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LOCALIZATION AND CHARACTERIZATION BY QUANTITATIVE AUTORADIOGRAPHY OF [^{125}I]LSD BINDING SITES IN RAT BRAIN

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The binding of [^{125}I]LSD in rat brain was characterized and localized by quantitative autoradiography. Frozen 32 μm thick brain sections were labeled in vitro with [^{125}I]LSD and exposed for 24 h against LKB Ultrofilm to generate autoradiograms. Nonspecific binding was defined as the labelling in the presence of 1 μM D-LSD. Scatchard analysis by densitometry indicated that the binding of [^{125}I]LSD was saturable, with a K_d of 0.2 nM. In agreement with the results of Hartig and co-workers [5,6], [^{125}I]LSD showed a high affinity for 5-HT$_2$ serotonin receptors with some overlap with D-2 dopamine receptors. High concentrations of [^{125}I]LSD sites were observed in layer IV of the cerebral cortex, caudate-putamen, claustrum, olfactory tubercle, nucleus accumbens, ependyma, mammillary nucleus and inferior olive. Co-incubation of sections with sulpiride to block binding to D-2 receptors resulted in a uniform 20–30% reduction in the amount of specific [^{125}I]LSD binding, with no qualitative difference in the pattern of labeling. However, co-incubation with ketanserin to block 5-HT$_2$ receptors resulted in a pattern of binding that was similar to previous descriptions of the location of D-2 receptors, with high levels of residual binding in caudate-putamen, olfactory tubercle, nucleus accumbens and inferior olive. Our results indicate that [^{125}I]LSD is a suitable ligand for quantitative autoradiography of both 5-HT$_2$ and D-2 receptors, and that there is a strong anatomical correspondence between these receptor subtypes, perhaps implying a functional interaction.

Hartig and co-workers [5,6] have recently reported that [^{125}I]LSD binds with a high affinity to 5-HT$_2$ serotonin receptors, and with a moderate affinity to D-2 dopamine receptors. [^{125}I]ligands are very useful for quantitative autoradiography because their high specific radioactivities and energies of decay allow the production of autoradiograms in hours, as opposed to weeks or months for [^{3}H]ligands. There is also little or no differential grey–white matter absorption for ^{125}I decay, a problem with ^{3}H that complicates the measurement of radioactivity by quantitative autoradiography [1,7]. We report here the use of [^{125}I]LSD to localize 5-HT$_2$ serotonin receptors and D-2 dopamine receptors in rat brain. A striking overlap is observed in the distribution of these receptors in rat brain, perhaps indicating some sort of functional relationship.

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Our procedure was to label 32 μm thick sections of rat brain in vitro with [^{125}I]LSD, and then to apply sections against LKB Ultrofilm to generate autoradiograms. Sections 32 μm thick of rat brain were cut with a cryostat and thaw-mounted onto subbed glass slides. The sections were either used immediately or stored at $-70°C$ until use. We labeled sections for autoradiography with 0.1–2 nM [^{125}I]LSD (1600 or 2000 Ci/mmol; Amersham) in 150 μl of 50 mM Tris-HCl buffer, pH 7.4, for 1 h at 23–25°C. Co-incubation with the selective antagonists ketanserin and sulpiride [10] was used to resolve 5-HT$_2$ and D-2 sites. Non specific binding was defined as the labeling in the presence of 1 μM D-LSD. After incubation, the sections were washed 4 $\times$ 20 min in Coplin slide jars containing 30–50 ml of 50 mM Tris-HCl buffer, 4°C. They were then given a brief dip in 4°C distilled water to remove buffer salts, quickly dried on a 60°C slide-warmer, and apposed as described [15] against LKB Ultrofilm for 24 h in spring-loaded aluminum X-ray cassettes. After development and fixation as described [15], density values of autoradiograms were determined with a microcomputer-assisted densitometer that referenced a [^{125}I]brain-mash standard curve to convert density values into molar quantities of bound ligand. 8–10 readings were taken from a single brain structure and averaged for the determination of the concentration of radioactivity. Total and nonspecific binding were determined separately and subtracted for the measurement of specific binding. Densitometric saturation and inhibition data were analyzed by non-linear least-square curve-fitting procedures available on the NIH-supported PROPHET time-sharing system. For Scatchard analysis, we used the program PENNBINKIN2. For analysis of inhibition curves, we used the program FITSITES.

