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2-[^{125}Iodo]LSD, a new ligand for the characterisation and localisation of 5-HT_2 receptors

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Summary. 1. LSD was iodinated with Na^{125}I and chloramine T, to get the radioligand [^{125}I]LSD (^{125}IOL) and with N-I-succinimide to obtain the nonradioactive compound 2-I-LSD (IOL) for comparative pharmacological studies.

2. The introduction of iodine in position 2 of LSD leads to an increase in selectivity for $5HT_2$ receptors. In rat cortex membranes, ^{125}IOL possesses a $K_D = 0.9 \pm 0.1$ nmol/l, $B_{max} = 240 \pm 20$ fmoles/mg, and a nonspecific binding of 30–40% in presence of 100 nmol/l ketanserin.

3. In competition experiments, 5HT antagonists showed monophasic displacement curves. Their K_I-values correlate well with pD'_2-values for inhibition of 5HT-induced contraction of canine basilar artery. It can be concluded that the sites labelled by ^{125}IOL have pharmacological properties in common with central $5HT_2$ receptors, which are identical with vascular postjunctional 5HT receptors.

4. The high specific radioactivity of ^{125}IOL permits detection of even small $5HT_2$ receptor densities which exist in the guinea pig ileum. These ^{125}IOL binding sites are pharmacologically different to those found in the brain or on the vessels and might be a special subpopulation of $5HT_2$ sites. For example, ketanserin has a high affinity to the sites labelled by ^{125}IOL in the brain and a 100 times lower affinity to the sites labelled in the ileum.

5. In a routine binding screen with various ligands, the inhibition constants of IOL for α_1, α_2, β, histamine and muscarinic receptors are > 100 nmol/l with the exception for dopamine receptors, 40 nmol/l.

6. ^{125}IOL was employed for the autoradiographic localisation of its binding sites after in vitro labelling of microtome rat brain sections. ^{125}IOL labelled $5HT_2$ sites in the cortex and dopamine receptors in the nucleus caudatus. The exposure times required were very short, compared to those of other $5HT_2$ ligands available.

Key words: [^{125}Iodo]LSD – $5HT_2$ receptors – Dopamine receptors – Canine basilar artery – 5HT-D receptors

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Abbreviations. The abbreviation IOL was chosen in analogy to 2-Br-LSD which is known under the synonym BOL-148 (Rutschmann and Stadler 1978). Part of this work has been presented at the Winter Meeting of the British Pharmacological Society London, January 1983 (Engel et al. 1983a) and at the Spring Session of the German Pharmacological Society in Mainz, March 1983 (Engel et al. 1983c).

Introduction

According to Peroutka and Snyder (1979), two distinct types of 5-hydroxytryptamine (5HT) binding sites have been postulated. $5HT_1$ binding sites are labelled by [^{3}H]5HT and $5HT_2$ binding sites can be identified by [^{3}H]spiperone. In the present study, the different 5HT recognition sites are termed receptors as recently functional correlates have been proposed for both $5HT_1$ and for the $5HT_2$ sites. Thus, the 5HT-induced contraction of peripheral blood vessels (Leysen et al. 1981; Cohen et al. 1981; Bradley et al. 1983) as well as behavioural responses (Peroutka et al. 1981; Leysen et al. 1981; Ortmann et al. 1982) are mediated by receptors which have pharmacological properties in common with $5HT_2$ sites. In addition, $5HT_2$ receptors have been demonstrated on feline and human platelets (Leysen et al. 1983; Geaney et al. 1983).

[^{3}H]5HT seems to be the only selective ligand for labelling $5HT_1$ receptors, which have been recently subdivided by Pedigo et al. (1981) into $5HT_{1A}$ and $5HT_{1B}$ subtypes. The physiological role of $5HT_1$ receptors (for review see Fozard 1983) is not yet completely elucidated. Relationships between the $5HT_1$ receptors and the autoreceptors in central and peripheral nervous system (Göthert and Schlicker 1983; Martin and Sanders-Bush 1982; Engel et al. 1983b) have been established by comparing the rank order of potencies of different 5HT agonists and antagonists. However, discrepancies exist in the literature concerning the potencies of 5HT antagonists at 5HT autoreceptors and at $5HT_1$ binding sites, probably due to the recently discovered inhomogeneity of the $5HT_1$ binding sites as pointed out by Nelson et al. (1983). Divergent opinions also exist about a possible coupling of $5HT_1$ receptors to adenylate cyclase (Peroutka et al. 1981; Bockaert et al. 1981).

The aim of the present investigation was to prepare a speicific radioiodinated ligand for the characterisation of $5HT_2$ receptors in the periphery where the receptor density is normally not so high as in the brain. A major drawback of other existing ligands for $5HT_2$ receptors, e.g., ^{3}H-spiperone, ^{3}H-methergoline, ^{3}H-mianserin, ^{3}H-methiothepin, ^{3}H-ketanserin (for review see Hamon 1983) and ^{3}H-mesulergine (Closse 1983), was partly their lack of selectivity for $5HT_2$ receptors and their inability to bind in an appreciable amount to peripheral $5HT_2$ receptors (Leysen et al. 1981).

As a starting material for the radioligand synthesis, *d*-LSD was used for two reasons: (1), it binds with an appreciable high affinity to $5HT_1$ and $5HT_2$ receptors (Peroutka and Snyder 1979) and (2) *d*-LSD can be iodinated by the chloramin T-method.

In this study, the pharmacological properties and binding characteristics of radioiodinated *d*-LSD to membranes from rat brain and guinea pig ileum will be described. Furthermore, the distribution of ^{125}IOL binding sites in rat brain will be discussed. Independently of this investigation, ^{125}IOL was synthesised by Hartig et al. (1983), using an alternative synthesis as indicated in a preliminary communication.

Materials and methods

Preparation of ^{125}IOL and IOL. *d*-LSD was synthesised as described by Stoll and Hofmann (1943). The iodination reaction of the radioligand was performed with chloramin T by a modified procedure according to Engel et al. (1981).

10 µl 13.5 mmol/l HCl containing 50% (v/v) methanol and ca. 50 µg *d*-LSD tartrate, 20 µl 0.3 mol/l K-phosphate buffer (pH = 7.2), 2 mCi Na^{125}I (as carrier free Na^{125}I from the Radiochemical Centre, Amersham, GB, Cat. No. IMS 30 in 20 µl 0.1 M NaOH) and 20 µl chloramin T (0.34 mg/ml) were combined in that order in a polypropylene test tube at room temperature and allowed to stand for 15 min in a heating bath at 65° C. The reaction was stopped by addition of 300 µl aqueous $Na_2S_2O_3$ solution (1 mg/ml). An electrophoresis of the reaction mixture on a thin layer silicagel plate (Merck, Darmstadt, FRG, No. 5724-0100), using as solvents (v/v) pyridine-glacial acetic acid water (0.33:0.6:9.07) was performed and showed a 50 – 70% incorporation of ^{125}I into *d*-LSD. It is important that all reaction solutions are freshly prepared immediately before the reaction starts and that *d*-LSD is used as a pure white crystalline powder as a reaction partner. 10 µl 0.1 mol/l NaOH are finally added to the reaction mixture before the iodinated product is extracted 3 times with 300 µl ethylacetate, containing 0.01% phenol. A Pasteur pipette is used for phase separation. The 3 washes are combined and spotted on a strip (25 × 40 cm) of Whatmann chromatography paper and chromatographed in a descending manner at 4° C with 0.1 mol/l ammonium formate, pH = 8.5, 0.01% phenol, for 16 h. The chromatogram is removed from the tank and the paper is cut while still wet into 1 cm strips which are immediately placed into 2.5 ml of methanol. The ^{125}IOL is recovered as a single narrow peak 8 cm from the origin while *d*-LSD runs through.

