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Rapid communication

Autoradiographic localization of binding sites for ^{125}I-DOI, a new psychotomimetic radioligand, in the rat brain

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The selective, high-affinity binding of various psychotomimetic phenylisopropylamine derivatives to $5HT_2$ receptors has been previously reported. Optimal stereospecific selectivity was detected for 4-bromo- or 4-iodo-R-(−)-2,5-dimethoxy phenylisopropylamines. Both of these derivatives are potent hallucinogenic agents in man (Glennon et al., 1986). Using membrane binding techniques, these workers have characterized the $5HT_2$ agonist properties of [^{3}H](+)-DOB, which selectively labels a guanylate-sensitive high-affinity state of the receptor (Titeler et al., 1985). The low specific activity of [^{3}H]DOB renders it impractical for use in autoradiographic receptor localization. The iodine-containing analog DOI (4-iodo-2,5-dimethoxyphenylisopropylamine), however, possesses a stereospecific $5HT_2$ selectivity comparable to DOB (Glennon et al., 1986), suggesting that ^{125}I-labelled DOI could be used for autoradiographic localization of $5HT_2$ receptors. Although ^{131}I- or ^{123}I-labelled analogs of DOI have been used in vivo for brain imaging and metabolic studies (Sargent et al., 1984), the utilization of ^{125}I-DOI for in vitro autoradiography has not been previously reported. We have initiated investigations of ^{125}I-DOI as a ligand for autoradiography and report here our preliminary results.

The R-(−) enantiomer of ^{125}I-DOI was synthesized to a radiochemical purity of 99 + %. The specific activity determined by HPLC quantitation was 1700 Ci/mmol. Details of the radioiodination procedure and the synthesis of the precursors will be given elsewhere (A. Hoffman et al., in preparation). Saturation curves and Scatchard plots for the R-(−)-^{125}I-DOI were determined in rat cortical membranes. Parietal-frontal cortices obtained from male Sprague-Dawley rats were homogenized in ice-cold 0.32 M sucrose. Following centrifugation at $900 \times g$ for 10 min, the supernatant was collected and centrifuged at $35\,000 \times g$ for 20 min, and the pellet resuspended in 50 mM Tris buffer, pH 7.4, containing 0.1% ascorbate, 0.1% BSA, 4 mM $CaCl_2$. Protein content of the membrane suspension was determined with the Biorad assay. Incubations were conducted in triplicate at 37 °C for 30 min in 0.5 ml final volume containing 0.2 mg protein and 12 concentrations of R-(−)-^{125}I-DOI (0.1-7 nM). Nonspecific binding was defined with 1 μM R-(−)-DOI.

^{125}I-R-(−)-DOI showed saturable, specific binding to the membrane preparations. The compound had K_d 1.41 ± 0.19 nM, $B_{max} = 112 \pm 15$ fmol/mg protein, calculated by linear regression analysis (mean of three independent determinations). Analysis with the LIGAND program indicated that the data agreed with a one-site model. Autoradiographic localization showed the highest density of specific binding in the cortex (layer IV), claustrum and lateral olfactory tracts; lower densi-

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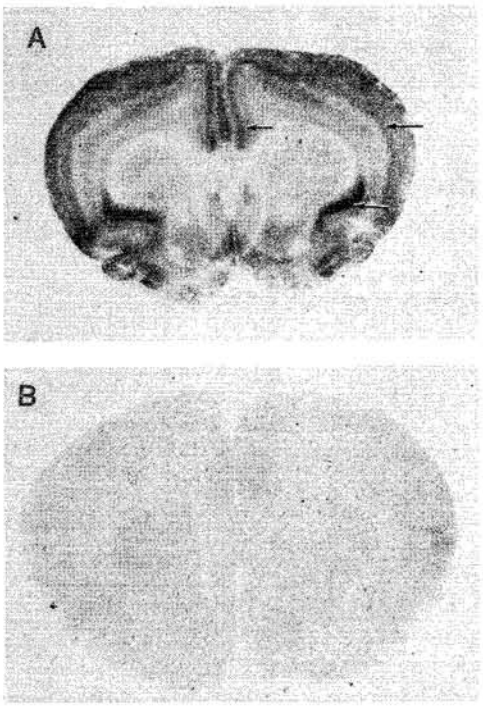

Fig. 1. (A) Specific binding of ^{125}I-R-(−)-DOI in rat forebrain sections. Arrows indicate the claustrum, parietal cortex, and cingulate cortex, the most densely labelled regions. (B) Non-specific binding. For autoradiography, 16 micron brain sections, obtained from 250 g male Sprague-Dawley rats, were thaw-mounted on gelatin-coated glass slides and preincubated 30 min at room temperature in 50 mM Tris buffer, pH 7.4 containing 4 mM $CaCl_2$, 0.1% ascorbate and 0.1% percent BSA. Sections were incubated 60 min (room temperature) in the same buffer containing 200 pM radioligand, washed in ice-cold buffer (3×10 min), rinsed in ice-cold distilled water (1 min), and dried under a cold air stream. Non-specific binding was defined with 1 μM R-(−)-DOI. Sections were then apposed to ^{3}H-sensitive Ultrofilm (LKB Industries, Rockville, MD) in X-ray cassettes at room temperature for 3 days and developed at 0°C for 4 min with undiluted Kodak D-19 developer.

ties were detected in the nucleus acumbens and the nucleus of the vertical limb of the diagonal band (fig. 1). Specific binding was also detected in the choroid plexus, locus coeruleus and medial vestibular nuclei (not shown). The R-(−)-DOI was completely displaced from all sites by 1 μM R-(−)- or S-(+)-DOI (only displacement by R-(−)-DOI is shown in fig. 1). In contrast to ^{125}I-LSD (Nakada et al., 1984), the labelled DOI showed virtually no specific binding in the caudate-putamen, indicating a lack of affinity for dopamine receptors. The areas showing high specific binding contain high densities of $5HT_2$ receptors as shown by previous studies (Nakada et al., 1984). ^{125}I-DOI thus appears to be more selective than ^{125}I-LSD for the localization of $5HT_2$ receptors. The claustrum has extensive afferent and efferent connections to sensory cortical regions and is involved in the processing of somatosensory, visual and auditory information (Sloniewski et al., 1986). The high-density binding of ^{125}I-DOI in the claustrum reported here, and that previously reported for LSD (Nakada et al., 1984), suggests that the actions of these structurally different hallucinogens may involve common receptors located in the claustrum.

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