Preliminary experiments performed by scintillation counting established that the binding of 0.1 nM [^{125}I]LSD was at equilibrium by 1 h, and that optimal levels of specific binding (70%) were obtained with 4×20 min washes. All subsequent characterization experiments were done with densitometry. Scatchard analysis of the binding of [^{125}I]LSD to layer IV of area 2 as defined by Krieg [8] indicated that the binding was to a single population of sites, with a K_d of 0.17 ± 0.1 nM and a B_{max} of 84.8 ± 3.8 fmol/mg protein ($n = 3$). In accord with the results of Hartig and co-workers [5,6], the binding of [^{125}I]LSD to layer IV was potently inhibited by the specific 5-HT$_2$ antagonist ketanserin (IC_{50} at 0.5 nM [^{125}I]LSD $= 26 \pm 3.9$ nM, $n = 3$) and by D-LSD ($IC_{50} = 3.5 \pm 0.9$ nM) but only weak inhibition by the selective D-2 antagonist sulpiride (15 ± 1% inhibition at 1 μM). Computer-assisted analysis of ketanserin inhibition curves showed a significant improvement in fit for a two-site binding model ($P < 0.03$ for all three curves) with one site accounting for 80% of the binding with an IC_{50} of 8 nM, and the smaller site accounting for 20% of the binding having an IC_{50} of 7 μM ($B_{max_1} = 83 \pm 1$%, $IC_{50_1} = 8 \pm 1$ nM; $B_{max_2} = 17 \pm 1$%, $IC_{50_2} = 7 \pm 1$ μM, $n = 3$). Layer IV of the cerebral cortex is known to have a high concentration of 5-HT$_2$ receptors [12]. The characterization of [^{125}I]LSD binding to the caudate-putamen, a region high in D-2 dopamine receptors [2,12] gave different results showing a similar B_{max} in Scatchard analysis (84.8 ± 3.8 fmol/mg protein,

n = 3) but a higher K_d (0.30 ± 0.03 nM), and a lower affinity for ketanserin (IC_{50} = 4.4 ± 2 μM, n = 3), with no significant improvement in fit to a two-site binding model. No difference was seen in the affinity for D-LSD to compete for [^{125}I]LSD binding in the caudate-putamen, while slightly greater inhibition was seen with 1 μM sulpiride (26 ± 6%, n = 3) with no evidence that the inhibition was dose-dependent. Based on these results, the binding of [^{125}I]LSD to slide-mounted sections appears to be preferentially to 5-HT_2 receptors with some binding to D-2 dopamine receptors, in general agreement with the findings of Hartig and co-workers [5,6].

Autoradiograms of [^{125}I]LSD binding are shown in Figs. 1 and 2. We observed a heterogeneous distribution of [^{125}I]LSD binding sites with the highest levels of binding (50–60 fmol/mg protein at 0.5 nM) found in the ependymal lining of the ventricles, in the nucleus accumbens, the claustrum, and throughout layer IV of the cerebral cortex. Within layer IV, the concentration of [^{125}I]LSD sites was unevenly distributed, with higher levels in prefrontal cortex (Krieg area 10) and parietal area 2 than in areas 17–18 (Fig. 1). High but slightly lower levels of binding (40 fmol/mg protein) were found throughout the olfactory tubercle, in the caudate-putamen, the inferior olive, the remainder of the cerebral cortex, with higher binding in layer V than in the other remaining layers (Figs. 1 and 2), throughout the olfactory bulb, in the olfactory nucleus and in the lateral septum. Moderate levels of binding (20–30 fmol/mg protein) were observed in the superficial layer of the superior colliculus, throughout the preoptic area and hypothalamus, the inferior colliculus, the globus pallidus and the laterodorsal thalamic nucleus. Lower levels of binding were found in all other brain regions, including all the regions of the hippocampus, the re-

