Response of single raphé neurons to (+)-LSD: correlation with (+)-LSD binding in brain

JAMES LEROY BENNETT*, GEORGE K. AGHAJANIAN**, *Department of Pharmacology, Michigan State University, East Lansing, Michigan 48824 and **Departments of Psychiatry and Pharmacology, Yale University School of Medicine, Connecticut Mental Health Center, 34 Park Street, New Haven, Conn. 06508, U.S.A.*

We recently reported the use of a rapid filtration technique to demonstrate the presence of stereospecific, high affinity (+)-lysergic acid diethylamide, (+)-LSD, binding (half saturation, 4×10^{-9}M) in brain fractions from a number of subcortical as well as cortical brain regions (Bennett & Aghajanian, 1974). In that report we provided evidence that the (+)-LSD binding site may be a 5-hydroxytryptamine (5-HT) receptor. In addition, part of that investigation included a preliminary result which indicated that a correlation might exist between (+)-LSD binding *in vitro* and concentrations of the drug *in vivo* which produce physiological effects (e.g., inhibition of the firing of the 5-HT-containing neurons in the midbrain dorsal raphé nucleus). Neurons within the raphé nucleus were selected because their response to (+)-LSD is uniform and occurs at low doses (Aghajanian, Foote & Sheard, 1968; Aghajanian & Haigler, 1974). To investigate physiological correlates of *in vitro* (+)-LSD binding further we attempted to determine if there was a relation between the properties of *in vitro* (+)-LSD binding as previously reported and two *in vivo* measures: (1) the dose-response curve of single raphé neurons to (+)-LSD; and (2) the distribution of ^{3}H-(+)-LSD in rat brain after the administration of ^{3}H-(+)-LSD at a dose just sufficient to inhibit raphé neurons.

Male albino rats (Charles River, 220-200 g) were used. For purposes of single unit recording, animals were anaesthetized with chloral hydrate (400 mg kg^{-1}), and mounted in a stereotaxic instrument. A burr hole was drilled in the midline of the skull with its centre slightly anterior to the lambdoidal sutures. A micropipette approximately 1 μm at its tip and filled with 2M $CaCl_2$ and green dye (*fast green*), was then lowered through the burr hole 0·5 mm anterior to lambda. The *in vitro* impedence of the pipettes (measured at 60 Hz) ranged from 4 to 6 megohms. Raphé units were tentatively identified by the characteristic slow rates (·2-2 spikes s^{-1}), their regular rhythm and a location in or near the midline ventral to the aqueduct (Aghajanian & others, 1968; Aghajanian & Haigler, 1974). The latter was indicated by a zone of relative electrical silence. Integrated unit rates were followed with an electronic counter whose analogue output was plotted on a potentiometric recorder. Units exhibiting a stable firing rate were used for the experiments. After a baseline rate had been observed for about 5 min, ^{3}H-(+)-LSD (3·0 Ci mmol^{-1}; New England Nuclear) was administered through a tail vein. The firing rate of raphé units decreased rapidly in response to a dose of 3 μg kg^{-1} and recovery was slow; e.g. raphé firing, after being depressed by 50%, would require 20 to 30 min to return to baseline. During the recovery phase, rats were decapitated at particular stages when the firing rate had been maintained at a constant percentage of baseline for at least 1 min. Brains were taken during the recovery rather than during the rapid onset of inhibition to allow for drug equilibration. The midbrain as well as other brain regions from these rats was homogenized in 6 volumes of a 0·05 M tris buffer (pH = 7·4) and (+)-LSD was extracted by the method of Axelrod, Brody & others (1957). The amount of ^{3}H-(+)-LSD was determined by liquid scintillation spectrometry. Also, (+)-LSD and metabolites were extracted from these homogenates and analysed by the methods of Niwaguchi, Inoue & Nakahara (1974). Protein was measured by the method of Lowry, Rosebrough, & others (1951) with bovine serum albumin as a standard. The percent inhibition of raphé cell firing in each rat just before death was plotted against the concentration of ^{3}H-(+)-LSD in the midbrain. The amount of free (+)-LSD (X_F) was estimated at a particular percent inhibition of raphé firing from the following two equations: $X_F = X_T - fR_T$ and $X_F = X_T - (X_{NS} + fR_T)$ where X_T is the total amount of (+)-LSD in the midbrain, f is the percent inhibition of raphé firing observed at various X_T valves, R_T is the total number of specific (+)-LSD binding sites, and X_{NS} is the amount of (+)-LSD which is non-specifically bound at various X_T values. The values in the above equations were determined as follows: X_T was determined by direct measurement of the ^{3}H-(+)-LSD, as described above, in the midbrain of each rat at a particular f value or percent inhibition of raphe cell firing. X_{NS} (non-specifically bound (+)-LSD) was extrapolated from our previous published *in vitro* work which dealt with the binding of (+)-LSD to crude brain homogenates (Bennett & Aghajanian, 1974). In our *in vitro* ^{3}H-(+)-LSD binding study we observed that a fraction of the bound ^{3}H-(+)-LSD could be displaced from the brain homogenate by adding excess unlabelled (+)-LSD while the non-displaceable ^{3}H-(+)-LSD (or non-specifically bound (+)-LSD) increased in a linear fashion as the concentration of ^{3}H-(+)-LSD in the homogenate was increased, e.g. when the concentration of (+)-LSD in the brain homogenate was 5 nM 18% of the bound (+)-LSD could not be displaced when coincubated with 5000 nM unlabelled (+)-LSD. R_T was also extrapolated from the *in vitro* binding study and equalled 6·4 p mol g^{-1} midbrain. X_F values were determined using both equations (Table 1) and plotted against the percent inhibition of raphé firing or the f value (Fig. 1).