Specific radioactivity of the labelled compound of the peak fraction in the final methanol solution is 1.9×10^6 cpm/10 µl methanol. The ligand is very stable when stored in methanol at −20° C and can be used without any loss of binding activity for at least 4 months. The decay rate of ^{125}I was taken into account for the calculation of ^{125}IOL concentration.

Nonradioactive IOL was prepared as described by Troxler and Hofmann (1957). IOL crystallizes in a pure form as a white product with a melting point of 202 – 204° C. The NMR spectrum devoid of the signal of the hydrogen atom in position 2 of the indol nucleus clearly showed that the iodine atom occupied position 2 of the molecule. A gaschromatographic method was used to prove the identity of ^{125}IOL and IOL. The compounds were analyzed on a Varian 3200 equipped with a QFl, siliconized Chromosorb packed column with approximately 2500 theoretical plates at a working temperature of 250° C. It could be shown that the retention time of the thermoionic detector signal and that of radioactivity were the same for both compounds, 13.2 min.

In other chromatographical systems like high pressure liquid chromatography, high performance thin layer chromatography, the radioactive and the nonradioactive product showed identical elution profiles and/or the same R_f-values. High pressure liquid chromatography was performed as a Lichrosorb RP 8 column (250 mm × 4.6 mm and 10 µm grain diameter) at room temperature under 84 bar pressure with a flow rate of 2.5 ml/min. The elution gradient consisted of phase A (1 l distilled water) and phase B (1 l acetonitrile). Both phases contained 1 ml formic acid and 1 ml triaethanolamine, pH = 3.0. 0 to 100% B was attained in 60 min. Under these conditions, the retention time for radioactive and nonradioactive IOL was 27.2 minutes as detected by UV-absorption (254 nm) or by γ-counting. The aera under the peak of IOL indicated a purity > 99.5%.

In the high performance thin layer chromatography on 10 × 10 cm Silicagel plates (60, Merck, Darmstadt, FRG), using as solvents n-hexane:acetone 1:1 (v/v), including 1% ethanol, the R_f-value for IOL was 0.88, whereby the nonradioactive and the radioactive compound (visualized by autoradiography) showed identical single spots. It can, therefore, be assumed that the radiolabelled and the nonradioactive IOL are structurally identical.

In order to estimate the degree of purity of ^{125}IOL and its stability during our storage or incubation conditions (37° C, 1 h), thin layer electrophoresis was performed. The content of free [^{125}I]iodine in the ^{125}IOL methanol solution or in the incubation buffer was less than 2% after 4 months of storage or after 1 h incubation at 37° C under our incubation conditions.

Preparation of particulate fractions. Crude membrane fractions from rat cerebral cortex were prepared as follows: rats (Sandoz own bred, weight about 250 g) were killed by decapitation. The cortex was removed from the rat brains and its weight recorded. The tissue was homogenised (Polytron homogeniser, position 6, 10 s) in ice-cold sucrose solution (0.32 mol/l) and centrifuged for 10 min, at 900 × *g*. The supernatant was ultracentrifuged for 20 min at 70000 × *g*. The pellet was resuspended in Tris-HCl buffer (50 mmol/l), pH 7.5, and incubated at 37° C for 15 min. The suspension was ultracentrifuged once more, as before (20 min, 70000 × *g*). The final pellet was suspended in 10 ml Tris-HCl buffer, and stored in liquid nitrogen. Just before beginning the binding experiment, 10 ml homogenate suspension were diluted with about 120 ml incubation buffer (Tris-HCl 50 mmol/l, pH 7.7, $CaCl_2$ 4 mmol/l, 0.1% ascorbic acid, and pargyline 10 µmol/l) as described by Peroutka et al. (1979) and briefly re-homogenised.

Guinea pigs (300 g) were killed with a blow on the head and about 1 m long pieces of ileum were removed from the ileo caecal end. The ileum was carefully rinsed with 37° C warm buffer solution, containing 10 mmol/l Tris-HCl, pH 7.4, and 150 mmol/l NaCl and cut in 10 cm long pieces. These ileal segments were put over a glass rod (0.5 cm diameter) and the longitudinal muscle was separated from the circular muscle by rubbing past the muscle with the forefinger. The isolated muscle strips were combined and the wet weight determined. The muscle was homogenised in a ten fold volume of the above mentioned ice-cold buffer with a Polytron (position 7) for 10 s. After centrifugation of the homogenate for 10 min at 700 × *g*, the fatty buffy coat on the top of the supernatant was totally removed with a spatula. The supernatant and pellet were re-homogenised and passed through a double layer of cheese cloth and centrifuged for 30 min at 40000 × *g*. The pellet was washed and recentrifuged

once more. The final pellet was re-homogenised in the same buffer and deep frozen in liquid nitrogen.

In vitro studies. Spiral strips from canine basilar arteries were investigated in vitro as described by Müller-Schweinitzer (1982).

Binding experiments with ^{125}IOL. For rat cortex membranes, binding assays were routinely carried out in disposable polypropylene test tubes (Sarstedt Cat. No. 55538). An aliquot (150 µl) of the membrane preparation containing 25 µg protein (rat cortex), 50 µl ^{125}IOL (ca. 12.5 pmol/l) and 50 µl of the competing drug at various concentrations were incubated for 60 min at 37° C. Bound and free ligand were separated by addition of 10 ml incubation buffer at 37° C, followed by rapid filtration through Gelman AE glass fibre filters. Each filter was rapidly washed with 10 ml of the same buffer solution. Nonspecific binding was determined in presence of 100 nmol/l ketanserin or 5 µmol/l cinanserine. All binding experiments were performed such that bound ligand was less than 10% of the total ligand and that the specific binding was a linear function of the protein concentration. The protein content was determined according to the method of Lowry et al. (1951). The radioactivity of the wet filter discs was determined in a gamma counter (Kontron, WW 1032) at 80% counting efficiency. In some binding experiments with rat brain cortex membranes, the incubation was performed in Microtiter plates (Sterilin, 96 holes, in a store, Julabo, WS 60) at 37° C for 90 min. Then the bound ligand was separated from the free ligand by using a cell harvester (Flow laboratories, Titertek, TM). The incubation solution was soaked under reduced pressure through glass fiber filter paper (Gelman, AE) for 10 s, and simultaneously washed with 50 mmol/l ice-cold Tris-HCl buffer, pH = 7.4. Afterwards, the filters were dried under reduced pressure with a stream of air for 10 s. Under these conditions, the total and nonspecific binding of ^{125}IOL were identical to the values obtained by the above described classical binding methodology using a Millipore filter device.

