〔J. Natural Toxins, **1**, 93-103 (1992)〕 [Lab. of Molecular Biology]

Effects of Neurotoxins and Carbamylcholine on Tyrosine Phosphorylation of Nicotinic Acetylcholine Receptor.

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We found that nicotinic acetylcholine receptor (AChR) prepared from *Torpedo californica* is phosphorylated on its tyrosine residues of β and δ subunits *in vivo* with the immunoblotting technique using specific antiserum or monoclonal antibody. The phosphorylation of the β and δ subunits was found to be enhanced by incubation of the AChR-rich membrane with α-bungarotoxin, cobrotoxin, or carbamylcholine. However, the modes of phosphorylation reactions were different between neurotoxins and carbamylcholine. The present results show the possibility that a cholinergic agonist physiologically regulates phosphorylation of the AChR *in vivo*, which suggests the reaction is included in the physiological mechanisms of the receptor desensitization.

〔Eur. J. Biochem., **207**, 631-641 (1992)〕 [Lab. of Molecular Biology]

Detailed Structural Analysis of Asparagine-Linked Oligosaccharides of the Nicotinic Acetylcholine Receptor from *Torpedo Californica*.

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The structures of major oligosaccharide moieties of the nicotinic acetylcholine receptor (AChR) protein from *Torpedo californica* have been reported to be high-mannose types. Here we report detailed analyses of structures of the remaining oligosaccharides in this receptor. After removal of sialic acid from each fraction, the resulting neutral oligosaccharides were separately pyridylaminated and were analyzed by a combination of sequential exoglycosidase digestion and HPLC, then identified on a two-dimensional sugar map. Each oligosaccharide was composed of species containing varying numbers of sialic acids. The desialylated complex-type oligosaccharides of AChR consisted of ten, eight and one different biantennary, triantennary and tetraantennary oligosaccharides, respectively.

〔Biochem. Int., **25**, 1087-1093 (1992)〕 [Lab. of Molecular Biology]

Immunological Detection of the Dystrophin Molecule with Antibody Directed Against the Synthetic Peptide.

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We synthesized a peptide designated R8 (amino acid residues 1157-1201) based on the primary structure presumed from the nucleotide sequence of the cDNA clone from the gene for Duchenne nuscular dystrophy. Antibody to the synthetic R8 generated by immunization of rabbit was tested on human and mouse skeletal muscle by Western blotting analysis. The antibody reacted with a component of the 400K dystrophin of normal human and mouse skeletal muscles, but not with components of the muscles of Duchenne muscular dystrophy patients and mdx mice. Thus we established that this peptide sequence is in fact missing in the protein product "dystrophin" encoded by the DMD gene. The antibody may prove useful for the diagnosis of the Duchenne types of muscular D.