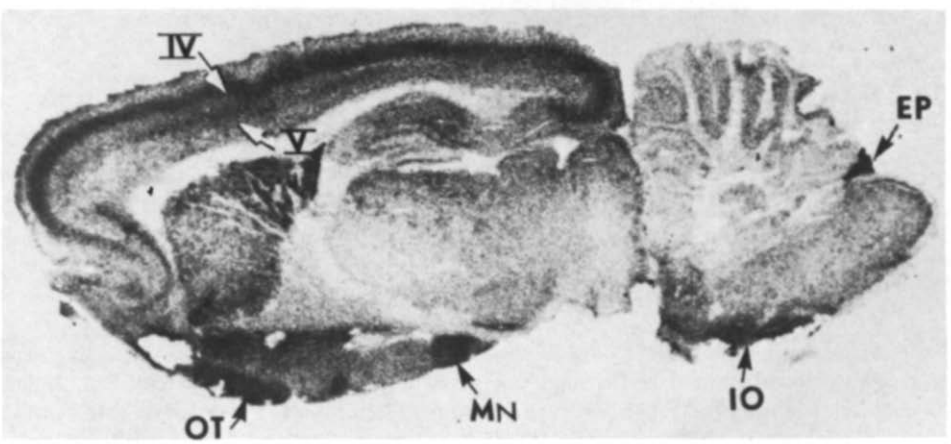

Fig. 1. LKB Ultrofilm autoradiogram of [^{125}I]LSD binding sites. A 32 μm thick sagittal brain section was labeled in vitro as described in the text with 0.5 nM [^{125}I]LSD and exposed against LKB Ultrofilm for 24 h. This section corresponds to plate 47 in the stereotaxic atlas of Paxinos and Watson [13]. EP, ependyma; IV, layer IV; V, layer V; IO, inferior olive; Mn, mammillary nucleus; OT, olfactory tubercle.

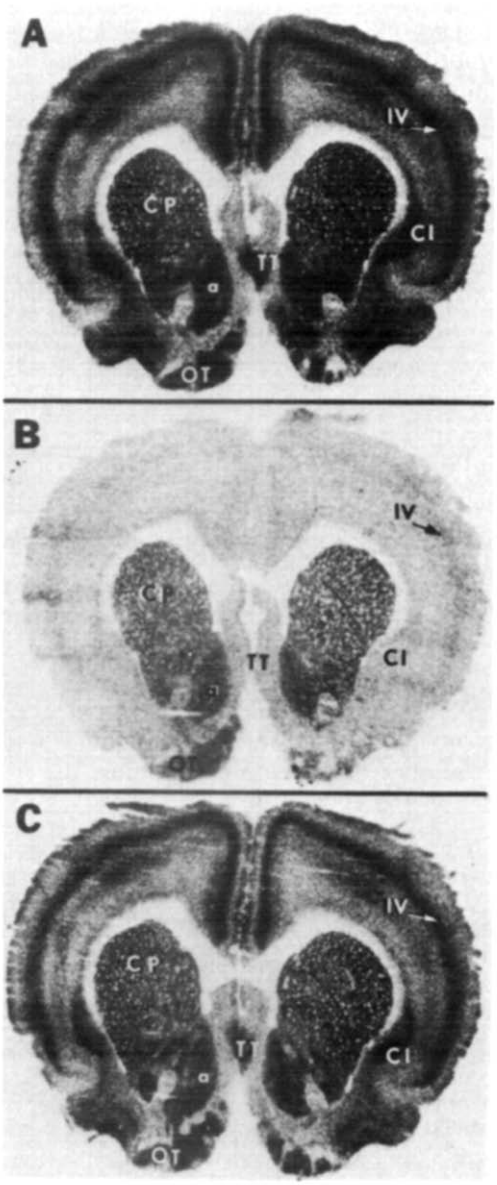

Fig. 2. Autoradiography of 5-HT_2 and D-2 receptors with [^{125}I]LSD. Consecutive 32 μm thick sections were incubated as described in the text with either 0.5 nM [^{125}I]LSD alone (A), 0.5 nM [^{125}I]LSD plus 300 nM ketanserin to block 5-HT_2 receptors (B), or 0.5 nM [^{125}I]LSD plus 1 μM sulpiride to inhibit D-2 receptor binding (C). Sections were taken at level 10 in Paxinos and Watson [13]. a, accumbens nucleus; CP, caudate-putamen; CL, claustrum; IV, layer IV; TT, taenia tecta.

mainder of the thalamus, the substantia nigra, the nuclei and cortex of the cerebellum and throughout the brainstem. Low but detectable levels of specific binding were found throughout the spinal cord and in white matter regions. Nonspecific binding was an anatomically uniform 20–30% of total binding.