The binding experiments with ileum membrane preparations were performed with the Millipore filter device in the same way as the rat cortex membranes with the modification that the incubation and washing buffer consisted of Tris-HCl buffer 10 mmol/l with 154 mmol/l NaCl. For the nonspecific binding, 10 µmol/l ketanserin or pizotifen were used.

Binding experiments with [^{3}H]5HT. The experiments were performed as described by Engel et al. (1983b).

Data analysis. The untransformed data from equilibrium binding experiments were analysed by nonlinear regression analysis using the program SAAM 27 (Berman and Weis 1967) and were fitted using a model for one or two classes of binding sites, according to Engel et al. (1981). By *F*-test analysis, the goodness of fit between different models was used to determine the most appropriate model (Snedecor and Cochran 1973). Some of the data were also numerically fitted, using the method "affinity spectrum" as described by Tobler and Engel (1983).

Autoradiography. Autoradiographic experiments were performed, according to Palacios (1983a) and Xue et al. (1983). Shortly, microtome cryostat sections of 10 µm thickness were mounted onto gelatine coated microscope slides and stored for at least a day at −23° C in order to improve the adhesion of the tissues to the slide. The mounted tissue sections were thawed, brought to room temperature and preincubated in Tris-HCl buffer 0.17 mol/l, pH = 7.4, containing 1% BSA, in order to remove endogenous catecholamines. Routinely the slides were incubated for 120 min at room temperature in the same buffer, containing 150 mmol/l NaCl and 50 pmol/l ^{125}IOL. For comparison of nonspecific binding, adjacent sections were incubated with 1 µmol/l cinanserin or 100 nmol/l spiperone. After incubation, the sections were washed twice in ice-cold buffer for 15 min to remove excess radioactivity. Slides were dried in a stream of cold air and were then apposed to LKB ^{3}H-Ultrafilm (LKB, Sweden) or Kodak X-Omatic film (Kodak, USA). Exposure time was 12 h at 4° C. Films were developed as previously described (Palacios 1983a).

Substances. Ergotamine tartrate, indoramine hydrochloride, methysergide hydrogen maleate, nicergoline hydrogen fumarate, perlapine, pizotifen hydrogen maleate and the enantiomers of LSD were synthesised in Sandoz, Basel, Switzerland.

The sources of the various other 5HT antagonists are: ketanserin and spiperone (Janssen, Beerse, Belgium); metitepine maleate (Hoffmann-La Roche, Basel, Switzerland); mianserin hydrochloride (Organon, Oss, Netherlands); cyproheptadine hydrochloride (Merck, Darmstadt, FRG); phentolamine hydrochloride (Ciba-Geigy, Basel, Switzerland); cinanserin hydrochloride (Squibb, Princeton, NJ, USA) and (+)- and (−)butaclamol (Ayerst Laboratories, Montreal, Canada); 5-hydroxytryptamine creatinine sulfate (Fluka, Buchs, Switzerland).

Results

Binding of ^{125}IOL to $5HT_2$ sites in rat cortex

Binding of ^{125}IOL to rat cerebral cortex membranes was saturable and reached a plateau between 1 and 2 nmol/l (Fig. 1). The graphical transformation of the saturation curve in a Scatchard-plot (inset of Fig. 1) gives a straight line. Nonlinear regression analysis also showed a single class of high affinity binding sites ($F < 0$). The binding constants from three independent experiments were $K_D = 0.9 \pm 0.1$ nmol/l and $B_{max} = 240 \pm 20$ fmol/mg protein. The nonspecific binding at the K_D concentration was between 30−40% of total binding.

In kinetic binding experiments (Fig. 2), specific binding was rapid and reached a plateau phase after 60 minutes which remained stable for at least 80 min. The ratio of the rate constants (k_{off}/k_{on}) yields a K_D-value of 0.7 nmol/l which compares well with the thermodynamic equilibrium constant obtained from Fig. 1.

The pharmacological identity of the sites labelled by ^{125}IOL was analysed in rat brain cortex by competition experiments performed with a variety of 5HT antagonists. As can be seen from Fig. 3, the competition curves of ketanserin, cinanserin and methysergide reach a plateau at about 30% of total binding. However, IOL displaces nearly 100% of the tracer from its binding sites due to self competition. Therefore, we used ketanserin (100 nmol/l) or cinanserin (5 µmol/l) if not otherwise indicated for the estimation of nonspecific binding. The enantiomers of LSD and butaclamol competed with ^{125}IOL in a strong stereoselective way. The

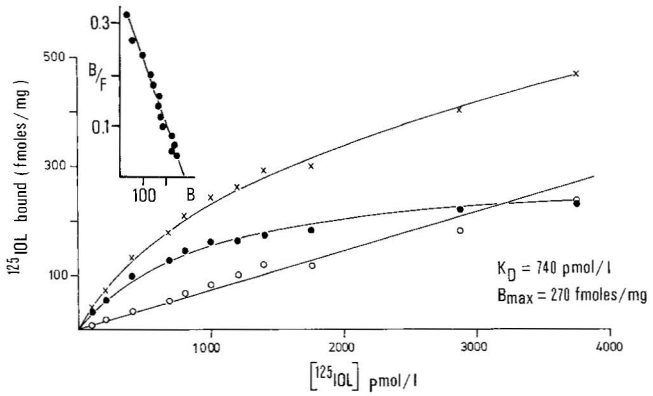

Fig. 1. Binding of ^{125}IOL to rat cortex membranes. Membrane fragments (100 µg protein/ml) were incubated with different concentrations of ^{125}IOL in the presence and absence of 10^{-7} mol/l ketanserin. *Ordinate:* total (X), specific (●) and nonspecific (○) radioligand bound in fmol/mg. *Abscissa:* concentration of free ligand. B_{max}- and K_D-values were determined by nonlinear regression analysis for a model with one class of binding sites. There was no improvement of fit for a two sites model ($F < 0$). *Inset:* Scatchard plot. *Ordinate:* Bound/Free (10^{-3} l/mg). *Abscissa:* Bound (fmol/mg). Each point of the binding curve in this and the following figures represents means of triplicate determinations from a representative experiment. The SEM is smaller than 3%

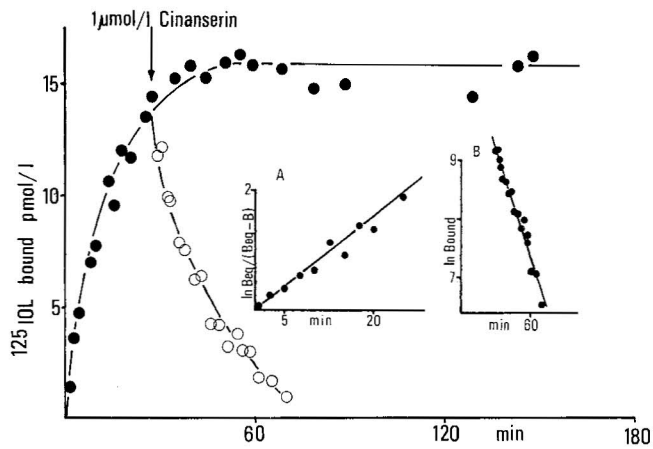

Fig. 2. Forward and reverse kinetics of specific ^{125}IOL binding to rat cortex membranes. Membranes (108 µg/ml protein) were incubated with ^{125}IOL (231 pmol/l) in a total volume of 313 ml. Total and nonspecific binding were determined simultaneously and substracted for specific binding. At the indicated times, triplicate aliquots of 250 µl were sampled and filtered as described in Materials and methods. After 45 min incubation time (*arrow*), dissociation was induced by addition of an excess of competitor. *Inset A:* The association rate constant was calculated from the pseudo first order plot of the association reaction: $k_{on} = (k_{obs} - k_{off})/L_{tot}$, where k_{obs} is the slope of the plot, B_{eq} = bound radioligand in the equilibrium state, L_{tot} is the free ligand at time zero and B = occupied receptor. *Inset B:* Semilogarithmic plot of a first order dissociation reaction. For this particular experiment, $k_{on} = 8.6 \times 10^7$ lx (mol/min)$^{-1}$ and $k_{off} = 0.06$ min^{-1}