* Correspondence.

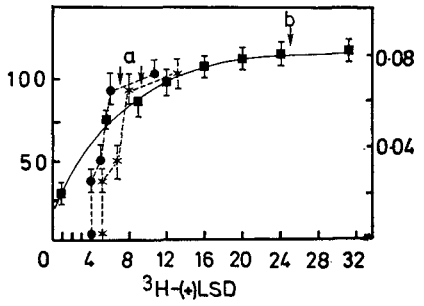

FIG. 1. The response of midbrain raphé neurons to various concentrations of ^{3}H-(+)-LSD in the midbrain compared to the previously published *in vitro* binding curve of ^{3}H-(+)-LSD to midbrain homogenates. The concentration of free ^{3}H(+)-LSD (X_F) was determined by two equations (see Table 1). X_F values determined by equation $X_F = X_T - fR_T$ were plotted (●----●) and compared to X_F values (*-----*) determined by equation $X_F = X_T - (X_{NS} + fR_T)$. Left ordinate represents the percent inhibition of rat midbrain brain units after intravenous administration of (+)-LSD. Right ordinate represents the amount of "specifically" bound (+)-LSD (pmol mg^{-1} protein) in homogenates of rat midbrain. Abscissa —^{3}H-(+)-LSD (M × 10^9). Each point on the response curve represents the mean of 4 to 5 experiments. a—raphé firing. b—*in vitro* (+)-LSD binding.

Fig. 1 summarizes our results and compares them to our previously published *in vitro* (+)-LSD binding curve (Bennett & Aghajanian, 1974). When raphé neurons were completely inhibited the concentration of free-(+)-LSD in the midbrain, as determined by the two equations, was estimated to be between 9·7 and 13·5 nM, while the concentration at 50% inhibition was between 4·6 and 5·8 nM. The 50% inhibition values are slightly greater than the half-saturation value of (+)-LSD for the *in vitro* binding site (Bennett & Aghajanian, 1974). Virtually no inhibition was observed when the concentration of (+)-LSD was between 4·1 and 5·0 nM. The higher of the two values was obtained from the equation $X_F = X_T - fR_T$ while the lower values were obtained from the equation $F_F = X_T - (X_{NS} + fR_T)$ (see Table 1) The latter equation is an extension of the former since it includes not only the specific (fR_T) but also the non-specifically bound (X_{NS}) (+)-LSD. These equations are similar to those developed by Goldstein for analysis of dose response curves (Goldstein, Aronow & Kalman, 1974).

