HIGH-AFFINITY BINDING OF L-GLUTAMATE TO CHICK RETINAL MEMBRANES

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Binding of L-[3H]glutamate to membranes from whole chick retina and from subcellular fractions enriched with photoreceptor terminals (P₁), or terminals from the inner plexiform layer (P₂) was studied. Na⁺-dependent and Na⁺-independent binding to these membranes was demonstrated. Na⁺-independent binding was stereospecific. Kinetic analysis of the binding process indicated a single high-affinity system (K_B = 0.55 μM) with a capacity of approximately 20 pmoles/mg protein in all the membrane fractions. [3H]Glutamate binding to P₁ and P₂ fractions was effectively displaced by several structural analogues of glutamate. Glutamate diethyl-ester appreciably displaced binding, whereas kainic acid did not displace bound glutamate. Data indicate the binding of [3H]glutamate to physiologically relevant receptors in the chick retina.

INTRODUCTION

L-Glutamate is likely to be a major excitatory transmitter in the mammalian central nervous system (1, 2) exerting its actions through specific membrane receptors (3). In the retina, it has been suggested that glutamate and/or aspartate could be the excitatory transmitter substance released from the photoreceptor terminals (4). It has been postulated that an excitatory neurotransmitter must be released continuously from the photoreceptor terminals in the dark, maintaining bipolar and horizontal cells depolarized. Upon light stimulation, the inward sodium current would be blocked, causing the hyperpolarization of photoreceptors and the arrest
of the excitatory neurotransmitter release (5). There is inferential evidence, that the hyperpolarizing response of Horizontal Cells to stimulation by light might be due to the decrease of an excitatory transmitter substance. The identity of this excitatory transmitter is unknown, but in the vertebrate retina it is unlikely to be acetylcholine or catecholamines, since these are not present in the outer retinal layers (6), and acetylcholine has been shown to be without effect when applied to the Horizontal Cells of the carp retina (7).

The evidence for postulating glutamate or aspartate as neurotransmitter candidates in the retina is mainly indirect, and is derived from electrophysiological studies, which clearly establish their capacity for depolarizing horizontal, bipolar, and amacrine cells (4, 8, 9). On the other hand, glutamate and aspartate have been found mainly localized in the external layers of the retina (10). A high-affinity, sodium-dependent uptake mechanism shared by glutamate and aspartate has been characterized in the rat retina (11, 12) as well as in synaptosomal fractions from the chick retina (13).

Recently, Neal et al. (14) have demonstrated in a superfusion system, the arrest of aspartate release following illumination of the rabbit retina, which backs up aspartate as the transmitter at this level. Based on studies of kainic acid lesions in the Goldfish retina, it has been proposed that while glutamate could be the transmitter released by rods; cones could release aspartate as the transmitter (15). Recently, pharmacological evidence has been provided as to propose glutamate as the neurotransmitter of the ON pathways in the retina (16).

The presence of specific postsynaptic receptors for a substance paralleling the distribution of its physiological effects, has been considered as evidence for the compound to play a neurotransmitter role. Binding of highly labeled ligands to membrane preparations is now a well established technique for the direct study of drug-receptor interactions, and has been applied extensively to the study of neurotransmitters and their physiological receptors. The binding of amino acids to synaptic membranes has been largely studied for GABA (17–21) and glycine (22, 23), which are considered as major neurotransmitter candidates in the CNS. In these studies, it has been established that the binding of amino acids to synaptic membranes in the absence of sodium involves an interaction with postsynaptic receptor sites, while binding to pre and postsynaptic uptake sites is a temperature and sodium-dependent phenomenon (17–23). Using these techniques, postsynaptic receptors for L-glutamate have been characterized in brain homogenates (24), synaptic membranes (25), isolated membrane glycoproteins (26), and proteolipid membrane fractions (27), as well as, in cerebellar membranes (28, 29).