pK_D-values of the (*d*) and (*l*) enantiomers were 8.8 ± 0.03 and 4.6 ± 0.03, respectively for LSD and 8.0 ± 0.03 and 5.2 ± 0.8, respectively for (+)- and (−)butaclamol.

Since all competition curves of the antagonists were monphasic, pK_D-values were calculated for 15 compounds which are included in Table 1. These biochemically derived

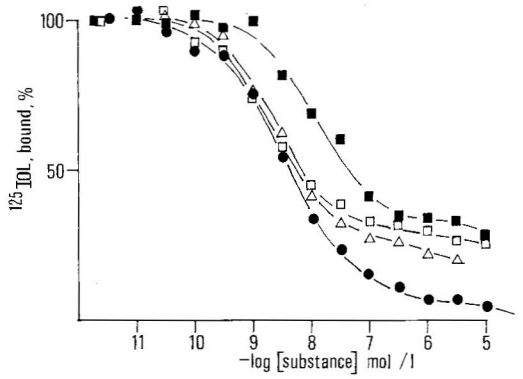

Fig. 3. Inhibition of ^{125}IOL binding to rat cortex membranes. The incubation mixture contained 0.12 nmol/l ^{125}IOL and ca. 0.12 mg/ml protein. The experimental data are fitted for a one site model (pK_D-values are in Table 1). *Ordinate:* total amount of ^{125}IOL bound. ● ^{127}IOL; ■ cinanserine; △ methysergide; □ ketanserin

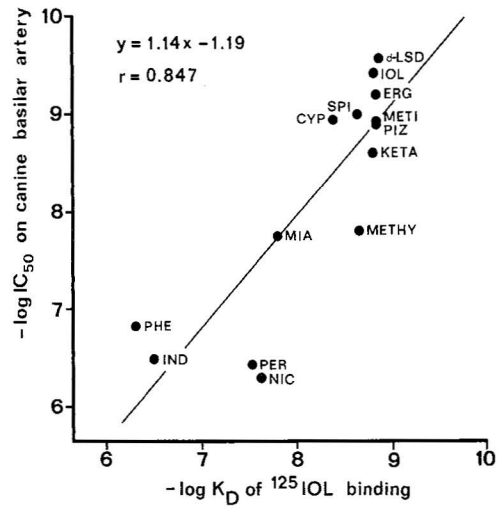

Fig. 4. Correlation between binding affinities of compounds from Table 1 (-log K_D-values, *abscissa*) measured for specific ^{125}IOL binding to rat brain cortex membranes and drug potencies in vitro (-log IC_{50}-values, *ordinate*) for inhibition of 5-HT-induced contraction of canine basilar artery. Data are compared by linear regression analysis. The regression line and the correlation coefficient are indicated; $P < 0.001$. The abbreviations correspond to the substances from Table 1. The log IC_{50}-values contain pA_2- and pD'_2-values

affinities (pK_D-values) correlated well with pharmacologically derived affinities (pA_2- and pD'_2-values) against serotonin-induced contractions in the canine basilar artery of the dog (Fig. 4 and Table 1).

Binding of ^{125}IOL to guinea pig ileum membranes

Due to the high specific radioactivity of the ligand, it was possible to label sites in membranes of the longitudinal muscle of the guinea pig ileum. In Fig. 5, a saturation curve is shown together with a Scatchard plot. The nonspecific binding was determined in the presence of 10 µmol/l pizotifen. The equilibrium binding constants from four experiments were $K_D = 0.9 \pm 0.2$ nmol/l and $B_{max} = 15 \pm 7$ fmol/mg protein. In competition studies (Fig. 6), the antagonists showed again monophasic competition curves. In particular, those of

Table 1. pD'_2-values for inhibition by various antagonists of contractile responses to 5HT of spiral strips from canine basilar artery and pK_D-values for inhibition of specific ^{125}IOL binding in rat cerebral cortex membranes and in guinea pig ileum membranes. Values are expressed as means ± SEM; number of determinations in parentheses

Antagonist	Cortex rat (pK_D)	Ileum guinea pig (pK_D)	Basilar artery dog (pD'_2)
d-LSD	8.87 ± 0.03 (6)	8.3 ± 0.2 (6)	9.58 ± 0.06 (20)
IOL	8.82 ± 0.12 (4)	8.8 ± 0.1 (6)	9.44 ± 0.07 (3)
Ergotamine	8.84 ± 0.05 (3)		9.21 ± 0.09 (12)
Methysergide	8.65 ± 0.15 (4)	8.5 ± 0.3 (3)	7.81 ± 0.06 (15)
Pizotifen	8.80 ± 0.15 (4)	7.3 ± 0.2 (3)	8.90 ± 0.08 (8)
Nicergoline	7.63 ± 0.03 (3)		6.30 ± 0.10 (9)
Ketanserin	8.80 ± 0.12 (4)	6.3 ± 0.1 (5)	8.62 ± 0.06 (9)
Metitepin	8.80 ± 0.17 (3)		8.94 ± 0.15 (10)
Spiperone	8.64 ± 0.24 (3)		8.99 ± 0.13 (8)
Perlapin	7.53 ± 0.02 (3)		6.43 ± 0.10 (9)
Mianserin	7.79 ± 0.06 (3)		7.74 ± 0.15 (8)
Cyproheptadin	8.38 ± 0.13 (3)		8.95 ± 0.06 (12)
Indoramine	6.48 ± 0.09 (3)		6.49 ± 0.12 (10)*
Phentolamine	6.30 ± 0.04 (3)		6.83 ± 0.04 (4)*
Cinanserin	8.40 ± 0.10 (4)	6.4 ± 0.1 (5)	

* pA_2-value

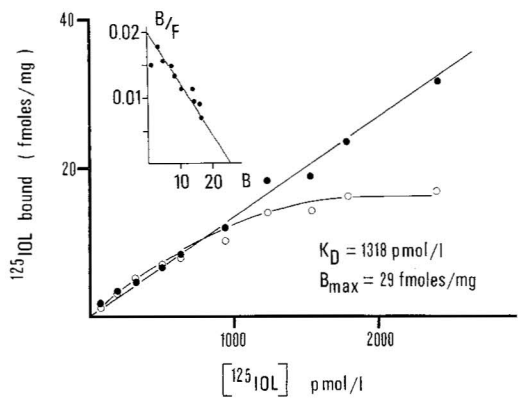

Fig. 5. Binding of ^{125}IOL to guinea pig ileum. Membrane fragments of smooth muscle of guinea pig ileum (1 mg protein/ml) were incubated with different concentrations of ^{125}IOL as indicated in legend to Fig. 1 in presence and absence of 10 µmol/l pizotifen. *Ordinate:* specific (○) and nonspecific (●) radioligand bound. *Inset:* Scatchard plot of the specific saturation curve

