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AUTORADIOGRAPHIC LOCALIZATION OF δ OPIOID RECEPTORS IN THE RAT BRAIN USING A HIGHLY SELECTIVE BIS-PENICILLAMINE CYCLIC ENKEPHALIN ANALOG

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A pioneering approach to the design of a ligand selective for the δ opioid receptors was implemented by Mosberg et al. (1983) who demonstrated that the cyclic conformationally restricted enkephalin analog (2-D-penicillamine,5-D-penicillamine)enkephalin (DPDPE) displayed extremely high specificity toward the δ opioid receptors. Recently Akiyama et al. (1985) provided the first in vitro characterization of tritium labeled DPDPE binding to δ opioid receptors in the rat brain and neuroblastoma-glioma hybrid (NG 108-15) cells.

In the present study we examined the light microscopic autoradiographic distribution of δ opioid receptors in the rat central nervous system employing the highly selective δ agonist [^{3}H]DPDPE (S.A. = 40 Ci/mmol, Amersham Corp, Arlington Heights, IL.).

Male Sprague-Dawley rats (150-200 g) were killed by decapitation, and the brain was removed and coated with plastic embedding medium (OCT Compound, Lab-Tek Products) onto microtome chucks and frozen by immersion into liquid nitrogen. Saggital and coronal sections (20 μm) were cut, thawed and mounted onto chrome-alum/gelatin coated glass slides and air dried at room temperature. Slide-mounted sections were preincubated for 15 min at 25°C in 50 mM Tris-HCl buffer (pH 7.4 at 25°C) containing 5 mM $MgCl_2$, 2 mg/ml BSA, 20 μg/ml bacitracin and 100 mM NaCl, to reduce any endogenous opioids that might be present. After two consecutive 5 min rinses (to eliminate NaCl) in Tris-HCl buffer (pH 7.4 at 25°C) containing 5 mM $MgCl_2$, 2 mg/ml BSA and 20 μg/ml bacitracin, the slide-mounted sections were incubated for 1 h at 25°C in the same buffer (pH 7.4 at 25°C) containing 5 nM [^{3}H]DPDPE in the absence or presence of 1 μM [Met^5]enkephalin. Adjacent sections were used for the determination of nonspecific binding. After the incubation was terminated, the slide-mounted sections were given three rinses of 10 min each in 50 mM Tris-HCl buffer (pH 7.4 at 4°C) containing 5 mM $MgCl_2$ and 2 mg/ml BSA at 0-4°C. This rinse time resulted in the maximal specific binding (about 83%). The sections were then dried for 15 min by a stream of cold air and stored overnight in a dessicated box at 4°C. Autoradiographs of the slide-mounted sections were prepared by opposing the sections to tritium sensitive film (LKB Ultrofilm, LKB Products) for 4-7 weeks at 4°C. After exposure the film was developed in Kodak D-19 at 18°C for 7 min.

The regional distribution of [^{3}H]DPDPE binding sites in a saggital section of rat brain is shown in fig. 1. Areas containing the highest densities of receptors are the superficial (II, III) and deep (VI) layers of the cerebral cortex, the caudate-putamen and forebrain structures such as the external plexiform layer of the olfactory bulb, the olfactory tubercle and the nucleus of the lateral olfactory tract. Moderate receptor density was characteristic of the hippocampal formation, where grains were seen throughout the stratum oriens from CA1 to CA4 region. Generally low density of receptors

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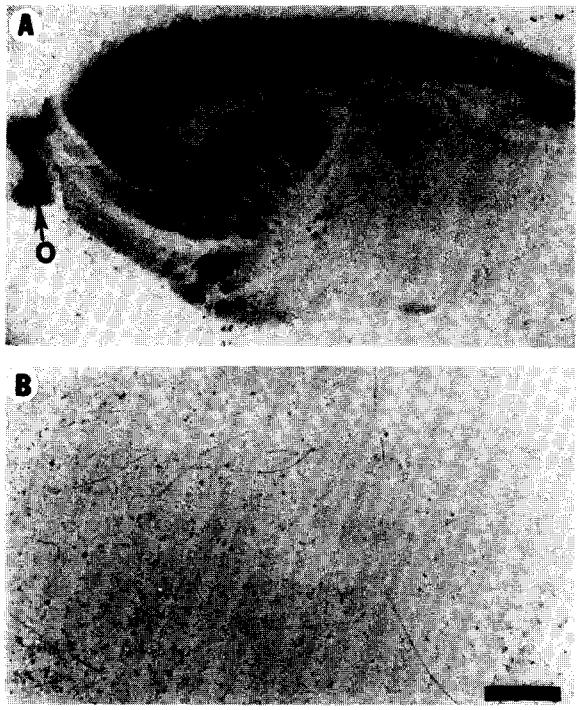

Fig. 1. [^{3}H](2-D-penicillamine,5-D-penicillamine)enkephalin ([^{3}H]DPDPE) binding sites in a saggital section of the rat brain. (A) Autoradiograph showing [^{3}H]DPDPE binding sites in the olfactory bulb (O), cerebral cortex (ctx), caudate-putamen (cp), nucleus accumbens (a), hippocampus (h) and thalamus (T). (B) Non-specific binding of [^{3}H]DPDPE in the presence of 1 μM of [Met^5]enkephalin in a rat brain slice adjacent to that seen in A. Bar = 500 μm.

was found over the thalamus and septal nuclei. Low specific binding was seen in the cerebellum and the medulla oblongata. There was no demonstrable specific [^{3}H]DPDPE binding over the white matter areas such as corpus callosum or the white matter area of the cerebellum.

In summary this study shows for the first time the unique regional distribution of specific δ opioid receptors in the rat brain using the highly selective opioid agonist [^{3}H]DPDPE.

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References

Akiyama, K., K.W. Gee, H.I. Mosberg, V.J. Hruby and H.I. Yamamura, 1985, Characterization of [^{3}H](2-D-penicillamine,5-D-penicillamine)enkephalin ([^{3}H]DPDPE) binding to delta opiate receptors in the rat brain and neuroblastoma-glioma hybrid (NG 108-15) cells, Proc. Natl. Acad. Sci. U.S.A. (in press).

Mosberg, H.I., R. Hurst, V.J. Hruby, K. Gee, H.I. Yamamura, J. Galligan and T. Burks, 1983, Bis-penicillamine enkephalins possess highly improved specificity toward delta opioid receptors, Proc. Natl. Acad. Sci. U.S.A. 80, 5871.