Table 4 Phosphorylation of Thymidine with Various Mixtures heated for 44 Days

Days	T (1)	T (2)	T (3)	Tp and pT (1)	Tp and pT (2)	Tp and pT (3)	pTp (1)	pTp (2)	pTp (3)
1	97.4	91.3	89.4	2.6	8.7	10.6	—	—	—
2	92.9	84.6	78.0	7.1	15.4	16.8	—	—	5.2
3	89.9	77.4	73.9	10.1	20.0	20.0	—	2.6	6.1
4	86.6	73.4	67.2	12.9	22.4	22.4	0.5	4.2	10.4
5	83.9	67.1	61.3	14.8	26.1	25.1	1.3	6.8	13.6
7	80.2	59.0	55.8	17.8	31.2	25.9	2.0	9.8	18.3
12	73.0	48.1	38.9	23.4	37.5	36.1	3.6	14.4	25.0
20	65.3	36.3	24.9	29.3	42.9	41.4	5.4	20.8	33.7
22	64.0	36.3	23.4	30.9	44.1	42.4	5.1	19.6	36.2
31	59.4	31.2	20.3	34.2	43.6	41.1	6.4	25.2	38.6
44	52.2	29.2	18.0	38.8	46.1	38.7	9.0	24.7	43.3

(1) Thymidine, Na_2HPO_4, NH_4Cl and urea, molar ratio 1 : 3 : 10 : 10; (2) thymidine, $Na_4P_2O_7$, NH_4Cl and urea, molar ratio 1 : 1.5 : 10 : 10; and (3) thymidine, $Na_5P_3O_{10}$, NH_4Cl and urea, molar ratio 1 : 1 : 10 : 10 at 65° C. Yields are given in % of total T present.

We have recently demonstrated that inorganic polyphosphates are formed from urea and orthophosphate (L .E. O. and R. Osterberg, in preparation), so it may be significant that mixtures of urea with inorganic polyphosphates are excellent phosphorylating agents. The failure of mixtures of urea and trimetaphosphate to react with nucleosides is surprising.

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Binding of *d*-Lysergic Acid Diethylamide to Subcellular Fractions from Rat Brain

We have examined some of the characteristics of LSD (*d*-lysergic acid diethylamide) binding to subcellular fractions from rat brain, on the assumption that whatever the mode of action of LSD may be, the drug must first bind to LSD receptors. Studies on a model LSD receptor, that is, the receptor site of an antibody to *d*-lysergamide, have already been described[1].

The binding of generally labelled ^{3}H-LSD (1.9 Ci/mmol^{-1}, New England Nuclear) to tissue fractions was studied by suspending 8 mm dialysis bags, containing 0.4 ml. of sample, in a bath of 50 ml. of a modified Krebs Ringer solution (*p*H 7.25). The bath was flushed with N_2 and contained 0.01% ascorbic acid to reduce oxidation of ^{3}H-LSD. The bath also contained varying concentrations of ^{3}H-LSD and unlabelled LSD, and in some cases other drugs as well[2,3]. After dialysis of the sample overnight in the dark at 4° C, we measured in triplicate, by liquid scintillation techniques, the radioactivity in equal volumes of bag and bath.

The excess radioactivity in the dialysis bag over that in the bath represents the amount of ^{3}H-LSD taken up at equilibrium by the contents of the bag and it is this excess that is plotted in the figures. By this measurement, we determined the amount of LSD taken up by synaptosomes, myelin and mitochondria at LSD concentrations ranging from 10^{-10} M to 10^{-4} M in order to detect and characterize various types of LSD binding (Fig. 1).

The known lipid solubility of LSD might be expected to preclude detection of a small population of specific LSD-binding macromolecules. Indeed, equilibrium dialysis of the myelin and mitochondrial fractions showed binding directly proportional to LSD concentration between 10^{-10} M to 10^{-4} M (Fig. 1). This is equivalent to and indistinguishable from a large concentration of non-specific LSD binding sites (dissociation constant = $K_D > 10^{-4}$ M). The synaptosome fraction also showed this type of binding, but at low LSD concentrations there was additional binding which did not occur in myelin or mitochondria (Fig. 1). We have seen these results in five or more preparations of each of the three fractions. The additional specific binding in synaptosomes shows two classes of LSD binding sites, with $K_{D_1} = 9.0 \times 10^{-9}$ M and $K_{D_2} = 1.2 \times 10^{-6}$ M (Fig. 2). The concentration of the two sites is 6.4 and 340 pmol g^{-1} fresh weight of tissue, respectively. The concentration of the high affinity sites is thus exceedingly low. We did not detect binding sites of still higher affinity, which limits their concentration to 5×10^{-11} M during dialysis.

