IMMUNOHISTOCHEMICAL LOCALIZATION OF THE KAPPA$_1$ OPIOID RECEPTORS

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Using antibodies generated to the C-terminal 41 amino acids of the cloned rat kappa receptor, the present study examines the distribution of the kappa$_1$ receptor-like immunoreactivity (li) using immunohistochemical techniques. The localization of the kappa$_1$ receptor staining generally corresponds well to previous receptor binding and *in situ* hybridization studies, with stained fibers and/or cells observed in such regions as the endopiriform nucleus, claustrum, nucleus accumbens, olfactory tubercle, bed nucleus stria terminalis, medial preoptic area, paraventricular, supraoptic and lateral hypothalamus, median eminence, thalamus, medial amygdala, parabrachial nucleus, nucleus of the solitary tract, and the superficial layers of the spinal cord. This localization is consistent with the wide range of functions associated with kappa$_1$ receptors, including analgesia and hormonal regulation.

The kappa opioid receptor, one of three receptor types found in the central nervous system, has been implicated in a number of behaviors and functions, including analgesia, electrolyte balance, hormonal regulation, and the modulation of reward pathways. The recent cloning of the kappa$_1$ receptor (1,2) suggests it is a member of the seven transmembrane family of receptors that is negatively linked to adenylate cyclase. *In situ* hybridization studies (3) suggest an excellent correspondence between the kappa$_1$ mRNA and receptor binding, with differences in localization due in part to receptor transport. To further examine the anatomical distribution of the kappa$_1$ receptor in the rat, selective antibodies were raised and immunohistochemical studies were performed to localize the kappa$_1$ receptor protein.

Antibody Production. A 242bp fragment (1098-1340) of the rat kappa$_1$ receptor (1) was subcloned into the pGEX-kg protein expression vector (4). This plasmid, when expressed in bacteria (JM101), produces a glutathione S-transferase (GST)-kappa$_1$ receptor fusion protein which can be purified from crude bacterial extracts using glutathione affinity chromatography (5). The portion of the kappa$_1$ receptor that is expressed with this construct corresponds to the terminal 41 amino acids of the rat kappa$_1$ receptor, a region which shows little amino acid homology to the cloned mu and delta receptors (6-9). Antibodies were generated by inoculating rabbits (New Zealand White) with 250 μg of the kappa$_1$ receptor-fusion protein suspended in Freund's adjuvant using a standard injection schedule. The resulting rabbit serum was affinity purified using the terminal 41 amino acid kappa$_1$ 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 skull, postfixed in Zamboni's fixative (24 h), sectioned on a Jung microtome (30 μ) and immunohistochemically stained using standard methods (10). Floating sections were washed in 50 mM KPBS, incubated with 0.3% H_2O_2 (30 min), rinsed in 50 mM KPBS and incubated for 96 h with the kappa$_1$ 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 kappa$_1$ antibody with an excess of kappa$_1$ receptor-fusion protein (1.2 μM).

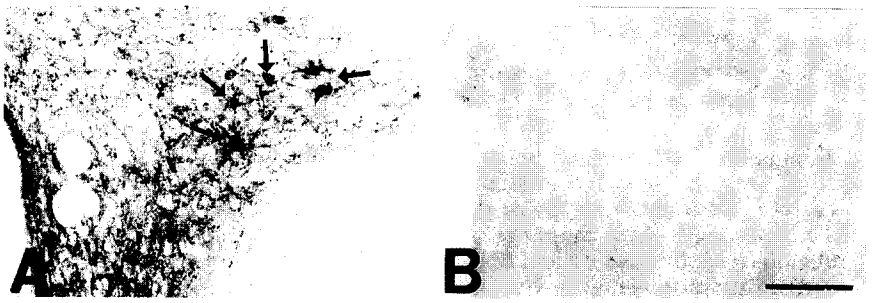

Figure 1. Kappa$_1$ receptor immunohistochemical staining in the paraventricular nucleus (PVN) of the hypothalamus (A). Kappa$_1$ receptor staining is observed in fibers and terminals of the PVN, as well as in the magnocellular cells indicated by arrows. Panel B shows a hypothalamic section in which the kappa$_1$ antibody was coincubated with 1.2 μM of the fusion protein. This control demonstrates that the immunohistochemical staining is specific and can be blocked by the kappa$_1$ receptor fusion protein. Size bar = 100 μm.

The distribution of the kappa$_1$ receptor immunoreactivity is consistent with previous receptor binding and *in situ* hybridization studies (3). Immunohistochemical staining was observed in regions including the endopiriform nucleus, claustrum, nucleus accumbens, olfactory tubercle, bed nucleus stria terminalis, medial preoptic area, paraventricular, supraoptic and lateral hypothalamus, paraventricular and central nucleus of the thalamus, medial amygdala, superior and inferior colliculi, parabrachial nucleus, the nucleus of the solitary tract and the superficial layers of the spinal cord. In the nucleus accumbens, differences in staining were seen in the accumbens core vs. shell regions, with more intense kappa$_1$ receptor staining in the lateral and septal pole regions of the nucleus accumbens shell. In the paraventricular nucleus of the hypothalamus (Fig. 1), kappa$_1$ staining is observed in scattered fibers and in the magnocellular neurons. These neurons project directly to the neural lobe via the internal layer of the median eminence, which also demonstrates dense kappa$_1$ receptor immunohistochemical staining. The localization of kappa$_1$ receptor staining in the paraventricular and supraoptic nuclei as well as the internal layer of the median eminence, provides an anatomical basis for the inhibitory effects of kappa agonists on vasopressin and oxytocin release.

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