Many immunofluorescent studies of the µ Opioid Receptor (OPRM1) also referred to as MOR, use antisera directed against the carboxy (C)-terminal region to localize OPRM1 in tissue sections. These antisera share common epitopes, are usually not species-selective, and no labeling is observed with them in mice that do not express functional OPRM1. We have developed an antisera directed against the rat OPRM1 amino (N)-terminal sequence (AMAB8629) directed at the receptor’s C-terminus, and a guinea pig anti-rat OPRM1 monoclonal antibody directed against the first 22 N-terminal amino acids of the rat OPRM1. We developed two-color immunofluorescence to determine the spatial relationships in spinal cord between OPRM1 labeling obtained with the N- and C-terminal epitopes in rats and mice. Animals were perfused with Lanu’s fixative and then sections were stained using a Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629) directed at the receptor’s C-terminus, and a guinea pig anti-rat OPRM1 monoclonal antibody directed against the first 22 N-terminal amino acids of the rat OPRM1. These were followed by cyanine Cy5 2-conjugated donkey anti-rabbit and cyanine Cy3 3-conjugated donkey anti-guinea pig secondary antibodies. We observed that virtually all cell bodies, fibers, and puncta were double labeled in rat spinal cord. Additionally, we observed that, in rats, the two antibodies appeared to label identical subcellular structures. In contrast, only the C-terminal antibody stained cell bodies, fibers, and puncta in tissue sections of mouse spinal cord. With both antibodies, staining was abolished in tissue sections where the antisera were pre-incubated with their respective immobilizing peptides. The basis for this species-specificity is unclear since the amino acid sequences of the N-terminal region of rat and mouse OPRM1 are nearly identical. These studies were supported by R&D Systems, Inc. and the Department of Neuroscience at the University of Minnesota.

**Materials and Methods**

**Perfusion**

Sprague Dawley rats (Harlan Laboratories, 200 g) and C57BL/6 mice (Harlan Laboratories, 40 g) were anesthetized with sodium phenobarbital and transcardially perfused with a phosphate-buffered saline (PBS) solution, followed immediately by Lanu’s fixative and 10% sucrose in PBS. Spinal cords were removed and stored in 10% sucrose at 4°C prior to sectioning. Spinal cords were sectioned at 10 µm on a cryostat. Animals were perfused with Lanu’s fixative and then sections were stained using a Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629). Monoclonal antibody directed against the first 22 N-terminal amino acids of the rat OPRM1.

**Anti-Rabbit µ Opioid Receptor/OPRM1 Monoclonal Antibody**

Rabbit Anti-Rat µ Opioid Receptor Antibody (R&D Systems, Catalog # MAB8629). Monoclonal antibody directed against the first 22 N-terminal amino acids of the rat OPRM1.

**Secondary Antibodies**

- **Cy3-conjugated Donkey Anti-Rabbit IgG Secondary Antibody** (Jackson ImmunoResearch, Catalog # 711-225-152).
- **Cy5-conjugated Donkey Anti-Guinea Pig IgG Secondary Antibody** (Jackson ImmunoResearch, Catalog # 706-185-146).

**Immunohistochemistry**

Tissue sections were incubated in 2% normal goat serum for 30 minutes at room temperature. They were then incubated overnight at 4°C with a mixture of the two primary antibodies, each diluted 1:2000. Following a series of washes in PBS, tissue sections were then incubated for 2 hours at room temperature with a mixture of the two secondary antibodies, each diluted 1:500. To investigate specific binding of the OPRM1 antibodies, the primary antibody mixture was pre-absorbed with synthetic peptides from either the N-terminal region or C-terminal region (amino acids 384–398) of rat OPRM1 at a concentration of 50 µg of peptide per ml of diluted antibody prior to incubation with the spinal cord tissue. Images were collected using an Olympus Fluview FV1000 confocal microscope using 40X, 1.2 N.A. objective and 488 and 543 nm lasers.

**Results**

Within the first 22 amino acids of the rat OPRM1, the N-terminal region of OPRM1 proteins differ by 3 amino acids. These subtle differences can lead to non-specific labeling in tissues. As a result, researchers are forced to modify immunohistochemistry protocols to enhance the specific signal and reduce non-specific labeling. We utilized a proprietary antibody development protocol to successfully develop a rabbit monoclonal antibody to the N-terminal region of OPRM1. The OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (A), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; guinea pig anti-rat OPRM1 polyclonal antibody (Jackson ImmunoResearch, Catalog # 706-185-146), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (B), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; guinea pig anti-rat OPRM1 polyclonal antibody (Jackson ImmunoResearch, Catalog # 706-185-146), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (C), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; donkey anti-guinea pig secondary antibody (Jackson ImmunoResearch, Catalog # 711-225-152), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (D), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, Catalog # 711-150-152), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (E), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; Cyanine Cy5-conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, Catalog # 711-225-152), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (F), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; Cyanine Cy3-conjugated donkey anti-guinea pig secondary antibody (Jackson ImmunoResearch, Catalog # 706-185-146), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (G), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; Cyanine Cy3-conjugated donkey anti-guinea pig secondary antibody (Jackson ImmunoResearch, Catalog # 706-185-146), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (H), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; Cyanine Cy5-conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, Catalog # 711-225-152), red. As a control, the OPRM1 was detected in perfusion-fixed frozen sections of rat cervical spinal cord using a (I), Rabbit Anti-Rat µ Opioid Receptor/OPRM1 Monoclonal Antibody (R&D Systems, Catalog # MAB8629), green; donkey anti-guinea pig secondary antibody (Jackson ImmunoResearch, Catalog # 711-225-152), red.