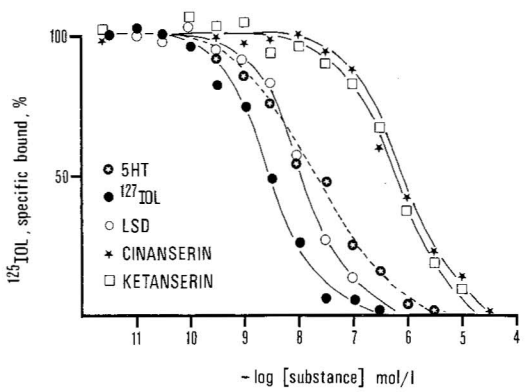

Fig. 6. Inhibition of ^{125}IOL binding to guinea pig smooth muscle membrane preparations. The incubation mixture contained 0.2 nmol/l ^{125}IOL and ca. 1 mg protein/ml. The experimental data of the antagonists are fitted for a one site model and the corresponding pK_D-values are enclosed in Table 1. The 5HT competition curve is fitted for a two site model and the parameters describing the high and low affinity site are collected in Table 2

ketanserin, cinanserin and pizotifen (Table 1) were markedly weaker competitors than in rat cortex membranes. The differences between the pK_D-values of various antagonists in the different membrane preparations are given in Table 1.

5HT competition with ^{125}IOL binding sites

As can be seen from Fig. 7, in rat cortex, 5HT competes in a biphasic way with sites labelled by ^{125}IOL, producing a shallow competition curve. The shape of the displacement curve is insensitive to a high amount of guanine nucleotides. A similar shallow competition curve can be obtained at the sites labelled by ^{125}IOL in guinea pig ileum as depicted in Fig. 6. The high and low affinities of 5HT recognition sites in both membrane preparations together with their distribution ratios are summarised in Table 2.

Specificity of IOL binding

In order to determine the specificity of the new radioligand, we have synthesised IOL, the corresponding nonradioactive compound. IOL was tested in a variety of binding assays in so far as radioligands with specificity for certain receptors were used. For comparison, spiperone and ketanserin were included in the binding screen. The affinity spectra of the three compounds are summarized in Table 3. The most prominent observation was the fact that $5HT_1$ sites are not a homogenous class of binding sites (Fig. 8). All three competitors recognise at least two $5HT_1$ sites with a high and a low dissociation constant (Table 3). Thus, IOL possessed a very high affinity to $5HT_2$ receptors and, except for $5HT_1$ sites, there was a moderate affinity to dopamine receptors. As previously reported, spiperone has an equally high affinity to

$5HT_2$ and to dopamine receptors, whereas ketanserin binds to $5HT_2$, α_1 and histamine receptors.

Autoradiographic localisation of ^{125}IOL binding sites in the rat brain

The binding characteristics of ^{125}IOL to mounted tissue sections of rat brain were similar to those described in membrane preparations. The binding presented nanomolar affinity was reversible, saturable and blocked by serotoninergic and dopaminergic drugs. Conditions for the autoradiographic studies were selected from these experiments (see Materials and methods).

The highest density of ^{125}IOL binding sites were seen in the rat frontal cortex and parts of the basal ganglia (Fig. 9A), in particular, the nucleus caudatus, nucleus accumbens and olfactory tubercles. The binding in other brain areas was much lower; in particular, the globus pallidus, hippocampus, thalamus and hypothalamus, brain stem and cerebellum presented very low density of autoradiographic grains, similar to that seen in white matter tracts.

The binding of ^{125}IOL in the rat cortex presented a laminar distribution with a band of high density of receptors localized over lamina IV and a second band of a lower density over lamina I (Fig. 9A).

The addition of 10^{-7} mol/l ketanserin to the incubation mixture resulted in the blockade of the binding of ^{125}IOL to the cortex, while at the same level, binding to the nucleus caudatus was very little affected (Fig. 9B). Spiperone, at a

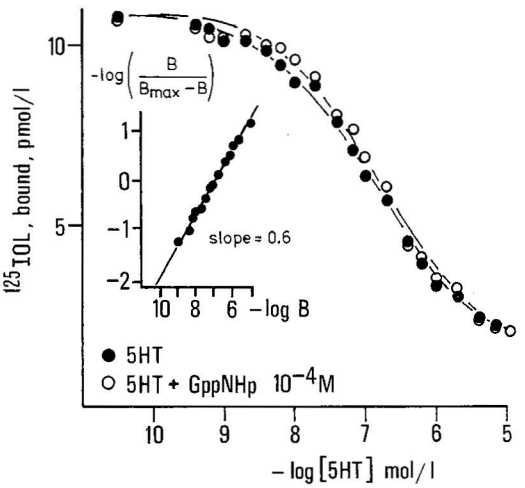

Fig. 7. Inhibition of ^{125}IOL binding by 5HT to rat cortex membranes. The competition between ^{125}IOL and 5HT resulted in a shallow competition curve. Inset: Hill plot with slope = 0.6; GppNHp (10^{-4} mol/l) had no significant influence on the shape of the displacement curve. The inhibition constants of 5HT, calculated by nonlinear regression analysis for two independent binding sites, are demonstrated in Table 2

Table 2. pK_D-values for inhibition of ^{125}IOL binding by 5HT to rat cortex and to guinea pig ileum membranes. The competition curves were analysed by a nonlinear regression analysis for the model of two independent binding sites ($P < 0.01$ by F-test). The dissociation constants of the high (H) and low (L) affinity sites (pK_D-values) and their distributions in % are indicated as means ± SEM of (n) determinations

Membranes	n	$pK_{D,H}$	$pK_{D,L}$	% (H)	% (L)
Cerebral cortex, rat	(4)	8.7 ± 0.6	6.6 ± 0.4	28 ± 5	72 ± 5
Guinea pig ileum	(4)	8.3 ± 0.1	6.8 ± 0.3	70 ± 9	30 ± 9

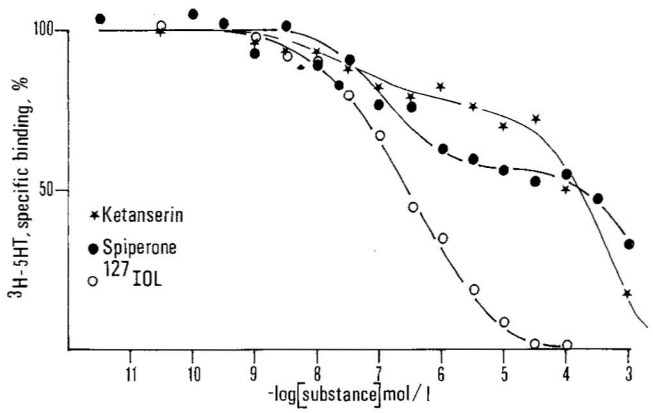

Fig. 8. Competition curves of ketanserin, spiperone and IOL with [^{3}H]5HT in rat cortex membranes. The radioligand binding assay was performed as described under Materials and methods. The lines through the data points represent the computer-generated best fit based on a model of two independent binding sites. By F-test analysis, the two site fit was highly significant, $P < 0.01$. The corresponding binding parameters are included in Table 3. Binding in the absence of competitor was 90–130 pmol/l and nonspecific binding in presence of 50 µmol/l 5HT was ca. 50%

