist returned MMCs and subsequent motility 23% faster than control after intestinal atony [40]. At lower doses, NKA has been shown to dose-dependently increase amplitude and frequency of small intestinal contractions of humans, increasing the fraction of phase II of the MMC [38]. In the small intestine, Nepadutant (an NK2 antagonist) when given 15 minutes prior to GIT surgery, decreased post-operative time for return to motility by 39 % in the rat jejunum [41]. So, it is anticipated that, while receptor mRNA expression is

highest orally, their effect may continue abnormally and also affect the small intestine more generally.

In the large intestine, the cecum showed the highest levels of NK2 mRNA expression compared to other segments. High levels of NK1 receptor mRNA in the right and left ventral colons, and moderate levels in the cecum have also been reported [19]. It is reasonable to expect then that the cecum would respond well to therapy targeting neurokinin receptors. Motilin receptors, to which the prokinetic agent erythromycin lactobionate binds, were also found to be at high levels in the cecum of the horse [26]. While cecal disease is not common in the horse (consisting 3.7% of reported surgical referrals [42]), case management can be challenging [43]. Tachykinin therapy may be a viable tool in cases of colic involving the caecum, many of which are managed medically [43]. NK3 receptor mRNA levels proved to be more variable between individual horse samples than that of NK2. An individual showed very high concentrations, while others showed low to undetectable levels in both the small and large intestine. Standard error was high between individual samples in the present study as well. Low detectable amounts of mRNA may account for this problem. A larger sample size may be useful in determining the true role of NK3 in the equine intestinal tract. Perhaps NK3 expression is greater in different layers of the intestinal lining, as the smooth muscular layer was only evaluated here. NK3 therapy may also have a greater effect if given centrally versus locally, as has been noted in the rat colon [30]. Based on the current study, it is expected that they would have low therapeutic value, or at least as an unreliable therapeutic target. Potential use may be as adjunctive therapy in some individuals. Species differences have been noted in the relative importance of one receptor compared to another in a particular pathological process [6, 27]. The current study may support NK3 as a less physiologically relevant receptor than NK2 and NK1 for the horse. Changes seen in models of ILD and ISO have been previously described, and are consistent with acute inflammatory changes [44]. It was found that NK1 receptor mRNA tended to decrease after distension. Similarly, NK2 receptor mRNA concentrations were lower for ILD and ISO when compared to control/sham operated samples. Other equine enteric receptors have shown similar trends; motilin receptors were seen to decrease by 10% after intraluminal distension [25]. Also similar to motilin receptors, the decrease in NK2 receptor mRNA was more pronounced after 2 hours of ILD, compared to 120 minutes of ischemia and subsequent reperfusion. Distension has a greater effect on the seromuscular layer [45], when compared to ischemia/reperfusion. Since seromuscular layers were evaluated here, the present results are thus not unexpected. The relative importance of this decrease is in NK mRNA expression is unknown. The efficacy of NK2 agonist therapy may be hampered in postoperative ileus due to the decreased expression of NK2 receptors in the jejunum following intraluminal distension and ischemia.

In contrast to the current study, SP, as measured by immunoreactivity, was found to be unchanged in a model of impaction colic [15] and ischemic injury [18]. Studies evaluating electrical activity in the GIT, such as isometric studies [48] or vivo studies using electrogastrography [47, 49], may be useful in determining the true biologic significance of this decrease in receptor concentration. It is important to note, for all the results described here, that the levels reported are for receptor mRNA which, although obviously important in determining receptor level, may not always correlate directly with functional receptor expression.

The present study confirms the presence of NK2 receptor mRNA in the smooth muscle of the equine intestinal tract. Distribution of NK2 receptor mRNA was not uniform throughout the intestinal tract, with highest concentrations in the small intestine being in the duodenum, while the

cecum had the highest levels of the large intestine. Additional study is indicated to determine the true role of NK3 receptors in the horse; current analysis suggests that they may not play an important physiological role. Tachykinin agonists and antagonists may be of therapeutic value in the horse for both mediating motility post-operatively in the case of ileus, and decreasing the destructive effects of inflammation; however, additional research concerning ligand and receptor physiology in the horse is warranted. Receptor mRNA concentrations were affected by intraluminal distension and ischemia/reperfusion injury, however, the consequence of this decrease in concentration requires further investigation. Tachykinins have a complex mechanism of action in the gastrointestinal tract [4], and thus additional research and *in vivo* studies in the horse are indicated.

