Veterinary Research Scholars Program
University of Missouri

Expression of Mu Opioid Receptor in the Equine Hoof Lamellar Interface in Health and Laminitis

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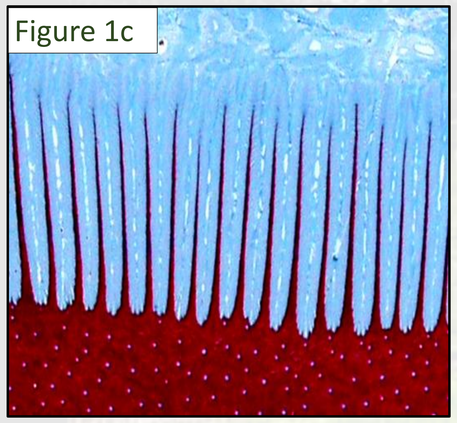
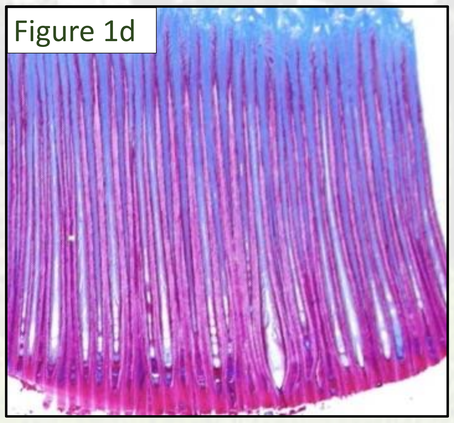
Introduction

- Laminitis is a common, painful affliction of the equine hoof
- Failure of analgesic strategies to control pain often necessitates euthanasia
- Opioids may provide both analgesic and anti-inflammatory effects





The above images depict normal hoof growth (Fig. 1a); abnormal hoof growth as a result of chronic laminitis (Fig. 1b); normal hoof lamellar interface (HLI) (Fig. 1c); appearance of HLI in chronic laminitis (Fig. 1d) (low power magnification)

Opioid Receptors (OR)

- G-protein coupled receptors
  - Three primary pharmacologically distinct types: mu (MOR), kappa (KOR) & delta (DOR)
- MOR density is highest within the CNS
  - Demonstrated in other equine tissues
- High degree of interspecies homology
  - Major divergence at N- and C- terminals

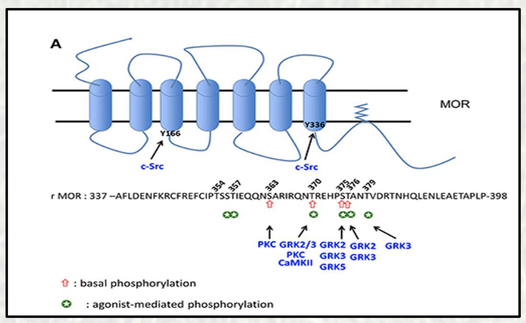


Figure 2. Mu opioid receptor (MOR)

Objectives

- Validation of an antibody for equine tissue (western blot)
- Immunohistochemical evaluation of archived hoof lamellae to evaluate the presence and distribution of MOR

Hypothesis

Mu opioid receptors are constitutively expressed in healthy lamellae and the level of expression is increased during laminitis.

Antibody Selection

- Few available antibodies validated for equine tissue
- *OPRM1* gene analysis to select promising primary antibody candidate

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human     361   IEQQNSTRIRQNTRDHPSTANTVDRTNHQLENLEAETAPLP
horse           IEQQNSTRVRQNTRDHPSTANTVDRTNHQLENLEAETAPLP
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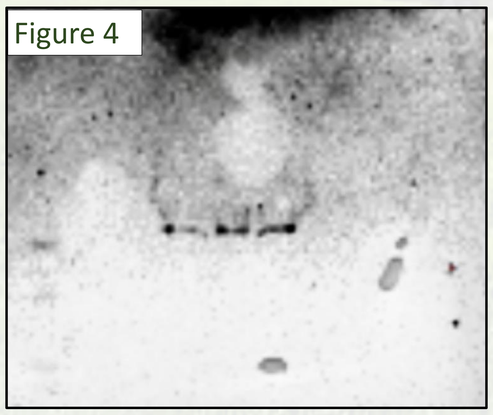
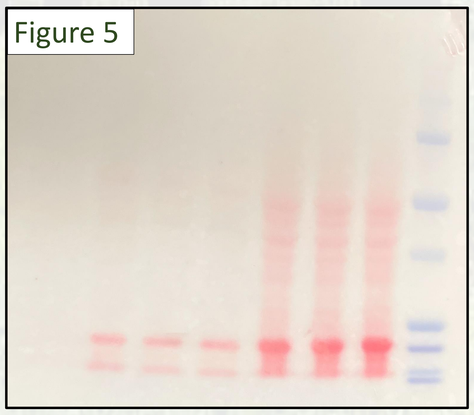
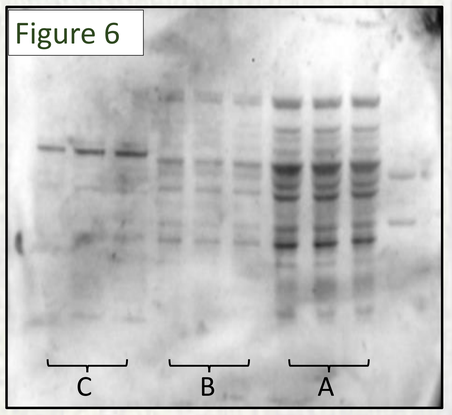
Figure 3. Comparison of *OPRM1* gene (human and horse) depicting only one discrepancy. Highlighted sequence is the target site for Neuromics™ antibody RA10104.

- Rabbit MOR IgG selected for validation (Neuromics RA10104)
- Goat anti-rabbit HRP antibody used as secondary antibody (Invitrogen A16104)

Western Blot Analysis for Validation







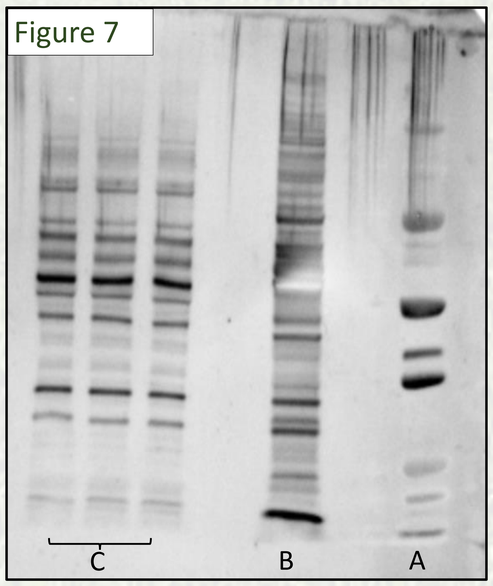


Figure 4. Initial test depicting incomplete transfer. Some prominent bands, but an incomplete protein ladder.
Figure 5. Ponçeau stained membrane post transfer. Depicts improvements in transfer protocol (compared to Fig. 4).
Figure 6. Imaging of membrane from Figure 5. Protein ladder is incomplete (transfer protocol still requires refinement). All samples were run in triplicate. Lane A, 1/10th dilution of equine thalamus/hippocampus. Lane B, 1/20th dilution of same tissue. Lane C is rat PC12 cells (positive control).
Figure 7. Western blot using Neuromics RA10104 and Invitrogen A16104 showing non-specific bands. Lane A, protein ladder. Lane B, control rat PC12 cells. Lane C, triplicate of a 1/10th dilution using equine tissue.

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