Table 3. In vitro receptor binding profiles of compounds with specificity for $5HT_2$ receptors (K_i-values in nmol/l)

Compound	$5HT_2$	$5HT_1$		H_1	α_1	α_2	β	D_2	ACh muscarinic
		high	low						
Spiperone	1.2	50	79,000	inactive	10	inactive	–	0.16	inactive
Ketanserin	2.1	50	39,000	10	10	inactive	–	220	inactive
IOL	0.9	32	3,500	1,200	100	100	620	40	inactive

For spiperone and ketanserin, the K_i-values are taken from Leysen et al. (1981), where all details of receptor binding expriments are described. **a.** The affinities to $5HT_1$ receptors were determined from competition experiments using [^{3}H]5HT as ligand and rat cortex membranes as described by Engel et al. (1983b). Since all three 5HT antagonists showed shallow displacement curves (see Fig. 8) with Hill slopes smaller than unity, the curves were analysed by nonlinear regression analysis for two independent binding sites. The high and low affinity sites are indicated. The corresponding distribution ratios for the high and low affinity part of the competition curves are in %: spiperone 43/56, ketanserin 23/77 and IOL 52/48. **b.** For IOL, the various K_D-values are obtained under the following experimental conditions, according to Closse (1983), if not otherwise indicated: H_1: [^{3}H]mepyramine, rat brain without cerebellum; α_1: [^{125}I]BE 2254, rat cortex, according to Engel and Hoyer (1981); α_2: [^{3}H]clonidine, rat brain without cerebellum; D_2: [^{3}H]spiperone, calf striatum; ACh muscarinic: [^{3}H]QNB, rat brain without cerebellum; β: [^{125}I]Cyanopindolol, guinea pig left ventricle, according to Engel et al. (1981)

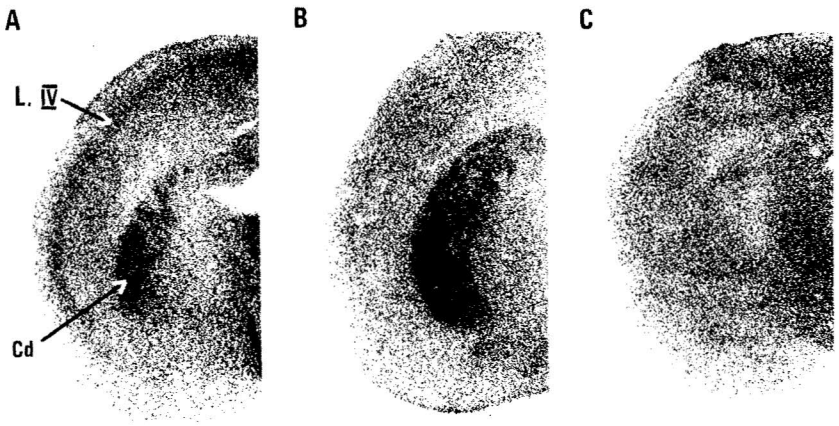

Fig. 9A—C. Autoradiographic localization of ^{125}IOL binding sites in the rat brain. The pictures are photograpies from the autoradiograms of rat brain coronal sections (corresponding to the A 5780 level of the atlas of König and Kippel 1953). **A** was incubated with ^{125}IOL alone, and represents the "total binding". Enrichment of binding sites (*dark areas*) can be seen in lamina IV (*L. IV*) and nucleus caudatus (*Cd*). The section in **B** was incubated with ^{125}IOL and 10^{-6} mol/l cinanserin, to block binding of ^{125}IOL to $5HT_2$ sites. Note the inhibition of the binding to L. IV. **C** is from a section incubated with 10^{-7} mol/l spiperone which blocks binding to dopamine and $5HT_2$ sites. The autoradiographic grains seen under this condition correspond to the "nonspecific binding"

concentration of 10^{-7} mol/l, completely blocked the binding of ^{125}IOL to the cortex and nucleus caudatus, letting only a homogeneous low density of autoradiographic grains corresponding to nonspecific binding (Fig. 9C).

Discussion

The observation that *d*-LSD can be iodinated at position 2 by an electrophilic substitution prompted us to synthesise the radioligand ^{125}IOL by the chloramine T procedure. Using this iodination method, 50–70% radioactivity could be incorporated. Nonradioactive IOL was synthesised as described by Troxler and Hofmann (1957). It was used in the structural analysis of ^{125}IOL and in comparative pharmacological tests. According to NMR spectral analysis, only the position 2 of the ergot alkaloid is substituted by iodine. Since the radioactive and the nonradioactive coumpound showed the same elution profile in different chromatographic systems it is assumed that both compounds are structurally identical and that the specific radioactivity of the purified radioligand corresponds to the theoretical radioactivity of one ^{125}iodine atom which is 2175 Ci/mmol. Independently from us, Hartig et al. (1983) iodinated LSD with radioactive N-iodosuccinimide by the method of Troxler and Hofmann (1957) which resulted in a very low specific radioactivity of 23–360 Ci/mmol, requiring 5–50 times higher protein concentrations than used in the present assays.

To evaluate the pharmacological specificity of the sites labelled by ^{125}IOL, a number of 5HT antagonists were investigated for their binding properties. The rank order of potencies by which these compounds displace ^{125}IOL correlates well with their potencies to inhibit 5HT-induced contractions of the canine basilar artery. Furthermore, there exists a good correlation between our values obtained from ^{125}IOL displacement in rat cortex, and those from [^{3}H]ketanserin (Leysen et al. 1983) or [^{3}H]mesulergine displacement studies (Closse 1983). *l*-LSD was 39,000 fold less active than *d*-LSD, indicating that, in addition, stereoselectivity can be used as a characteristic of the interaction of radioligands with their receptors. Butaclamol, which has a high stereoselectivity for dopaminergic sites (Seeman 1980), also discriminates with a high stereoselectivity ratio at serotonergic sites (Enna et al. 1976). The ratio pK_D (+)butaclamol/pK_D (–)butaclamol was ~600.

The present data support the suggestion that ^{125}IOL labels sites in the rat brain which correspond to the $5HT_2$ sites as defined by Peroutka and Snyder (1979) and which have pharmacological properties in common with classical vascular D receptors (Gaddum and Picarelli 1957; Bradley et al. 1983).

In contrast to our findings, Peroutka et al. (1983) have reported that 5HT-induced contraction of canine basilar artery appears to be mediated by $5HT_1$ receptors. However, we (Müller-Schweinitzer and Engel 1983) have provided clear evidence based on the rank order of potencies of 19 antagonists and 8 indole derivatives that the constrictor responses to 5HT of canine basilar artery are mediated by $5HT_2$-like receptors.

