μ-opioid receptor expression of substantia gelatinosa neurons hyperpolarized by DAMGO

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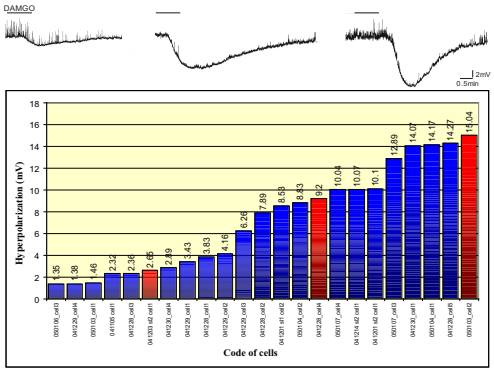
Introduction

Morphine and its analogs which act on μ-opioid receptors are potent analgesic substances. One of their major target area is the spinal substantia gelatinosa (SG) where many neurons express μ-receptors. Neurophysiological studies have demonstrated that at least 40-50% of SG neurons responded to μ-agonists. However detailed immunocytochemical mapping of μ-opioid receptor (MOR-1) identified only 10% of SG neurons as MOR-1 immunoreactive. These neurons have dense staining for MOR-1 receptor outlining the soma and dendrites as well. We have tried to reconcile this discrepancy by using a combined electrophysiological and morphological approach.

Methods

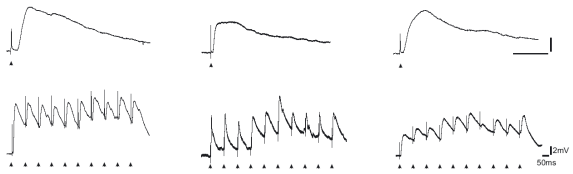
Responses to the μ-opioid agonist DAMGO were recorded with biocytin filled patch-electrode from SG neurons in rat spinal cord slices obtained from young (3-4 week old) animals. Following the electrophysiological recording slices were fixed, resectioned and reacted with antibodies to reveal MOR-1 receptors and other cell types in lamina II such as PKCγ and NOS containing neurons. The triple or quadrople immunofluorescent labelling was examined by confocal microscope.

DAMGO induced hyperpolarization in SG neurons



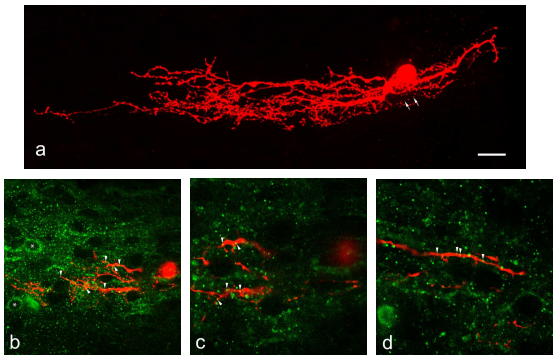
Extent of hyperpolarization of the 23 out of 42 neurons responding to bath application of DAMGO (1μM, 1 min). The graph shows individual records from neurons responding to DAMGO with small, medium and large hyperpolarization. These cells are marked with red columns.

Monosynaptic EPSPs evoked by dorsal root stimulation in DAMGO sensitive neurons

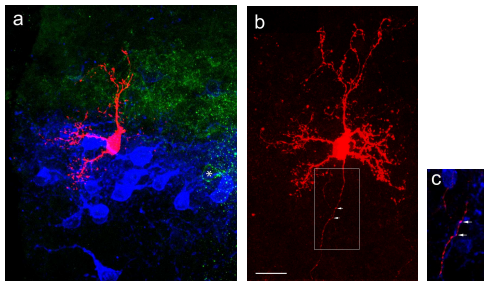


Dorsal root stimulation evoked monosynaptic EPSPs in all tested DAMGO sensitive neurons (n=5). An EPSP was considered monosynaptic if the latencies did not change with repetitive stimuli and if no failure was observed with a high-frequency (10Hz) stimulation. Upper row shows EPSPs evoked by single stimulus, lower row shows EPSPs evoked by repetitive stimulation at the time marked by the arrowheads. Traces were recorded from neurons responding with small (left column) medium (middle column) and large (right column) hyperpolarization to DAMGO (1 μM, 1 min).