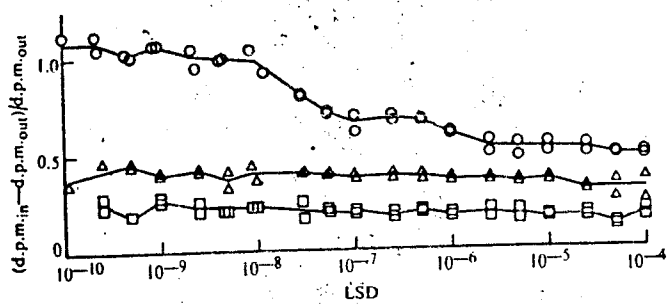

Fig. 1 High affinity LSD binding in synaptosomes and its absence in myelin and mitochondria. The amount of LSD taken up during equilibrium dialysis at various LSD concentrations (10^{-10} M to 10^{-4} M) by constant concentrations of synaptosomes (○), mitochondria (△) and myelin (□) derived from rat cerebral cortical grey matter was determined. At 4.5×10^{-9} M LSD, for example, the bath contained 1,860 d.p.m. 0.10 ml.$^{-1}$ ($d.p.m._{out}$) and the synaptosome sample contained 3,690 d.p.m. 0.10 ml.$^{-1}$ ($d.p.m._{in}$); $d.p.m._{in} - d.p.m._{out}$, the difference between the radioactivity in the dialysis bag and the radioactivity in the bath after dialysis (1,830 d.p.m. 0.10 ml.$^{-1}$ in this case), represents the amount of ^{3}H-LSD taken up by the tissue. This amount multiplied by $[LSD]_{total}/[^3H\text{-}LSD]$ represents total LSD taken up. If the ratio $(d.p.m._{in} - d.p.m._{out})/d.p.m._{out}$ is greater at a low LSD concentration than at higher LSD concentrations, high affinity binding is present. Myelin and mitochondria lack the high affinity binding sites present in synaptosomes. Subcellular fractions were prepared from rat brain cerebral cortical grey matter of 190–250 g Sprague–Dawley rats by a slight modification[4] of Gray and Whittaker's procedure[5]. The crude mitochondrial fraction in 0.32 M sucrose was layered on top of a discontinuous sucrose gradient (1.2 and 0.8 M sucrose) and centrifuged at 200,000*g* for 75 min in a Spinco SW-50 rotor to yield myelin, synaptosomes and mitochondria. The myelin and synaptosome fractions were diluted with water to 0.32 M sucrose and centrifuged at 200,000*g* for 45 min. The pellets, and the mitochondrial pellet, were suspended in the buffer mentioned. The purity of the ^{3}H-LSD was periodically checked by thin-layer chromatography on Eastman silica gel sheets. If more than 5–10% of the ^{3}H-LSD was degraded it was repurified by the same method. The solvent system was $CHCl_3$: ethanol : acetic acid (105 : 15 : 1.5) containing 0.01% ascorbic acid. The chromatograms were prepared and run in an N_2 atmosphere in the dark at 4° C. The myelin and mitochondria results plotted in this figure are each from four experiments and the synaptosome results are from two experiments. Protein concentrations are: myelin, 2.6 mg ml.$^{-1}$; mitochondria, 4.6 mg ml.$^{-1}$; synaptosomes, 11.4 mg/ml. Nitrogen concentrations are: myelin, 0.42 mg ml.$^{-1}$; mitochondria, 0.76 mg ml.$^{-1}$; synaptosomes, 1.38 mg ml.$^{-1}$. The protein content was determined by the method of Lowry *et al.*[6], using human serum albumin as a standard, and nitrogen content was determined by the method of Lang[7].

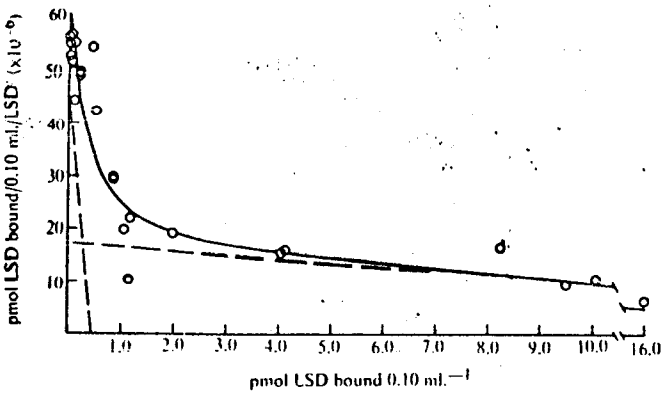

Fig. 2 The amount of LSD taken up by synaptosomes during equilibrium dialysis is expressed as a Scatchard plot[8] in order to determine the number, affinity, and concentration of high affinity binding sites. The points are experimental data. The curve is a theoretical curve for binding by two classes of binding sites with dissociation constants of 9.0×10^{-9} M and 1.2×10^{-6} M and present at concentrations of 0.40 pmol 0.10 ml.$^{-1}$ (6.4 pmol g^{-1} fresh weight of tissue) and 21 pmol 0.10 ml.$^{-1}$ (340 pmol g^{-1} fresh weight of tissue), respectively. The dashed lines indicate the two classes of binding sites.