Due to the high affinity and high specific radioactivity of ^{125}IOL, it was possible for the first time to identify biochemically serotonergic binding sites on the longitudinal muscle of guinea pig ileum.

Originally, Gaddum and Picarelli (1957) classified the 5HT receptors, which are located on the smooth muscle of the guinea pig ileum as 5-HT-D receptors. Methysergide possesses a high affinity to these D receptors. However, pharmacological findings of van Nueten et al. (1981) demonstrate that the 5HT antagonist ketanserin is ineffective in the rat fundus and in guinea pig ileum. These findings support our results showing for ketanserin ~100 times weaker affinity in the ileum than in the brain or on the blood vessels. It seems that ^{125}IOL identifies 5HT receptors in the ileum which might be $5HT_2$-like sites but which appear to be different from those in the brain.

Interestingly, both sites might belong to subclasses of $5HT_2$ receptors since they bind with equal affinities ^{125}IOL or methysergide as can be seen from Table 2. The probability that ^{125}IOL identifies dopamine receptors in the ileum can be ruled out at the moment, since the inhibition constants of the

competitors in Table 2 are quite dissimilar to their known antidopaminergic activities. In addition, there is so far no clear evidence for the occurrence of specific dopamine receptors in the guinea pig ileum (Goldberg et al. 1978). The existence of specific inhibitory prejunctional dopamine receptors in the ileum can also be excluded by the work of Görich et al. (1982).

The 5HT displacement curves of ^{125}IOL provoked our interest because the shallow displacement curves had Hill coefficients of 0.6. The same observation of complex 5HT competition curves was made by Leysen et al. (1981), using [^{3}H]ketanserin, and by Closse (1983), using [^{3}H]mesulergine as $5HT_2$ specific radioligands. By nonlinear curve fitting, two affinity states of the $5HT_2$ receptor for 5HT could be calculated. In both tissues (brain cortex and ileum), the high and low affinity state have the same pK_D-values, however, their percentages of distribution are different. Furthermore, the shallow displacement curves of 5HT could not be shifted to the right and transformed in a monophasic curve by addition of a high amount of guanine nucleotides as it is known for β-adrenoceptors. On the guinea pig ileum, no 5HT sensitive adenylate cyclase could be measured (Markstein, unpublished). The shallow 5HT competition curve in the presence of GTP and the absence of a 5HT sensitive cyclase should be further investigated for the understanding of the molecular pharmacology of this 5HT-D receptor on the guinea pig ileum.

In order to delineate the specificity of ^{125}IOL for $5HT_2$ receptors we established a binding profile of the compound in comparison to ketanserin and spiperone. The most difficult task is the differentiation between $5HT_1$ and $5HT_2$ specificity, since in CNS, at least two subclasses of of $5HT_1$ receptors exist as has been shown by detailled binding studies performed with [^{3}H]5HT as ligand and spiperone as competitor (Nelson et al. 1983) or with different 5HT antagonists as competitors (Engel et al. 1983b). The main conclusion from Table 3 is the fact that IOL possesses the highest affinity to $5HT_2$ receptors, succeeded by a moderate affinity to one subclass of $5HT_1$ receptors and to dopamine receptors. Likewise for ketanserin and spiperone, the difference between the pK_D-value at the $5HT_2$ receptor and at the high affinity $5HT_1$ receptor is not as large as generally assumed. In most cases in the literature, the pK_D-value of the $5HT_1$ affinity is calculated from an IC_{50}-value (concentration of competitor which inhibits 50 % of specific binding), irrespectively of an anomalous shallow competition curve with a Hill slope < 1.0. By this procedure, the estimation of the pK_D-value of a competitor is intermediate between the high and low affinity state of the 5HT receptor and, hence, leads to a misleading low affinity value.

The autoradiographic visualization of ^{125}IOL confirmed the binding of this ligand to $5HT_2$ and dopamine receptors. The laminar distributions of ^{125}IOL binding sites and its drug sensitivity is similar to that observed with ^{3}H-spiperone (Palacios et al. 1981), ^{3}H-LSD (Meibach et al. 1980; Palacios 1983a), and ^{3}H-ketanserin (Palacios 1983b). $5HT_2$ sites were highly concentrated in lamina IV and lamina I of the cortex, especially in the frontal cortex. In contrast with cortical ^{125}IOL binding sites, those seen in the nucleus caudatus were insensitive to cinanserin but blocked by spiperone, indicating that ^{125}IOL labels dopamine receptors in this brain area. Thus, ^{125}IOL appears as a useful ligand for the visualization of $5HT_2$ and dopamine receptors by autoradiography. Because of the high specific activity, only short exposure times are required (12 to 24 h compared with 3 to 6 weeks required with the ^{3}H-labelled ligands).

In conclusion, ^{125}IOL is a new ligand with specificity for $5HT_2$ binding sites and with the ability to identify subtypes of $5HT_2$ sites in peripheral tissues which are not accessible, for example with tritiated ligands, e.g. [^{3}H]ketanserin. One major advantage of iodinated ligands as receptor probes in autoradiography is the possibility to reduce the exposure time of the slices to the film to a couple of hours and to facilitate visualisation of receptors in areas with low receptor densities.

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References

Berman M, Weiss MF (1967) SAAM Manual NIH, PHS Publication 1703, Bethesda, Maryland, USA

Bockaert J, Nelson DL, Herbet A, Adrien J, Enjalbert A, Hamon M (1981) In: Haber B, Gabay S, Issidorides M, Alivisatos S (eds) Serotonin: Current aspects of neurochemistry and function. Plenum Press, New York, pp 327–345

Bradley PB, Humphrey PPA, Williams RH (1983) Are vascular 'D' and '$5HT_2$' receptors for 5-hydroxytryptamine the same? Br J Pharmacol 295P

Closse A (1983) [^{3}H]mesulergine, a selective ligand for serotonin-2-receptors. Life Sci 32:2485–2495

Cohen ML, Fuller RW, Wiley KS (1981) Evidence for $5HT_2$ receptors mediating contraction in vascular smooth muscle. J Pharmacol Exp Ther 218:421–425

Engel G, Hoyer D (1981) [^{125}I]BE 2254, a new high affinity radioligand for α_1-adrenoceptors. Eur J Pharmacol 73:221–224

Engel G, Hoyer D, Berthold R, Wagner H (1981) (±)[^{125}Iodo]-cyanopindolol, a new ligand for β-adrenoceptors: Identification and quantitation of subclasses of β-adrenoceptors in guinea pig. Naunyn-Schmiedeberg's Arch Pharmacol 317:277–285

Engel G, Müller-Schweinitzer E, Stadler P (1983a) (^{125}I)LSD, a new ligand with selectivity for $5HT_2$ receptors. Br J Pharmacol 78:83P

Engel G, Göthert M, Müller-Schweinitzer E, Schlicker E, Sistonen L, Stadler PA (1983b) Evidence for common pharmacological properties of [^{3}H]5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors in sympathetic nerves. Naunyn-Schmiedeberg's Arch Pharmacol 324:116–124

Engel G, v Amsterdam F, Palacios JM (1983c) ^{125}I-LSD, a new useful ligand for the characterisation and localisation of 5-HT receptors. Naunyn-Schmiedeberg's Arch Pharmacol 322:R92

