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Rapid communication

^{125}I-LSD: A HIGH SENSITIVITY LIGAND FOR SEROTONIN RECEPTORS

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^{125}I-labeled receptor ligands offer unique advantages over their ^{3}H-labeled counterparts. Carrier-free ^{125}I-labeled ligands can be synthesized with specific activities of up to 2170 Ci/mmol while (mono) tritium labeled ligands are limited to 29 Ci/mmol. Therefore, ^{125}I-labeled ligands can be approximately 70-fold more sensitive than ^{3}H-labeled ligands in detecting receptor sites. In addition, ^{125}I-labeled ligands emit relatively energetic X-rays and γ-rays which are readily detected by gamma counting equipment. We report here the serotonergic binding properties of ^{125}I-LSD the first reported ^{125}I-labeled ligand for serotonin receptors.

For all experiments, frontal cortex membranes were prepared by a slight modification of the method of Peroutka and Snyder (1979). All receptor binding assays were performed with 10 mg wet weight of tissue per ml. Nonspecific binding was defined by 1 μM (+)-butaclamol for [^{3}H]spiroperidol assays and 1 μM (+)-LSD for all other radioligands. I-LSD and ^{125}I-LSD were purified by thin-layer chromatography. The purity of I-LSD was greater than 95% as assayed by high performance liquid chromatography.

^{125}I-LSD (labeled at the 2 position) was first synthesized in a nonradioactive form by Troxler and Hofmann (1957). This ligand displays potent serotonin antagonist actions in peripheral (uterine) muscle tissue (Cerletti and Doepfner, 1958) but no reports of its CNS pharmacology or receptor binding properties have appeared. We synthesized I-LSD by the method of Troxler and Hofmann (1957) and characterized its serotonin receptor binding properties in rat frontal cortex membranes.

5-HT$_2$ serotonin receptor sites labeled by [^{3}H]spiroperidol are potently inhibited by both (+)-LSD and I-LSD whereas 5-HT$_1$ receptor sites assayed by [^{3}H]serotonin binding are much more readily inhibited by (+)-LSD than by I-LSD (ta-

TABLE 1

IC_{50} values (nM) in rat frontal cortex membranes. IC_{50} data for ^{125}I-LSD was obtained in 50 mM Tris-HCl, pH 7.6 (at 23°C) using 5 nM ^{125}I-LSD except in the case of serotonin and the catecholamine agonists in which the buffer was supplemented with 1 mM Na-ascorbate and 10 μM pargyline. Radioligand concentrations and buffer conditions for the tritiated ligands were the same as Peroutka and Snyder (1979). Nonspecific binding, tissue concentrations, and the membrane preparation method are described in the text. This data is the mean ± S.E.M. from three to six determinations using five different displacer concentrations assayed in triplicate. Fresh displacer dilutions were prepared for each experiment.

Displacer	Radioligand		
	[^{3}H]Serotonin	[^{3}H]LSD	[^{3}H]Spiroperidol
LSD	7.0 ± 0.3	8.3 ± 0.8	4.2 ± 0.3
I-LSD	99 ± 7	26 ± 3	6.4 ± 0.3
Displacer	**Radioligand: ^{125}I-LSD**		
Spiroperidol	3.1 ± 0.2		
(+)-LSD	5.5 ± 0.9		
I-LSD	7.1 ± 0.4		
Mianserin	15.4 ± 0.3		
Cinanserin	19 ± 6		
Haloperidol	290 ± 40		
Domperidone	910 ± 110		
(−)-LSD	> 1000		
Serotonin	1200 ± 400		
Dopamine	220000 ± 45000		
Epinephrine	> 400000		
Norepinephrine	> 400000		

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ble 1). These results demonstrate that I-LSD displays approximately 15-fold higher affinity for 5-HT_2 than 5-HT_1 serotonin receptor sites and is a much more selective ligand than (+)-LSD. The affinity of I-LSD for [^{3}H]LSD binding is intermediate between those displayed for [^{3}H]serotonin and [^{3}H]spiroperidol binding. This behavior is expected because [^{3}H]LSD labels both 5-HT_1 and 5-HT_2 sites in frontal cortex (Peroutka and Snyder, 1979).

Encouraged by these findings, we developed a method for the synthesis of ^{125}I-lSD which will be described in a separate communication. We have synthesized ^{125}I-LSD with specific activities ranging from 23 to 360 Ci/mmol. Purified ^{125}I-LSD co-migrates with I-LSD in several different TLC systems. The binding of ^{125}I-LSD to rat frontal cortex membranes is saturable and 70–80% specific as defined by displacement with 1 μM (+)-LSD. Scatchard plots of ^{125}I-LSD binding are linear with K_D 5.6 $\pm$ 0.8 nM ($\pm$ standard deviation, n = 7) and B_{max} 37 $\pm$ 7 fmol/mg tissue. The binding is reversible and is destroyed by boiling the membranes for 5 min. ^{125}I-LSD binding is potently displaced by the 5-HT_2 ligand, spiroperidol, and by the putative serotonin antagonists cinanserin and mianserin (table 1). Serotonin is by far the most potent neurotransmitter in inhibiting ^{125}I-LSD binding. ^{125}I-LSD binding does not appear to involve α- or β-adrenergic receptors under our assay conditions, since epinephrine and norepinephrine are very weak inhibitors. Dopamine and the dopamine antagonists haloperidol and domperidone are also weak inhibitors of ^{125}I-LSD binding in this tissue. Both I-LSD and (+)-LSD display a high affinity for ^{125}I-LSD binding and this inhibition is quite stereoselective as shown by the very weak affinity displayed by (−)-LSD, the biologically inactive stereoisomer.

Our finding that ^{125}I-LSD is a selective, high affinity serotonin 5-HT_2 ligand is in agreement with binding data for the closely related ergot, 2 Br-LSD. Br-LSD displays nearly the same affinity as I-LSD for both [^{3}H]serotonin binding (K_i 100 nM) and for [^{3}H]spiroperidol binding (K_i 2.5 nM) in rat frontal cortex (Peroutka et al., 1981). In a preliminary communication, [^{3}H]Br-LSD was reported to display a K_D of 6 to 10 nM and this binding was readily inhibited by LSD but weakly inhibited by serotonin in rat brain coronal slices (Beck et al., 1981).

^{125}I-LSD binding in rat frontal cortex membranes is reversible, saturable and stereospecific. This binding is destroyed by boiling the membranes and exhibits a dissociation constant of 5.6 nM. This ligand displays an excellent ratio of specific to nonspecific binding with 70–80% of the binding displaced by 1 μM (+)-LSD. In addition, other competing serotonergic ligands display a plateau phase after displacing 70–80% of the binding of ^{125}I-LSD. ^{125}I-LSD exhibits a preference for 5-HT_2 over 5-HT_1 serotonin receptor sites and should be a very sensitive, selective ligand for these receptor sites. We are currently characterizing the binding of ^{125}I-LSD to dopaminergic sites in bovine caudate and will report those findings in a separate communication.

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