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N_1-METHYL-2-[^{125}I]LSD ([^{125}I]MIL), A PREFERRED LIGAND FOR SEROTONIN 5-HT_2 RECEPTORS

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The most widely used ligands for studying serotonin 5-HT_2 receptors are [^{3}H]spiroperidol, [^{3}H]ketanserin and [^{125}I]LSD. Each of these ligands, however, exhibits some undesirable properties. The affinity of [^{3}H]spiroperidol for serotonin 5-HT_2 receptor sites is 10 fold weaker than its affinity for dopamine D-2 receptors. [^{3}H]Ketanserin has an excellent selectivity for 5-HT_2 compared to D-2 receptor sites but it exhibits only a 5 fold weaker affinity for histamine H_1 and α_1-adrenergic receptors (Leysen et al., 1982). [^{125}I]LSD has much greater sensitivity than tritiated probes however, previous work in our laboratory has indicated that its affinity for 5-HT_2 sites is only 8 fold stronger than its affinity for D-2 sites (Hartig et al., 1985). Thus, improved selectivity in combination with the high specific activity of an ^{125}I-ligand would greatly facilitate the study of 5-HT_2 receptors. Structure-activity studies have indicated that methylation of LSD derivatives at the N-1 position enhances serotonin antagonism in isolated rat uterus preparations (Gupta et al., 1983). Halogenation at the 2 position combined with methylation at the N-1 position enhances serotonin antagonism even further (Gupta et al., 1983). Consequently, we have synthesized N_1-methyl-2-I-LSD (MIL) in both radiolabeled and nonradioactive forms and now report its receptor binding properties. We have found that [^{125}I]MIL has the highest affinity yet reported for serotonin 5-HT_2 receptors, along with the best overall selectivity for these receptor sites.

[^{125}I]MIL was synthesized from 2-[^{125}I]LSD (2000 Ci/mmol) using methyl tosylate as the alkylating agent. [^{125}I]MIL was purified to greater than 98% chemical purity by HPLC. It is stable in ethanol for at least 3 months when stored at −20°C. We included 0.01% BSA in all assay buffers to minimize adsorption of the radioligand to container walls.

The binding of [^{125}I]MIL to rat frontal cortex membranes was saturable and reversible. Scatchard plots of the binding were linear with $K_d = 0.26 \pm 0.01$ nM (mean ± S.E.M.) and $B_{max} = 10.8 \pm 0.3$ fmol/mg tissue. Using 1 micromolar ketanserin to define nonspecific binding, the specific binding of 0.2 nM [^{125}I]MIL represented 75-90% of the total binding when tissue concentrations of 2-10 mg tissue per ml were employed. Inhibition of [^{125}I]MIL binding by various ligands (table 1) closely resembled inhibition of [^{125}I]LSD binding (Kadan et al., 1984) to serotonin 5-HT_2 receptors (Spearman rank correlation $P < 0.001$). [^{125}I]MIL binding was potently inhibited by the serotonergic antagonists ketanserin, mianserin and spiroperidol, whereas the dopaminergic antagonist haloperidol was much weaker. The most potent agonist was serotonin and stereospecific inhibition of binding was observed when the (+)- and (−)-isomers of LSD were tested.

We measured the affinity of MIL for a variety of receptor sites in the brain (table 1). The strongest secondary binding site for MIL was the dopamine D-2 receptor. The apparent affinity of MIL for D-2 receptors was 34 fold weaker than

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TABLE 1

Binding characteristics of MIL and [^{125}I]MIL. IC_{50} data for [^{125}I]MIL were obtained in 0.01% BSA, 50 mM Tris-HCl, pH 7.6 using 0.2 nM [^{125}I]MIL and 2 mg (wet weight) rat frontal cortex membranes per ml. For agonist assays, the buffer was supplemented with 1 mM Na-ascorbate and 10 μM pargyline. Rat frontal cortex membranes were prepared as described in Kadan et al. (1984). Nonspecific binding was defined by 1 μM ketanserin. For determinations of apparent K_i values, the following ligands and tissues were used according to published methods: 5-HT_1: [^{3}H]serotonin, rat frontal cortex; D-2: [^{3}H]spiroperidol, bovine caudate; α_1: [^{3}H]prazosin, rat whole cortex; α_2: [^{3}H]RX 781094, rat whole cortex; β: [^{125}I]cyanopindolol, rat whole cortex. Experimental details for K_i determinations will be provided in a separate communication. Data are presented as mean values ± S.E.M. from three to six experimental determinations.

Displacer	IC_{50} value (nM)
Spiroperidol	2.1 ± 0.2
(+)-LSD	2.4 ± 0.5
Ketanserin	2.9 ± 0.3
Mianserin	7.1 ± 0.8
Haloperidol	117 ± 22
Phentolamine	1170 ± 230
Propranolol	3920 ± 350
(−)-LSD	> 10000
Serotonin	1250 ± 217
Dopamine	402000 ± 66000
Epinephrine	1600000 ± 312000
	Apparent K_i (nM)
Serotonin 5-HT_1	48 ± 5
Dopamine D-2	8.9 ± 0.6
Adrenergic α_1	16 ± 0.6
Adrenergic α_2	74 ± 9
Adrenergic β	623 ± 18

the measured affinity of [^{125}I]MIL for serotonin 5-HT_2 sites. The apparent affinity of MIL for α_1-adrenergic receptors was stronger than the apparent affinity of I-LSD for these sites (Engel et al., 1984). Nevertheless, the affinity of MIL for serotonin 5-HT_2 sites ($1/K_d$) was 62-fold stronger than its interaction with α_1-adrenergic sites ($1/$apparent K_i). MIL exhibited a relatively weak affinity for serotonin 5-HT_1, α_2-adrenergic and β-adrenergic receptor sites.

In agreement with previous structure-activity studies on peripheral serotonin receptors (Gupta et al., 1983), our results indicate that methylation at the N-1 position increases the affinity of 2-I-LSD for central serotonin 5-HT_2 receptors but apparently does not affect is affinity for dopamine D-2 receptor sites. Preliminary in vivo binding studies in mice suggest that the increased affinity and lipophilicity of [^{125}I]MIL also make it a superior ligand for in vivo studies of serotonin 5-HT_2 receptor sites. These data indicate that [^{125}I]MIL is a preferred ligand for serotonin 5-HT_2 receptors, displaying the highest affinity of any serotonin 5-HT_2 ligand, the best overall selectivity and an excellent ratio of specific to nonspecific binding.

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