9. Footnotes

a. Tissue-Tek O.C.T.; Bayer Corporation, Pittsburgh, Pennsylvania, USA
b. Rompun, Bayer Animal health, Etobicoke, ON, Canada
c. Guafenesin powder; Rodia, Mississauga, ON, Canada
d. Bioniche Animal Health, Belleville, ON, Canada
e. Lactated Ringer's solution; Baxter, Mississauga, ON, Canada
f. Tripure Isolation Reagent; Roche Diagnostics Ltd., Basel, Switzerland
g. Mini BeadBeater; BioSpec Products Inc, Bartlesville, Oklahoma, USA
h. Polytron homogenizer; Kinematica AG, Litau, Switzerland
i. Ambion Inc, Austin, Texas, USA
j. Ambion DNA – free; Roche Molecular Biochemicals, Laval, QC, Canada
k. Invitrogen Corp, Carlsbad, California, USA
l. Superscript First Stand, Roche Molecular Biochemicals, Laval, QC, Canada
m. BioSpec Products Inc, Bartlesville, Oklahoma, USA
n. Lightcycler; Roche Diagnostics Ltd., Basel, Switzerland
o. T-Gradient Thermoblock; Biometra Corp, Göttingen, Germany
p. Roche Diagnostics Ltd., Basel, Switzerland
q. SYBR Green I; Invitrogen Corp, Carlsbad, California, USA
r. LightCycler Relative Quantification Version 1.0; Roche Diagnostics Ltd., Basel, Switzerland
s. SAS Institue Inc. 2004. SAS OnlineDOC (R) 9.1.3. Cary, NC: SAS Institute Inc

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10. References

1. Conlon JM. The tachykinin peptide family, with particular emphasis on mammalian tachykinins and tachykinin receptor agonists. *Tachykinins. Springer*. 2004; 25 – 61.
2. Regoli D, Drapeau G, Dion S, D'Orleans-Juste P. Pharmacological receptors for Substance P. *Life Sci* 1987; 40: 109 –117.
3. Holzer P. Implications of tachykinins and calcitonin gene-related peptide in inflammatory bowel disease. *Digestion*. 1998; 59: 269 – 283.
4. Holzer P, Holzer-Petche U. Tachykinins in the gut, part I: expression, release and motor function. *Pharmacol Ther* 1997; 73: 173 – 217.

5. Di Sebastiano P, Grossi L, Di Mola FF, Angelucci D, Freiss H, L. Marzio et al. SR140333, a substance P receptor antagonist, influences morphological and motor changes in rat experimental colitis. *Dig Dis Sci.* 1999; 44: 439 – 444.

6. Mazelin L, Theodorou V, More J, Emonds-Alt X, Fioramonti J, LionelBueno. Comparative effects of nonpeptide tachykinin receptor antagonists on experimental gut inflammation in rats and guinea-pigs. *Life Sci.* 1998; 63: 293 – 304.

7. Wallace JL, McCafferty DM, Sharkey KA. Lack of beneficial effect of a tachykinin receptor antagonist in experimental colitis. *Regul Peptides.* 1998; 73: 95 – 101.

8. Brierley SM, Nichols K, Grasby DJ, Waterman SA. Neural mechanisms underlying migrating motor complex formation in mouse isolated colon. *Brit J Pharmacol* 2001; 132: 507 – 517.

9. Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. *J Physiol.* 1931; 72 – 74.

10. Giuliani S, Barbanti G, Turini D, Quartara L, Rovero P, Antonio Giachettia et al. NK2 tachykinin receptors and contraction of circular muscle of the human colon - characterization of the NK2 receptor subtype. *Eur J Pharmacol*. 1991; 203: 365 – 370.

11. Onori L, Aggio A, Taddei G, Ciccocioppo R, Severi C, V Carnicelli et al. Contribution of NK3 tachykinin receptors to propulsion in the rabbit isolated distal colon. *Neurogastroent Motil* 2001; 13: 211 – 219.

12. Onori L, Aggio A, Taddei G, Loreto MF, Ciccocioppo R, Riccardo Vicini et al. Peristalsis regulation by tachykinin NK1 receptors in the rabbit isolated distal colon. *Am J Physiol - Gastr L.* 2003; 285: G325 - G331.

