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제목	Purification and Characterization of the β-Adrenergic Receptor from Rat Cerebral Cortex
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내용	

Catecholamines acting through β-adrenergic receptors regulate a wide range of metabolic activities in mammalian tissue. Of the various receptors coupled to adenylate cyclase, the β-adrenergic receptors are the most extensively characterized and have been purified from both nonmammalian and mammalian sources. However, most studies of the molecular properties of β-adrenergic receptors have been confined to peripheral tissues. Less progress has been achieved in characterizing the brain β -adrenergic receptor.

The goal of the present study was, therefore, to purify and characterize the neurotransmitter receptor proteins. To achieve this goal, the following stepwise experiments were performed. At first, the membrane-bound β-adrenergic receptors were solubilized from brain tissue. Secondly, conditions for affinity chromatography were determined to purify the solubilized receptors effectively. Finally, the large-scale purification was performed and the characteristics of the purified β -adrenergic receptor were examined.

The β-adrenergic receptor of rat cerebral cortex was successfully solubilized by sequential treatment with cholate and digitonin. About 50% of the total receptor pool was released by this solubilization procedure. The β-adrenergic receptors in the digitonin extract were identified using the β-adrenergic antagonist, (-)-[^{3}H]dihydroalprenolol ([^{3}H]DHA). The solubilized receptor retained all of the essential characteristics of membrane-bound receptor, namely saturability; stereoselectivity; high affinity to β-adrenergic drugs.

In order to facilitate effective purification, a highly selective affinity adsorbent was prepared. A β-adrenergic antagonist, (-)-alprenolol, was immobilized to Sepharose CL-4B by using a hydrophilic spacer arm, bisoxirane. The affinity gel showed a high capacity and biospecific adsorption and elution for the neurotransmitter receptor.

Finally, the β-adrenergic receptor of rat cerebral cortex was purified by sequential affinity chromatographic steps. Purified receptor preparations bind [^{3}H]DHA with a specific activity of 13.65 nmol/mg of protein, representing a 35,000 fold purification from cortical membranes with a 3% overall yield. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of purified receptor revealed a band of protein of 52,000 daltons and a minor band of low molecular weight. The binding of [^{3}H]DHA to purified receptor preparations displayed affinity, specificity, and stereoselectivity characteristic of solubilized receptor.