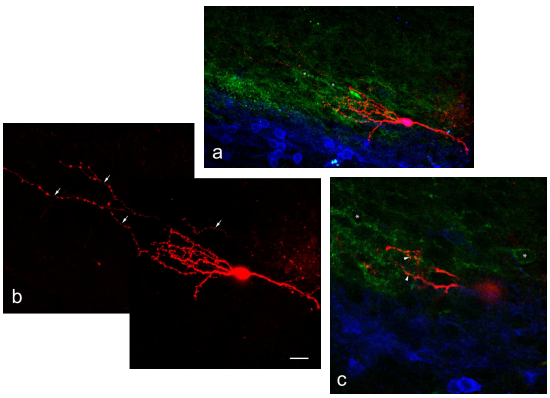
Morphology and MOR-1 receptor expression of biocytin labelled cells sensitive to DAMGO



a) Projected confocal image of a lamina II neuron (islet cell) responded to DAMGO with large hyperpolarization. **b), c), d)** Single optical sections of the same cell. (*red:* biocytin, *green:* MOR-1 receptor, arrows: axon of the patched and biocytin filled cell, *arrowheads*: MOR-1 receptors on the dendrites. *: „classical" MOR-1 immunoreactive neuron. Scalebar: 20 μm)



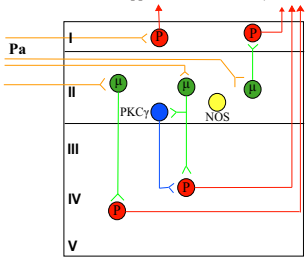
a) A small antenna like cell responded to DAMGO with intermediate hyperpolarization in its immunostained environment. **b)** Projected image of the same cell. **c)** Axon of the cell forms close appositions with PKCγ immunoreactive structures in a single confocal optical section. (*red:* cell filled with biocytin, *green:* MOR-1 receptors, *blue:* PKCγ immunopositive structures, *arrows:* axon of the neuron, *: „classical" MOR-1 cells. Scalebar: 20 μm)



a) A small islet cell responded to DAMGO with small hyperpolarization in its immunostained environment. **b)** Projected image of the same cell. **c)** Enlarged view of the dendrites with MOR-1 receptors in a single optical section. (*red:* cell filled with biocytin during the patch clamp recording, *green:* MOR-1 receptors, *blue:* PKCγ immunopositive structures, *arrows:* axon of the neuron, *arrowheads:* MOR-1 receptors, *: „classical" MOR-1 cells. Scalebar: 20 μm)

Summary

1. Patch-clamp electrophysiology combined with multiple fluorescent immunocytochemistry is a suitable method for morphological and receptor-chemical examination of cells identified physiologicaly.
2. 60% of lamina II neurons responded to DAMGO as it was reported in previous studies.
3. Monosynaptic EPSPs evoked by dorsal root stimulation indicate, that primary afferents may have direct input to DAMGO responding cells.
4. Cells hyperpolarized by DAMGO belonged to different morphological types and only two of them could be characterized as „classical" MOR-1 cells.
5. There was no correlation between the amplitude of hyperpolarization and the morphological appearance of the labelled neurons.
6. Surprisingly, the MOR-1 receptor density did not correlate with the magnitude of the hyperpolarizing effect of DAMGO.
7. Axons of biocytin filled neurons travelled in lamina II and terminated in lamina I or in deeper laminae (III--IV). Many of the labelled axons formed close appositions with PKCγ-immunoreactive structures.



P (red): projection neuron, **μ** (green): MOR-1 cell, **Pa** (orange): primary afferent fibres

Conclusion

Our data demonstrate that neurons hyperpolarized by DAMGO form more heterogenious cell population then was suggested previously, and most of them were not the classical MOR-1 type. The lack of correlation between the receptor density and the magnitude of hyperpolerization caused by DAMGO raises the possibility that other splice-variants of MOR-1 receptor, or different signaling pathways might be involved in the various responses of SG neurons to μ-agonists.