From Fig. 1, it appears that raphé cells respond in a strange fashion to low concentrations of free (+)-LSD. Thus we observed no change in the concentration of free (+)-LSD even though the inhibition of raphé firing went from 0 to 32% inhibition. If we compare the *total* (X_T) amount of drug present in the brain at 0% inhibition with 32% we observe (see Table 1, column 3 and compare line 1 with line 2) that 40% more (+)-LSD is present in the brain when the raphé is inhibited by 32%. This indicates (a) that a certain amount of (+)-LSD must be present before raphé cells are inhibited, and (b) that once this concentration is reached (4 to 5 nM) the cells become very responsive to the presence of additional (+)-LSD.

Fig. 2 shows the amount of (+)-LSD in various brain regions at various times after the intravenous administration of 15 μg kg^{-1} of ^{3}H-(+)-LSD. Two min after the administration of ^{3}H-(+)-LSD there was a marked difference in the distribution of the drug. The amount of ^{3}H-(+)-LSD dropped disportionately in most regions between the second and fifteenth minute after the administration of the drug. Thus, by the fifteenth minute the striatum contained about 3 times more (+)-LSD than the cerebellum or mind brain during the last 15 min we observed a small additional drop of ^{3}H-(+)-LSD concentrations in all brain regions. Analysis of the radioactivity in the brains of these rats 30 min after administration of ^{3}H-(+)-LSD, resulted in the indentification of a single compound whose chromatographic behaviour was identical to (+)-LSD.

Other investigators, who have been working with crude as well as pure preparations of drug or toxin binding sites have also demonstrated quantitative agreement between *in vitro* binding curves and various

Table 1. *Values used in deriving the concentration of free (+)-LSD in the midbrain at various levels of inhibition of raphé cell firing.*

% inhibition of raphé firing "f" value	[1]Number of p mol of (+)-LSD specifically bound or f × R_T	Total number of p mol of (+)-LSD mg^{-1} midbrain or X_T value	[2]Percent of (+)-LSD bound nonspecifically	[3]Number of p mol of (+)-LSD bound nonspecifically or X_{NS} value	$X_F = X_T - fR_T$	[4]X_F *if* $X_F = X_T - (X_{NS} + fR_T$
0	0	5 ± 1·2	18 ± 4	0·9	5	4·1
32 ± 8	2·0	7·0 ± ·7	19 ± 3	1·0	5	4·0
50 ± 8	3·2	9 ± ·8	21 ± 5	1·2	5·8	4·6
89 ± 9	5·7	14 ± 1·2	24 ± 2	2·2	8·3	6·1
100 ± 7	6·4	20 ± 1·4	29 ± 3	3·9	13·6	9·7

[1]R_T = 6·4 p mol g^{-1} midbrain as determined from *in vitro* binding data.
[2]These values were determined from *in vitro* binding data.
[3]$X_{NS} = (X_T - fR_T)$ times the % of (+)-LSD bound non-specifically.
[4]X_F values were multiplied by 1000 and then expressed as nmol litre^{-1} rather than nmol kg^{-1} midbrain (See Fig. 1).

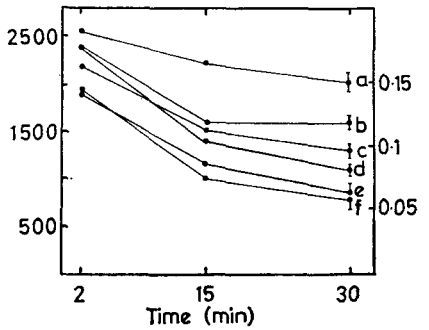

FIG. 2. (+)-LSD in various regions of the brain at various times after intravenous administration of 15 μg kg^{-1} (+)-LSD. Each point represents the mean of three experiments. Bracketed vertical lines represent s.e.m. a—striatum. b—cortex. c—hippocampus. d—diencephalon. e—midbrain. f—cerebellum. Left ordinate represents counts min^{-1} mg^{-1} protein. Right ordinate represents pmol (+)-LSD mg^{-1} protein.

