

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE MU OPIOID RECEPTORS

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Using antibodies generated to the C-terminal 63 amino acids of the cloned rat mu receptor, the present study examines the distribution of the mu receptor-like immunoreactivity (li) in perfused rat brain sections. Generally, the localization of the mu receptor-li compares well with previous receptor binding studies, with staining observed in such regions as the caudate-putamen, nucleus accumbens, hippocampus, amygdala, thalamus, superior and inferior colliculi, interpeduncular nucleus, parabrachial nucleus, locus coeruleus, raphe nuclei, nucleus tractus solitarius, and the dorsal horn of the spinal cord. This distribution is consistent with the broad number of functions associated with mu receptors, including inhibition of sensory transmission and the regulation of neuroendocrine and neurotransmitter release.

The mu receptor is one of three opioid receptor types localized in the central nervous system. Together with the other opioid receptor types, delta and kappa, these receptors play important modulatory roles in the control of neurotransmitter release, pain regulation and neuroendocrine control. The recent cloning of the mu opioid receptor (1,2) has allowed the visualization of the receptor, not only in terms of its binding sites, but also in terms of the mRNA encoding this receptor with *in situ* hybridization techniques (3,4). In addition, specific antibodies can be generated to the deduced protein sequences of the cloned receptor to directly visualize the mu receptor protein using immunohistochemical techniques. The present study examines the immunohistochemical localization of the mu opioid receptor protein in perfused rat brain sections and provides a better understanding of the cells and fibers expressing these receptors in the rat CNS.

Antibody Production. A 228 bp fragment (1175-1403) of the rat mu receptor (2) was subcloned into the pGEX-kg protein expression vector (5). This plasmid, when expressed in bacteria (JM101), produces a glutathione S-transferase (GST)-mu receptor fusion protein which can be purified from crude bacterial extracts using glutathione affinity chromatography (6). The portion of the mu receptor that is expressed with this construct corresponds to the C-terminal 63 amino acids of the rat mu receptor, a region which shows little amino acid homology to the cloned delta and kappa receptors (7,8). Antibodies were generated by inoculating rabbits (New Zealand White) with 250 µg of the mu receptor-fusion protein suspended in Freund's adjuvant using a standard injection schedule. The resulting rabbit serum was affinity purified using the C-terminal 63 amino acid mu receptor protein coupled to a sepharose-4B-cyanogen bromide column.

Immunohistochemistry. Male Sprague-Dawley rats (n = 4) were perfused transcardially with 0.9% saline followed by Zamboni's fixative. Brains were removed from the calvaria, postfixed in Zamboni's fixative (24 h), sectioned on a Jung microtome (30 µ) and immunohistochemically stained using standard methods (9). Floating sections were washed in 50 mM KPBS, incubated with 0.3% H₂O₂ (30 min), rinsed in 50 mM KPBS and incubated for 48 h with the mu receptor antibody (1:1000, diluted in 50 mM KPBS, 0.4% Triton, 1% BSA, 1% normal goat serum) at 4°C. After washing with 50 mM KPBS, sections were incubated with biotinylated goat anti-rabbit (1:200, 1 h, 22°C), followed by an avidin-biotin complex coupled to HRP (1:200, 1 h, 22°C, Vector Elite). The HRP reaction product was visualized by DAB with nickel chloride enhancement. Immunohistochemical controls included the co-incubation of the mu antibody with an excess of the mu receptor-fusion protein (4 µM).

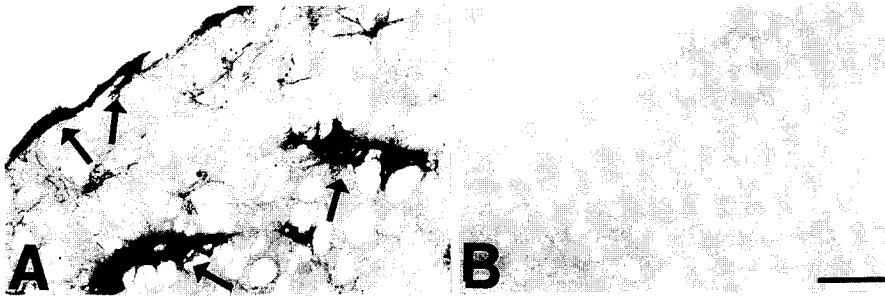


Figure 1. Mu receptor immunohistochemical staining in the caudate-putamen (A). Mu receptor staining is most dense in the striatal patches and subcallosal streak that are indicated by arrows. In addition, scattered cells and fibers are seen in the striatal matrix. Size bar = 200 μ m.

The distribution of the mu receptor immunoreactivity is consistent with previous receptor binding and *in situ* hybridization studies (3). Immunohistochemical staining was observed in regions including the caudate-putamen, nucleus accumbens, olfactory tubercle, lateral septum, medial preoptic area, bed nucleus of the stria terminalis, amygdala (intercalated nuclei, posteriocortical medial nucleus), hippocampus, thalamus (habenula, paraventricular nucleus, medial geniculate body), superior and inferior colliculi, interpeduncular nucleus, parabrachial nucleus, locus coeruleus, raphe nuclei (median, raphe magnus), pontine reticular nuclei, nucleus tractus solitarius, and in the superficial layers of the spinal cord. In the caudate-putamen, mu immunohistochemical staining is most dense in the striatal patches and subcallosal streak, with fewer fibers seen in the matrix compartment (Fig. 1). One region demonstrating dense mu immunohistochemical staining and little or no mu receptor binding is the external layer of the median eminence, where mu receptors may influence the release of anterior lobe hormones.

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