We next co-incubated sections with either ketanserin or sulpiride to determine the relative contributions of 5-HT_2 and D-2 receptors to the pattern of [^{125}I]LSD binding. Co-incubation of 0.5 nM [^{125}I]LSD with 1 μM sulpiride produced a consistent 15–30% decrease in the amount of [^{125}I]LSD binding in a variety of brain regions, but did not change the relative concentration of [^{125}I]LSD binding sites among brain regions (Fig. 2). This indicated that the qualitative pattern of labeling seen in autoradiograms was largely due to 5-HT_2 receptors. By contrast, co-incubation with 300 nM ketanserin produced a marked change in the appearance of [^{125}I]LSD autoradiograms (Fig. 2), reducing binding in all brain regions, and eliminating the labeling in the ependyma, layers IV and V of the cortex, the claustrum and the mammillary nucleus. The remaining pattern of labeling was concentrated in the caudate-putamen, the nucleus accumbens, the olfactory tubercle and the inferior olive. The distribution of binding strongly resembled previous descriptions of the location of D-2 dopamine receptors in rat brain [2,12]. To verify that this residual binding represented D-2 receptors, we performed inhibition curves with sulpiride in the presence of 300 nM ketanserin. In the presence of ketanserin to block 5-HT_2 receptors, there was a strong increase in the ability of sulpiride to compete for [^{125}I]LSD binding in the caudate-putamen, with half-maximal inhibition now occurring at 22 nM ($IC_{50} = 22 \pm 5$ nM, $n = 3$). Visual inspection of nucleus accumbens and olfactory tubercle indicated that the binding to these structures was similarly reduced by sulpiride. Thus, the component of [^{125}I]LSD binding that is not blocked by ketanserin corresponds anatomically and in pharmacological selectivity to a D-2 dopamine receptor.

The autoradiographic localization of [^{125}I]LSD binding to 5-HT_2 and D-2 receptors is in general agreement with the findings of Palacios et al. [12] and Altar et al. [2] in which the binding of [^{3}H]spiroperidol was separately localized to serotonin and dopamine sites. High concentrations of presumed 5-HT_2 receptors were found in layer IV of frontal cortex and in the claustrum, while high concentrations of D-2 receptors were observed in the caudate-putamen. High levels of total [^{3}H]spiroperidol binding were observed in the olfactory tubercle and nucleus accumbens, but because of high levels of nonspecific binding in these regions, it was difficult to determine if these sites represented 5-HT_2 or D-2 receptors. The greater specificity of [^{125}I]LSD for 5-HT_2 receptors allowed us to observe that the caudate-putamen, nucleus accumbens and olfactory tubercle all contained a high concentration of 5-HT_2 receptors, in agreement with homogenate measurements of [^{3}H]ketanserin binding [9]. In addition, these same regions had high levels of [^{125}I]LSD sites with a low affinity for ketanserin and a high affinity for sulpiride, which we infer to be D-2 receptors. The inferior olive, a structure not described in previous autoradiographic studies of [^{3}H]spiroperidol binding [2,12], also showed high levels of 5-HT_2 and D-2 receptors. Thus, quantitative autoradiography with [^{125}I]LSD has revealed a strong anatomical overlap in the locations of D-2 receptors and 5-HT_2 receptors, with 5-HT_2 receptors present in high concentrations in all regions that contain D-2 receptors.

This finding suggests that D-2 and 5-HT_2 receptors might have functional interac-

tions in rat brain, though little is currently known about what functions they might mediate. Drug potencies at 5-HT_2 receptors correlate with their effects on the head-twitch response produced by serotonergic compounds [11,14], while the potencies of anti-psychotic drugs correlate with their affinities for the D-2 receptor [4]. Given that the serotonin behavioral syndrome reflects in part the hallucinogenic actions of 5-HT_2 drugs [3], it may be that both D-2 and 5-HT_2 receptors in mesolimbic regions are involved in the integration of sensory information with limbic functions. The use of [^{125}I]LSD to rapidly obtain quantitative autoradiograms of both 5-HT_2 and D-2 receptors should help to test this or any other proposed explanation for the function of these receptors in the mammalian brain.

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NOTE ADDED IN PROOF

Engel et al. (Naunyn-Schmiedeberg's Arch. Pharmacol., 325 (1984) 328–335) have also used [^{125}I]LSD for in vitro binding and autoradiography.