L-[^3H]LYSINE BINDING TO RAT RETINAL MEMBRANE:
I. Quantitative Determination and Characterization of the Binding Sites

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A saturable reversible binding to membranes from rat retina has been found for L-[^3H]lysine. Specific binding is time, temperature and protein concentration-dependent, and shows stereospecificity. The best computer fits of the experimental data are obtained with a receptor model based on two independent binding sites, of which only one site with a Kd value of 229.4 ± 14.23 nM and a Bmax of 2.04 ± 0.11 pmol/mg protein could be characterized satisfactorily. Several compounds included putative neurotransmitters have moderate or no affinity for L-lysine binding sites. A different pattern of distribution of L-[^3H]lysine binding sites is observed among various regions of the brain, with the highest density in the occipital cortex, and the lowest density in ponsmedulla.

The existence of binding sites in rat retinal membranes for L-lysine, as well as in the areas involved in the visual pathway, suggests a role for this amino acid in the physiological mechanism of the visual function.

INTRODUCTION

Lysine is an essential amino acid for the growing rat and for the maintenance of nitrogen equilibrium in the adult animal. Several studies have
shown the presence of lysine in different regions of the nervous system, and in particular, a high level of this amino acid has been demonstrated in the retina (1, 2). The regional distribution of lysine levels has been described to be similar to that of the mechanism of uptake (2), e.g. the amount of uptake measured in the retina is higher if compared to the brain slices. Moreover, it has been reported that the transport of lysine in the rat retina is not glucose and oxygen dependent, suggesting that the uptake of this amino acid is not related to the photoreceptor layer, but to that retinal layer, probably ganglion cells, which is characterized by a low rate of oxygen consumption (3, 4). In agreement with this finding, autoradiographic studies have shown that the amino acid is taken up by the inner plexiform layer and by the ganglion cells (5). The function of highly concentrated amino acids in the brain (e.g. glutamic acid, GABA, etc.) as well as in the retina (taurine, GABA, etc.) has been correlated with synaptic activity (6-9). Since the observed major characteristics of the presence of lysine in the rat retina suggest a specific role for this amino acid, it would be of interest to study whether or not lysine may be involved in the visual function. In the present work, the existence and the characteristics of L-lysine binding sites to rat retinal membranes have been studied as a preliminary step in order to investigate this hypothesis.

EXPERIMENTAL PROCEDURE

**Materials.** L-[4,5-3H]lysine monohydrochloride (specific activity 79.3 Ci/mmol) and carboxy-[14C]-inulin (specific activity 13 mCi/mmol) were obtained from Radiochemical Centre, Amersham. Unlabeled L-lysine monohydrochloride, and all the other compounds were purchased from Sigma Chemical Co., St. Louis, Missouri.

**Tissue Preparation.** Male Wistar albino rats (weighing 175-200 g) bred in our laboratory were used. The animals were killed by rapid decapitation, their eyes incised and both retinas dissected within 1 min. The retinas were immediately weighed and homogenized in 20 vol (w/v) of 50 mM Tris-HCl buffer, pH 7.4, using a Polytron PT 10 ST “OD” (Kinematica G.m.b.H., Luzern, Switzerland), at a setting of 5 for 30 sec. The homogenate was centrifuged at 16,000 g (L 50 rotor, 4°C) for 20 min and the pellet was suspended 1:20 (w/v) in the same buffer and recentrifuged at 48,000 g for 10 min. The resulting pellet was resuspended and recentrifuged twice, finally stored at -20°C overnight. In experiments in which regional distribution of L-[3H]lysine binding sites was studied, the brain areas, dissected as described by Glowinski and Iversen (10), were homogenized in 20 vol (w/v) of ice-cold 0.32 M sucrose with a Teflon-glass homogenizer. The homogenates were centrifuged at 1000 g for 10 min, and the supernatants were collected and centrifuged 20 min at 20,000 g. The resulting crude synaptosomal pellets were suspended in 5 vol of ice-cold distilled water and sedimented by centrifugation at 48,000 g for 10 min. The pellets were resuspended in 20 vol of Tris-HCl (50 mM, pH 7.4) and centrifuged for 10 min at 48,000 g. The membranes were once again resuspended in 20 vol of the buffer and frozen at -20°C overnight.

**L-[^3H]lysine Binding Assay.** Just before binding assay the crude membrane fraction, after thawing to room temperature, was washed three times by resuspension and recentrifugation.