physiological dose-response curves for a particular drug or toxin (Paton & Rang, 1966; Kasiai & Changeux, 1971; Changeux, Meunier & Huchet, 1971). Such observations are used in support of the idea that a particular binding site identified by *in vitro* techniques may represent the receptor which mediates the *in vivo* effects of the particular drug or toxin. We have found quantitative correspondence between the *in vitro* half saturation values for (+)-LSD binding and *in vivo* concentrations at which raphé neurons are inhibited by this drug. There is a difficulty, however, in directly comparing *in vitro* and *in vivo* concentrations since the amount of (+)-LSD bound in dilute *in vitro* suspensions is negligible in relation to the amount in the medium whereas *in vivo* a substantial proportion of the (+)-LSD may be in the bound form. We have attempted to overcome this difficulty by extrapolating from our *in vitro* binding data the amount of drug that could be bound to both specific ($f \times R_T$) as well as non-specific binding sites (X_{NS}) at each level of raphé inhibition. We realize the inherent problems associated with determining the *exact* amount of free (+)-LSD within the brain, but feel that our estimates are well within the range of the actual concentration of free (+)-LSD within the brain. It is possible that these estimates are too low because the amount of non-specific binding in brain homogenates would probably be higher than in the intact brain since brain homogenates probably have a greater number of exposed charged surfaces that would bind (+)-LSD non-specifically.

We previously reported that (+)-LSD binding was associated with areas known to receive a 5-HT input but not in the cerebellum which has little or no 5-HT input (Bennett & Aghajanian, 1974; Dahlstrom & Fuxe, 1965). Thus, the distribution of (+)-LSD binding sites, from the brain region with the highest concentration of binding sites to the region with few binding sites, was as follows: striatum > cortex > hippocampus > diencephalon > midbrain > cerebellum. We observed this same distribution of (+)-LSD in the brain 30 min after the intravenous administration of the drug. Thus, (+)-LSD appears to be retained more effectively in areas of the brain where there is a higher concentration of (+)-LSD binding sites. It should be noted, however, that the raphé neurons, whose physiological response we monitored, are located in the region with the second lowest concentration of (+)-LSD binding sites (i.e., midbrain). It is possible that the raphé (5-HT) neurons bind (+)-LSD avidly but represent too small a fraction of the midbrain for this to be reflected in binding measured in the total midbrain. Another apparent anomaly is the fact that midbrain raphé neurons are much more sensitive to (+)-LSD (applied microiontophoretically) than are neurons in various other regions of the brain (Haigler & Aghajanian, 1974). Since some of these regions have relatively large amounts of (+)-LSD binding, it appears that the occurrence of high-affinity (+)-LSD binding by itself is insufficient to predict the physiological efficacy of any presumed drug-receptor complex which may be reflected by the binding. Nevertheless, at least one physiological response to (+)-LSD (i.e., inhibition of raphé neurons) has been shown to occur in a concentration range which corresponds to high affinity (+)-LSD binding *in vitro*.

This work was supported by National Institute of Mental Health Grants MH-17871, MH-14459, MH-26599-01, by the State of Connecticut, by the Biological Science Training Program Grant STOIMH07114 and by the Pharmaceutical Manufacturers Foundation.

December 30, 1975

REFERENCES

AGHAJANIAN, G. K., FOOTE, W. E. & SHEARD, M. H. (1968). *Science*, **161**, 706–708.
AGHAJANIAN, G. K. & HAIGLER, H. J. (1974). *Brain Res.*, **81**, 364–372.
AXELROD, J., BRODY, R O., WITKOP, B. & EVARTS, E. V. (1957). *Ann. N.Y. Acad. Sci.*, **66**, 435–444.
BENNETT, J. L. & AGHAJANIAN, G. K. (1974). *Life Sci.*, **15**, 1935–1944.
CHANGEUX, J. P., MEUNIER, J. P. & HUCHET, M. (1971). *Molec. Pharmac.*, **7**, 538–553.
DAHLSTROM, A. & FUXE, K. (1965). *Acta physiol. scand.* **62**, *Suppl.* 32, 1–55.
GOLDSTEIN, A., ARONOW, L. & KALMAN, S. (1974). *Principles of drug action*, 82–111.
HAIGLER, H. J. & AGHAJANIAN, G. K. (1974). *J. Pharmac. exp. Ther.*, **188**, 688–699.
KASIAI, M. & CHANGEUX, J. P. (1971). *J. Memb. Biol.*, **6**, 1–23.
LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. Chem.*, **193**, 265–275.
NIWAGUCHI, T., INOUE, T. & NAKAHARA, Y. (1974). *Biochem. Pharmac.*, **23**, 1073–1080.
PATON, W. D. M. & RANG, H. P. (1966). *Proc. R. Soc. B.*, **163**, 2–44.