

## **In vitro effects of 5-hydroxytryptamine on the Jejunum of Healthy and Colic Horses: Characterization of a Putative 5-HT<sub>1A</sub> Receptor in the Equine Gut.**

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Prokinetic use for intestinal stasis in horses is mainly extrapolated from human medicine, with limited scientific proof that the targeted enteric receptor populations are similar in the equine and human gastrointestinal tract. As the prokinetic cisapride acts at serotonin (5-HT) receptors, we aimed to characterize the 5-HT receptors mediating the contractile response to 5-HT in equine jejunum longitudinal muscle. We compared the results of healthy horses with those from colic patients, who developed non-responsive post-operative ileus after surgery for small intestinal strangulation.

Strips of the mid-jejunum of healthy horses were collected at the slaughterhouse. Colic patient material was obtained after euthanasia with T61<sup>a</sup>. Longitudinal muscle strips, deprived of mucosa were prepared for isotonic measurement. All strips showed basal phasic activity. After stabilisation the tissue was challenged twice with  $1 \times 10^{-6}$ M carbachol. The second carbachol-induced contraction was used as internal standard and all contractions to 5-HT are

expressed as % of this second response. Results are given as mean  $\pm$  SEM; the number of horses used is denoted by *n*.

5-HT induced a tonic contraction with an increase in the amplitude of the phasic activity. Cumulative administration of 5-HT in the same strip led to rapid fading of the contractile response at the higher concentrations of 5-HT, in contrast to what has been reported in former studies on equine ileum and pelvic flexure<sup>1,2</sup>. The isolated concentration-response curve for 5-HT ( $10^{-10}$ M –  $3 \times 10^{-5}$ M) has the features of a monophasic sigmoidal concentration-response curve, consistent with a single-site interaction. The curve parameters are given in Table 1.

Table 1: Curve fit parameters of the concentration-response curves to 5-HT and 5-CT in jejunum from healthy and colic horses

		$E_{\max}$	$pEC_{50}$	Hill slope
5-HT	Healthy (n=24)	$79.04 \pm 2.47$	$7.88 \pm 0.07$	$1.07 \pm 0.08$
	Colic (n=7)	$70.72 \pm 11.21$	$7.41 \pm 0.23^{**}$	$0.79 \pm 0.08$
5-CT	Healthy (n=6)	$85.86 \pm 3.78$	$6.02 \pm 0.18$	$0.76 \pm 0.09$
	Colic (n=7)	$66.95 \pm 9.31^{**}$	$6.44 \pm 0.11^*$	$1.17 \pm 0.11^{**}$

\* $P < 0.05$ , \*\* $P < 0.01$ : Significantly different from the response in healthy horses

Tetrodotoxin ( $3 \times 10^{-6}$ M), atropine ( $3 \times 10^{-6}$ M) and the combination of both, did not affect the responses to 5-HT, suggesting a non-neurogenic, non-cholinergic contractile effect mediated by 5-HT receptors located directly on smooth muscle cells. The specific 5-HT receptor antagonists ketanserin (5-HT<sub>2A</sub>;  $3 \times 10^{-7}$ M), granisetron (5-HT<sub>3</sub>;  $3 \times 10^{-7}$ M); GR 113808 (5-HT<sub>4</sub>;  $1 \times 10^{-7}$ M); SB 269970 (5-HT<sub>7</sub>;  $3 \times 10^{-7}$ M), GR 127935 (5-HT<sub>1B,D</sub>;  $1 \times 10^{-7}$ M), SB 204741 (5-HT<sub>2B</sub>;  $3 \times 10^{-7}$ M), RS 102221 (5-HT<sub>2C</sub>;  $3 \times 10^{-7}$ M) had no effect on the responses to 5-HT, excluding interaction at 5-HT<sub>1B,1D</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub> and 5-HT<sub>7</sub> receptors.

The 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>5</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub>-receptor antagonist methysergide ( $1 \times 10^{-9}$ M,  $1 \times 10^{-8}$ M,  $1 \times 10^{-7}$ M) antagonised non-surmountably the concentration-response curve of 5-HT.

NAN 190 (5-HT<sub>1A</sub>; 1x10<sup>-7</sup>M, 3x10<sup>-7</sup>M, 1x10<sup>-6</sup>M ) behaved as a competitive antagonist. WAY 100635 (3x10<sup>-9</sup>M) shifted the concentration-response curve to 5-HT to the right in a parallel way without a change in E<sub>max</sub>, but the higher concentrations of WAY 100635 (3x10<sup>-8</sup>M and 3x10<sup>-7</sup>M) significantly depressed the E<sub>max</sub> of 5-HT. The pK<sub>b</sub> values of NAN 190 and WAY 100635 were estimated from the antagonist inhibition curve in the presence of a fixed concentration of 5-HT. The pK<sub>b</sub> values (NAN 190, 8.13 ± 0.06; WAY 100635, 8.69 ± 0.07) are in accordance with the pK<sub>b</sub> values of these antagonists for the 5-HT<sub>1A</sub> receptor reported in the literature.

The specific 5-HT<sub>1</sub>, 5-HT<sub>7</sub> agonist 5-carboxamidotryptamine (5-CT) elicited responses similar to those of 5-HT. The concentration-response curve parameters are given in Table 1. The responses to 5-CT were not influenced by application of tetrodotoxin plus atropine (both 3x10<sup>-7</sup>M), and the 5-HT<sub>7</sub>-receptor antagonist SB 269970 (5-HT<sub>7</sub>; 3x10<sup>-7</sup>M). The specific 5-HT<sub>1A</sub> receptor antagonist WAY 100635 (3x10<sup>-9</sup>M) antagonised the contractions to 5-CT. When WAY 100635 was applied at a concentration of 3x10<sup>-8</sup>M and 3x10<sup>-7</sup>M, the concentration-response curve to 5-CT was further shifted to the right but there was a clear concomitant suppression of the maximum effect elicited by 5-CT.

Responses to carbachol were not different in healthy horses (1.70 ± 0.08 cm) and colic horses (1.53 ± 0.21cm). 5-HT was less potent in colic horses than in healthy horses (Table 1).

The parameters of the concentration-response curves of 5-HT and 5-CT in colic horses are given in Table 1. The pEC<sub>50</sub> of 5-HT in colic horses was decreased. The E<sub>max</sub> of 5-CT was significantly decreased in colic horses, however pEC<sub>50</sub> and Hill slope were increased.

Our data clearly suggest that muscular 5-HT<sub>1A</sub> receptors mediate the contractions to 5-HT in equine jejunal longitudinal muscle. The receptor shows rapid desensitization, which could have important implications in horses with colic or ileus, where a status of hypercoagulability or necrotic intestinal segments could serve as a source of 5-HT overload.

## References and Footnotes

1. Nieto JE, Snyder JR, Kollias-Baker C, et al. In vitro effects of 5-hydroxytryptamine and cisapride on the circular smooth muscle of the jejunum of horses. *AJVR* 2000;61:1561-1565.
2. Weiss R, Abel D, Scholtysik G, et al. 5-Hydroxytryptamine mediated contractions in isolated preparations of equine ileum and pelvic flexure: pharmacological characterization of a new 5-HT<sub>4</sub> agonist. *J Vet Pharmacol Ther* 2002;25:49-58.
3. T61<sup>®</sup> ad us. Vet. Embutramide, mebenzoniumiodide, tetracain hydrochloride, dimethylformamide, Intervet Belgium, Ragheno Parc, Mechelen, Belgium