[J. Natural Toxin, **2**, 103-116 (1993)] [Lab. of Molecular Biology]

Carbohydrate in Nicotinic Acetylcholine Receptor : Effects of Exogenous, and of Removal of Endogenous Carbohydrate and Sialic Acid on Ligand Binding.

HIROSHI NOMOTO, HIROKI SHOJI, YASUHIRO NAGAKI, MITSUHIRO OHTA, KYOZO HAYASHI*

The role of carbohydrate moieties of nicotinic acetylcholine receptor (AChR) in the binding of ligands such as α-bungarotoxin and carbamylcholine was investigated. First we examined the effects of various carbohydrate compounds on the binding of the ligand to intact AChR by our solid-phase binding assay system. Sialic acid, ganglioside, and ovalbumin inhibited the binding, though relatively high concentrations of them were necessary. High mannose-type carbohydrates, $Man_9GlcNAc_2$ and $Man_8GlcNAc_2$, which are the main oligosaccharides of AChR did not show any inhibitory effect on the ligand-binding ability of AChR. Then, carbohydrate moiety or sialic acids were removed enzymatically, but the digested AChRs showed the same affinity for the ligands as the intact AChR. This suggests that the integral sialic acids or carbohydrate moieties were unnecessary for binding of AChR to the ligands.

[Biochem. Biophys. Res. Commun., **194**, 1269-1275 (1993)] [Lab. of Molecular Biology]

Nicotinic Acetylcholine Receptor mRNAs in Myasthenic Thymuses: Association with Intrathymic Pathogenesis of Myasthenia Gravis.

HIROKAZU HARA, KYOZO HAYASHI*, KIYOE OHTA, NOBUYUKI ITOH, MITSUHIRO OHTA

Reverse transcription and the polymerase chain reaction showed that the α-, β- and γ-subunit genes of nicotinic acetylcholine receptor (AChR) in human skeletal muscle were expressed in myasthenia gravis (MG) thymuses (3 thymomas and 7 hyperplastic thymuses). A study of the expression of these subunit mRNAs in a variety of human tissues showed that α-subunit mRNA was expressed in human thymus and cerebral cortex, and that all three subunits were expressed only in human child thymus. Our results suggest that a complete AChR similar to extrajunctional (embryonic) muscle AChR is expressed in MG thymuses and that intrathymic AChR might be the primary antigen which induces autoimmune responses to muscle AChR.

[Clin. Chem., **39**, 2053-2057 (1993)] [Lab. of Molecular Biology]

Detection and Characterization of Blocking-Type Anti-Acetylcholine Receptor Antibodies in Sera from Patients with Myasthenia Gravis.

HIROKAZU HARA, KYOZO HAYASHI*, KIYOE OHTA, NOBUYUKI ITOH, HIROSHI NISHITANI, MITSUHIRO OHTA

We developed a highly sensitive, convenient assay for measuring blocking-type anti-acetylcholine receptor (AChR) antibodies, which inhibit the binding of ^{125}I-labeled α-bungarotoxin (α-BuTx) to the AChR. This procedure detected inhibitory activities in sera from 76 % patients with myasthenia gravis. Results of an experiment done with synthetic peptide corresponding to the α-BuTx binding region in the α-subunit of Torpedo AChR suggested that this inhibition is due to nonspecific steric hindrance caused by the binding of antibodies to a region other than the α-BuTx site, rather than by direct binding to the latter site. The inhibitory activities of the blocking-type antibodies and the titers of non-blocking-type antibodies were correlated. Moreover, the blocking-type antibodies could dissociate ^{125}I-labeled α-BuTx from ^{125}I-labeled α-BuTx-human AChR complex, and their dissociation activities showed good correlation with the inhibitory activities.