We previously reported the autoradiographic localization of specific, high-affinity binding sites for R-(−)-4-[125I]-2,5-dimethoxyphenylisopropylamine (125I-DOI), a new psychotomimetic radioligand, in the rat brain (McKenna et al., 1987). DOI and its 4-brominated and 4-alkylated congeners (DOB, DOM and DOET) are potent halucinogenic agents in humans. [3H]DOB has recently been characterized as an agonist that selectively labels a guanylate-sensitive high-affinity state of the 5HT2 receptor (Lyon et al., 1987). Our previous results support these findings by consistently showing the highest binding densities in regions that a previous autoradiographic study, utilizing radio-iodine-labeled LSD (125I-LSD), characterized as containing high densities of 5HT2 receptors (Nakada et al., 1984).

In the present study, we report that two psychotomimetic ligands, LSD and the halogenated phenylalkylamine DOB, mutually and specifically cross-displace 125I-LSD and 125I-DOI in specific brain regions.

Incubation of rat forebrain sections in 200 pM 125I-LSD (fig. 1A) gave high binding values in several brain regions. The binding values (expressed in units of fmol/mg protein ± S.E.) in each region were as follows: nucleus acumbens (40.3 ± 1.7), caudate nuclei (35.7 ± 0.8), claustrum (33.8 ± 1.1), olfactory tubercle (31.9 ± 1.0) and frontal cortex (31.0 ± 0.8).

125I-(−)DOI (200 pM) bound to the same regions labeled by 125I-LSD in adjacent brain sections (fig. 1D) but yielded somewhat lower total binding values. The highest density of 125I-(−)DOI binding was in the claustrum (33.4 ± 1.6 fmol/mg protein), followed by the frontal cortex (22.9 ± 1.6 fmol/mg), nucleus acumbens (18.1 ± 1.0 fmol/mg) and olfactory tubercle (15.6 ± 2.6 fmol/mg). The lowest binding density was found in the caudate nuclei (10.1 ± 0.4 fmol/mg).

125I-LSD showed the highest binding density in the caudate nuclei and nucleus acumbens; however, 125I-(−)DOI had a much lower binding density in these same regions. The highest density of bound 125I-(−)DOI was localized in the claustrum. In the claustrum, the binding density of both iodinated ligands at the same concentration was nearly equivalent.

Addition of 500 nM unlabeled LSD displaced more than 90% of the 125I-LSD binding in all regions measured (fig. 1C). This concentration of unlabeled LSD also displaced 97-99% of the 125I-(−)DOI binding in the cortex, claustrum, nucleus acumbens and olfactory tubercle; and 78% in the caudate nuclei (fig. 1F).

In addition, we found that 500 nM unlabeled (−)DOB displaced both 125I-(−)DOI and 125I-LSD in specific regions. (−)DOB displaced 90%...
Fig. 1. (A-E) Cross-displacement of \( {^{125}}I \)-LSD and \( {^{125}}I \)-DOI binding in rat brain by (-)DOB and LSD. The upper panel (A-C) shows (A) total binding of \( {^{125}}I \)-LSD; (B) displacement of \( {^{125}}I \)-LSD by 500 nM (-)DOB; and (C) displacement of \( {^{125}}I \)-LSD by 500 nM LSD. The lower panel (D-F) shows (D) total binding of \( {^{125}}I \)-DOI; (E) displacement of \( {^{125}}I \)-DOI by 500 nM (-)DOB; and (F) displacement of \( {^{125}}I \)-DOI by 500 nM LSD. The arrows in (A) and (B) indicate the cortex (cx), claustrum (cl) and caudate nucleus (ca). Details of the autoradiographic methods have been previously described (McKenna et al., 1987). Briefly, consecutive 16 \( \mu \)m sections of rat forebrain were thaw-mounted on gelatin-coated glass slides and incubated in 50 mM Tris buffer, pH 7.4, containing either 200 pM \( {^{125}}I \)-LSD (specific activity: \( \sim 2000 \) Ci/mmol) or 200 pM \( {^{125}}I \)-DOI (specific activity: \( \sim 1500 \) Ci/mmol). For displacement treatments, adjacent sections were incubated in the same concentration of each radioligand together with 500 nM concentrations of unlabeled LSD or (-)DOB (see text). Following incubation, sections were apposed to \( ^3 \)H-sensitive Ultrofilm (LKB Instrument Co., Rockville, MD) for 5 days and then developed. Autoradiograms were quantified against brain-paste standards containing known amounts of \( {^{125}}I \) using computerized densitometry, as described previously (Israel et al., 1985). In this method, molar units of bound ligand per mg protein in the tissue samples are determined by interpolation from the linear standard curve determined by densitometry (ln optical density vs. ln d.p.m./mg protein of standards). Quantitation was based on the mean (\( \pm \)S.E.) of individual sections from 3 separate animals (\( n = 3 \)) for all experiments.

of total \( {^{125}}I \)-DOI binding in the claustrum and cortex, while displacing 81\% of total binding in the nucleus acumbens and 47\% in the caudate nuclei (fig. 1E). (-)DOB displaced over 80\% of the total \( {^{125}}I \)-LSD binding in the claustrum and cortex, but only 48\% in the olfactory tubercles, 44\% in the nucleus acumbens and 26\% in the caudate nuclei (fig. 1B).

Unlabeled (-)DOB, the 4-bromo analog of DOI, displaced \( {^{125}}I \)-LSD most specifically from the claustrum and cortex, but displaced the radioligand to a lesser degree from the caudate nuclei, nucleus acumbens and olfactory tubercles. This observation is consistent with our measurement of the highest binding densities for \( {^{125}}I \)(-)DOI in the claustrum and cortex, with lesser amounts localized in the caudate nuclei, nucleus acumbens and olfactory tubercles. Unlabeled LSD, however, was much less selective in displacing both radioligands from specific brain regions; LSD displaced 80-99\% of the total binding of both \( {^{125}}I \)-LSD and \( {^{125}}I \)(-)DOI in all of the regions measured. These experiments were conducted at a single, non-saturating concentration, and hence do not resolve the issue of whether the observed differences in binding densities for these radioligands result from different receptor affinities, differences in the total number of receptors labeled by each ligand, or both.

Theoretical models for the hallucinogen receptor have been proposed based on structural analogies between LSD and phenylalkylamine hallucinogens (Nichols et al., 1978); however, to our knowledge, the present work represents the first utilization of autoradiography to demonstrate that a psychotomimetic phenylisopropylamine selectively displaces an anatomically discrete subset of the receptors occupied by the ergoline psychotomimetic, LSD. We report here that the radioligands \( {^{125}}I \)-LSD and \( {^{125}}I \)(-)DOI show high-den-
sity binding in the claustrum and cortex, and that the unlabeled hallucinogens LSD and (−)DOB preferentially displace the binding of both ligands in these regions. These findings support the speculation that the psychotomimetic actions of ergoline and phenylalkylamine hallucinogens may be mediated at common receptors located in the claustrum and cortex.

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References