Enna SJ, Bennet JP, Burt DR, Creese I, Snyder SH (1976) Stereospecificity of interaction of neuroleptic drugs with neurotransmitters and correlation with clinical potency. Nature 263:338–341

Fozard JR (1983) Functional correlates of $5HT_1$ recognition sites. Trends Pharmacol Sci 4:288–289

Gaddum JH, Picarelli ZP (1957) Two kinds of tryptamine receptor. Br J Pharmacol 12:323–328

Geaney DP, Schachter JM, Elliott JM, Graham-Smith DG (1983) Specific binding of [^{3}H]lysergic acid diethylamide (LSD) to human platelet membranes. Br J Pharmacol 78:82P

Görich R, Weihrauch TR, Kilbinger H (1982) The inhibition by dopamine of cholinergic transmission in the isolated guinea pig ileum. Naunyn-Schmiedeberg's Arch Pharmacol 318:308–312

Göthert M, Schlicker E (1983) Autoreceptor-mediated inhibition of ^{3}H-5-hydroxytryptamine release from rat brain cortex slices by analogues of 5-hydroxytryptamine. Life Sci 32:1183–1191

Goldberg LI, Volkman PH, Kohli JD (1978) A comparison of the vascular dopamine receptor with other dopamine receptors. Annu Rev Pharmacol Toxicol 18:57–79

Hamon M (1983) Radioactive ligand binding studies: Identification of central serotonin receptors. In: Marangos PJ (ed) Methods in neurobiology. Plenum Publication Corporation, New York, in press

Hartig PR, Kadan MJ, Evans MJ, Krohn AM (1983) ^{125}I-LSD: a high selectivity ligand for serotonin receptors. Eur J Pharmacol 89:321–323

König JFR, Klippel RA (1963) The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins, Baltimore, M.D., USA

Leysen JE, Awouters F, Kennis L, Laduron PM, Vandenberk J, Janssen PAJ (1981) Receptor binding profile of R 41 468, a novel antagonist at $5HT_2$ receptors. Life Sci 28:1015–1022

Leysen JE, Gommeren W, De Clerck F (1983) Demonstration of S_2-receptor binding sites on cat blood platelets using [^{3}H]-ketanserin. Eur J Pharmacol 88:125–130

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Martin LL, Sanders-Bush E (1982) Comparison of the pharmacological characteristics of $5HT_1$ and $5HT_2$ binding sites with those of 5HT autoreceptors which modulate serotonin release. Naunyn-Schmiedeberg's Arch Pharmacol 321:165–170

Meibach RC, Maayani S, Green JP (1980) Characterization and radioautography of (^{3}H)LSD binding by rat brain slices in vitro: The effect of 5-hydroxytryptamine. Eur J Pharmacol 67:371–382

Müller-Schweinitzer E (1982) Actions of Co-dergocrine mesylate and its components at vascular smooth muscle. Naunyn-Schmiedeberg's Arch Pharmacol 318:225–233

Müller-Schweinitzer E, Engel G (1983) Evidence for mediation by $5HT_1$ receptors of 5HT-induced contraction of canine basilar artery. Naunyn-Schmiedeberg's Arch Pharmacol 324:287–292

Nelson DL, Schnellmann R, Smit M (1983) ^{3}H-serotonin binding sites: Pharmacological and species differences. In: Segawa T, Yamamura HI, Kuriyama K (eds) Molecular pharmacology of neurotransmitter receptors. Raven Press, New York, pp 103–113

Nueten JM Van, Vanhoutte PM (1981) Selectivity of Ca^{++}-antagonism and 5HT-antagonism with respect to venous and arterial tissues. Angiology 32:476–484

Ortmann R, Bischoff S, Radeke E, Buech O, Delini Stula A (1982) Correlations between different measures of antiserotonin activity of drugs. Study with neuroleptics and serotonin receptor blockers. Naunyn-Schmiedeberg's Arch Pharmacol 321:265–270

Palacios JM (1983a) Quantitative receptor autoradiography: Application to the study of multiple serotonin receptors in rat cortex. In: Mandel P, De Feudis FV (eds) CNS receptors from molecular pharmacology to behavior. Raven Press, New York, 455–463

Palacios JM (1983b) Light microscopic autoradiographic localization of catecholamine receptor binding sites in brain: Problems of ligand specificity and use of new ligands. In: Usdin E (ed) Proceedings of the fifth international catecholamine symposium, Göteborg 1983. Alan R. Liss Inc., New York, in press

Palacios JM, Niehoff DL, Kuhar MJ (1981) [^{3}H]spiperone binding sites in brain: autoradiographic localization of multiple receptors. Brain Res 213:277–289

Pedigo NW, Yamamura HI, Nelson DL (1981) Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by neuroleptic spiperone in rat brain. J Neurochem 36:220–226

Peroutka SJ, Snyder SH (1979) Multiple serotonin receptors differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol. Mol Pharmacol 16:687–699

Peroutka SJ, Lebovitz RM, Snyder SH (1979) Serotonin receptor binding sites affected differentially by guanine nucleotides. Mol Pharmacol 16:700–708

Peroutka SJ, Lebovitz RM, Snyder SH (1981) Two distinct central serotonin receptors with different physiological functions. Science 212:827–829

Peroutka SJ, Noguchi M, Tolner DJ, Allen G (1983) Serotonin-induced contraction of canine basilar artery: Mediation by $5HT_1$ receptors. Brain Res 259:327–330

Rutschmann J, Stadler PA (1978) Chemical background. In: Berde B, Schild HO (eds) Handbook of experimental pharmacology 49, Ergot alkaloids and related compounds. Springer, Berlin Heidelberg New York, pp 29–86

Seeman P (1980) Brain dopamine receptors. Pharmacol Rev 32:229–313

Snedecor GW, Cochran WG (1973) Curvilinear regression. In: Snedecor GW, Cochran CG (eds) Statistic methods, chapt 15. The Iowa State University Press, 6th Edition, Ames, Iowa, USA, pp 447–471

Stoll A, Hofmann A (1943) Partialsynthese von Alkaloiden vom Typus des Ergobasins. Helv Chim Acta 26:944–965

Tobler HJ, Engel G (1983) Affinity spectra: A novel way for the evaluation of equilibrium binding experiments. Naunyn-Schmiedeberg's Arch Pharmacol 322:182–192

Troxler F, Hofmann A (1957) Substitution am Ringsystem der Lysergsäure. III. Halogenierung. Helv Chim Acta 40:2160–2170

Xue QF, Maurer R, Engel G (1983) Selective distribution of β- and α_1-adrenoceptors in rat lung visualized by autoradiography. Arch Int Pharmacodyn Ther 266:308–314

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Note added in proof

While this paper was in review Moretti-Rojas I, Ezrailson EG, Birnbaumer L, Entman ML, and Garber AJ published a report (J Biol Chem 258:12499–12508 [1983]) in which they described independently of us the synthesis of ^{125}IOL. In rat sarcolemma membranes, these authors found from competition experiments with ^{125}IOL as radioligand very low affinities (pIC_{50}-values) for d-LSD (7.9), cyproheptadine (6.1) and methysergide (6.5). It is difficult to classify these sites with the available nomenclatures and to compare their findings with the present investigation.