13. Patacchini R, Giuliani S, Turini A, Navarra G, Maggi CA. Effect of nepadutant at tachykinin NK2 receptors in human intestine and urinary bladder. *Eur J Pharmacol.* 2000; 398: 389 – 397.

14. Lecci A, De Giorgio R, Bartho L, Sternini C, Tramontana M, S Giuliani et al. Tachykinin NK1 receptor-mediated inhibitory responses in the guinea-pig small intestine. Neuropeptides 1999; 33: 91 – 97.

15. Cummings JF, Sellers AF, Lowe JE. Distribution of substance-P-like immunoreactivity in the enteric neurons of the large colon of normal and amitraz-treated ponies - an immunocytochemical study. *Eq Vet J*. 1985; 17: 23 – 29.

16. Burns G, Cummings JF. Neuropeptide distributions in the colon, cecum and jejunum of the horse. *Anat Rec*. 1993; 236: 341 – 350.

17. Pearson GT. Structural organization and neuropeptide distribution in the equine enteric nervous-system -an immunohistochemical study using whole-mount preparations from the small-intestine. *Cell Tissue Res.* 1994; 276: 523 – 534.

18. Sonea IM, Wilson DV, Bowker RM, Robinson NE. Tachykinin receptors in the equine pelvic flexure. *Eq Vet J*. 1997; 29: 306 – 312.

19. Solinger N, Sonea IM (2008) Distribution of the neurokinin-1 receptor in equine intestinal smooth muscle. *Eq Vet J.* 2008; 40: 321 – 325.

20. Belloli C, Arioli F, Beretta C, Madonna M . In vitro effects of tachykinins on the smooth musculature of the horse gut. *J Vet Pharmacol Therap.* 1994; 17: 379 – 383.

21. Malone ED, Kannan MS, Bown DR. Evaluation of substance P as a neurotransmitter in equine jejunum. *Am J Vet Res.* 2000; 61: 1178 – 1184.

22. Merritt AM, Blikslager AT. Postoperative ileus: To be or not to be? *Eq Vet J*. 2008; 40: 295 – 296.

23. Kalff JC, Schraut WH, Simmons RL, Bauer AJ. Surgical manipulation of the gut elicits an intestinal muscularis inflammatory response resulting in postsurgical ileus. *Ann Surg*. 1998; 228: 652 – 663.

24. Kalff KC, Carlos TM, Schraut WH, Billiar TR, Simmons RL, Anthony J Bauer. Surgically induced leukocytic infiltrates within the rat intestinal

muscularis mediate postoperative ileus. *Gastroenterol.* 1999; 117: 378 – 387.

25. Koenig J, Sawhney S, Cote N, Lamarre J. Effect of intraluminal distension or ischemic strangulation obstruction of the equine jejunum on jejunal motilin receptors and binding of erythromycin lactobionate. *Am J Vet Res.* 2006; 67: 815 – 820.

26. Koenig J, Cote N, Lamarre J, Harris WH, Trout DR, Daniel G Kenney et al. Binding of radiolabeled porcine motilin and erythromycin lactobionate to smooth muscle membranes in various segments of the equine gastrointestinal tract. *Am J Vet Res.* 2002; 63: 1545 – 1550.

27. Appell KC, Fragale BJ, Loscig J, Singh S, Tomczuk BE. Antagonists that demonstrate species differences in Neurokin -1 receptors. *Am Soc Pharm Exp Ther*. 1992; 41: 772 – 778.

28. Hardy J, Rakestraw PC. Postoperative care, complications, and reoperation. In: Auer A and A Stick. (4th edtn) Equine surgery. W. B. Saunders, *Oxford*. 2011; 514 – 529.

29. Blikslager AT, Marshall JF. Principles of intestinal injury and determination of intestinal viability. In: Auer A and A Stick. (4th edtn) *Equine surgery*. W. B. Saunders, Oxford: 2011; 411 – 415.

30. Eutamene H, Theodorou V, Fioramonti J, Bueno L. Rectal distention-induced colonic net water secretion in rats involves tachykinins, capsaicin sensory, and vagus nerves. *Gastroenterol.* 1997; 112: 1595 – 1602.

31. Pothoulakis C, Castagliuolo I, LaMont JT, Jaffer A, O'Keane JC. CP-96,345, a substance -P antagonist, inhibits rat intestinal responses to clostridium-difficile toxin-A but not cholera-toxin. *P Natl Acad Sci USA.* 1994; 91: 947 – 951.