We chromatographed a portion of the bath contents from myelin, synaptosomes and mitochondria dialysed against 10^{-6} M ^{3}H-LSD and found that 10–15% of the ^{3}H-LSD was degraded, but the degradation product did not exhibit high affinity binding to synaptosomes. ^{3}H-LSD was probably not metabolized to some chromatographically similar compound because brain tissue does not metabolize LSD[9].

We measured the inhibition by various compounds (all at 10^{-6} M) of 5.4×10^{-9} M ^{3}H-LSD binding to synaptosomes (Table 1). At 5.4×10^{-9} M, ^{3}H-LSD binds to both the high affinity site and the lower affinity site, in the ratio of 1.6 : 1. Among the synaptic transmitter candidates and their metabolites, serotonin, norepinephrine and tryptamine were inhibitory. LSD may interact with serotonin[10] or norepinephrine receptors[11]. Marchbanks reported 50% inhibition by 4×10^{-7} M LSD of ^{3}H-serotonin binding to synaptosomes (K_D for serotonin $=5\times10^{-7}$ M)[12]. Glutamate inhibited slightly, and the other three amino-acids tested were ineffective. Glutamate may function as a transmitter substance in the mammalian central nervous system[4,13]. Ergonovine, a lysergamide derivative, was a potent inhibitor.

Table 1 Inhibition of the ^{3}H-LSD Binding to Synaptosomes by Various Compounds

Inhibitor	Inhibition (%)	Inhibitor	Inhibition (%)
Transmitters and metabolites		Amino-acids	
Serotonin	70	L-Tryptophan	3
5-Hydroxyindole acetic acid	−7	L-Phenylalanine	0
Tryptamine	40	L-Tyrosine	5
Melatonin	14	L-Glutamate	16
L-Epinephrine	5	Others	
L-Norepinephrine	23	Ergonovine	70
DL-DOPA	−6	Tyramine	5
γ-Aminobutyric acid	2	d-Amphetamine	5
Hallucinogens		Morphine	−2
Mescaline	1	Spermidine	18
2,5-Dimethoxy-4-methyl-amphetamine (DOM)	25	Adrenochrome	34
Psilocybin	5	Histamine	−3
Psilocin	56		
5-Methoxytryptamine	55		
5-Methoxy-N,N-dimethyl-tryptamine	46		
N,N-Dimethyltryptamine (DMT)	27		
Bufotenine	52		
Harmaline	28		

In all cases the LSD was 5.4×10^{-9} M, all as ^{3}H-LSD, and the inhibitor concentration was 10^{-6} M. Both LSD and inhibitor were added simultaneously to the bath at the beginning of the experiment. For 100% inhibition, we used 10^{-4} M unlabelled LSD as inhibitor. Thus we measured only inhibition of specific LSD binding without interference by lipid/buffer partitioning or non-specific binding of LSD. The sources of the drugs and the preparation of psilocin from psilocybin have been previously described[1]. Data are mean values from two or three different synaptosome preparations. Mean values were all within 5% inhibition, except for the following: serotonin, ergonovine, amphetamine, ±12%; DOPA, DOM, tryptophan, ±20%; 5-hydroxyindole acetic acid, tyrosine, ±25%.

All of the hallucinogenic compounds we tested were inhibitory at 10^{-6} M except for mescaline and psilocybin. Mescaline at 10^{-4} M, however, inhibited 50% of the LSD binding. Psilocybin is probably not hallucinogenic but is rapidly dephosphorylated in the body[14] to the potent hallucinogen psilocin. Psilocybin did not compete for LSD binding, but psilocin did.

In spite of considerable differences in their structures, mescaline, psilocin and LSD show cross tolerance and produce symptoms similar in many respects, suggesting that their behavioural effects may be mediated at the same sites in the central nervous system, perhaps at a common set of post-synaptic receptors[15–17]. The potencies of these compounds in man are very different, however, for a variety of possible reasons (metabolism, passage across the blood-brain barrier, and so on). The ratio of potencies in man is 1 : 32 : 4,500–9,275 (mescaline : psilocin : LSD)[17,18]. Mescaline at 10^{-4} M and psilocin at 10^{-6} M inhibited 5.4×10^{-9} M LSD binding to synaptosomes by 50%, so the ratio of inhibitory potency is 0.32 : 32 : 6,400 (mescaline : psilocin : LSD). The strong similarity between the ratios for hallucinogenic potency and inhibitory potency implies that these three drugs act at the same synaptic sites.

Diab, Freedman and Roth have recently observed in autoradiographs ^{3}H-LSD of high specific activity bound to neurones of rat brain cortex[19]. Our results are complementary to and consistent with their observations on cerebral cortical tissue.

It is not possible to know which of the two LSD binding sites is related to the hallucinogenic properties of the drug, or whether both sites are involved, but the extremely low effective dosage of LSD leads us to favour the higher affinity site.

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