32. Castagliuolo I, Keates AC, Qui B, Kelly CP, Nikulasson S, Susan E. Leeman et al. Increased substance P responses in dorsal root ganglia and intestinal macrophages during clostridium difficile toxin A enteritis in rats. *Proc Natl Acad Sci USA.* 1997; 94:4788 – 4793.

33. Cutrufo C, Evangelista S, Cirilllo R, Ciucci A, Conte B, GiuseppeLopez et al. Effect of MEN 11467, a new tachykinin NK1 receptor antagonist, in acute rectocolitis induced by acetic acid in guinea-pigs. *Europ J Pharmacol.* 1999; 374: 277 – 283.

34. Mantyh C, Pappas T, Lapp J, Washington M, Neville L, J R Ghilardi et al. Substance P activation of enteric neurons in response to intraluminal clostridium difficile toxin A in the rat ileum. *Gastroenterol.* 1996; 111: 1272 – 1280.

35. Stucchi AF, Shofer S, Leeman S, Materne O, Beer E, J McClung et al. NK-1 antagonist reduces colonic inflammation and oxidative stress in dextran sulfate-induced colitis in rats. *Am J Physiol - Gastr L.* 2000; 279: G1298 - G1306.

36. Toulouse T, Fioramonti J, Maggi C, Bueno L. Role of NK2 receptors in gastric barosensitivity and in experimental ileus in rats. *Neurogastroent Motil*. 2001; 13: 45 – 53.

37. Goldhill JM, Shea-Donohue T, Ali N, Pineiro-Carrero VM. Tachykinergic neurotransmission is enhanced in small intestinal circular muscle in a rabbit model of inflammation. *J Pharmacol Exp Ther*. 1997; 282: 1373 – 1378.

38. Lordal M, Theodorsson E, Hellstrom PM. Tachykinins influence interdigestive rhythm and contractile strength of human small intestine. *Digest Dis Sci*. 1997; 42: 1940 – 1949.

39. Brierley SM, Nichols K, Grasby DJ, Waterman SA. Neural mechanisms underlying migrating motor complex formation in mouse isolated colon. *Brit J Pharmacol.* 2001; 132: 507 – 517.

40. Espat NJ, Cheng G, Kelley MC, Vogel SB, Sninsky CA, M P Hocking. Vasoactive-intestinal-peptide and substance-P receptor antagonists improve postoperative ileus. *J Surg Res*. 1995; 58: 719 – 723.

41. Toulouse T, Fioramonti J, Maggi C, Bueno L. Role of NK2 receptors in gastric barosensitivity and in experimental ileus in rats. *Neurogastroent Motil*. 2001; 13: 45 – 53.

42. Dart AJ, Hodgson DR, Snyder JR. Caecal disease in equids. *Aust Vet J*. 1997; 75: 552 – 557.

43. Smith LCR, Payne RJ, Boys Smith SJ, Bathe AP, Greet TRC. Outcome and long-term follow-up of 20 horses undergoing surgery for caecal impaction: a retrospective study (2000 – 2008). *Equine Vet J*. 2010; 42: 388 – 392.

44. Allen DJ, White NA, Tyler DE. Morphologic effects of experimental distention of equine small intestine. *Vet Surg*. 1988; 17: 10 – 14.

45. Dabareiner RM, White NA, Donaldson LL. Effects of intraluminal distention and decompression on microvascular permeability and hemodynamics of the equine jejunum. *Am J Vet Res*. 2001; 62: 225 – 236.

46. Moore JN. Diseases associated with colic by anatomic location. In: Kahn CM, Line S. (10th edtn) *The Merck Veterinary Manual. Merck Publishing Group*. 2010.

47. Sasaki N, Okamura K, Yamada H. Effects of mosapride, a 5-hydroxytryptamine 4 receptor agonist, on electrical activity of the small intestine and cecum in horses. *Am J Vet Res.* 2005; 66: 1321 – 1323.

48. Mule D, Serio T. Involvement of tachykinin NK2 receptors in the modulation of spontaneous motility in rat proximal colon. *Neurogastroentero Motil*. 2000; 12: 459 – 466.

49. Koenig J, Martin CEW, Dobson H, Mintchev MP. Use of multichannel electrogastrography for non invasive assessment of gastric myoelectrical activity in dogs. *Am J Vet Res.* 2009; 70: 11 – 15.

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