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STUDY ON THE BINDING BETWEEN BOTULINUM NEUROTOXIN  
AND RAT SYNAPTOSOMES

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The binding between botulinum neurotoxin and synaptosomes was studied by using *Clostridium botulinum* type D strain 1873 toxin labeled by  $^{125}\text{I}$  and rat forebrain synaptosomes, especially to the temperature dependency on the binding, the analysis of toxin receptor on the synaptosomal membrane and the structure change on the toxin after binding.

When the toxin was incubated with synaptosomes at  $37^\circ\text{C}$ , a unique peak in the increasing amount of binding toxin was observed within 5min and it decreased to a constant level by 10min after the binding. This specific binding depended on the temperature of incubation and was more clearly observed beyond  $25^\circ\text{C}$ , and not at all at  $0^\circ\text{C}$ . If this specific increase in the amount of toxin binding occurred, the release of  $^{125}\text{I}$ -toxin from the toxin-synaptosome complex decreased markedly with the addition of an excess amount of non-labeled toxin.

The toxin receptor on the synaptosomal membrane was analyzed by using  $^{125}\text{I}$ -toxin labeled by the photoaffinity cross-linker reagent, N-succinimidyl-6 (4'-azido-2'-nitrophenyl-amino) hexanoate. Substances with molecular weights of 160kDa or more, 145kDa, about 100kDa and 50kDa were detected on the radioautogram of SDS-PAGE gel beside large aggregates. Considering the molecular weight of toxin (140kDa), the substances with weight of 20kDa or more and 145kDa substances, whereas the substances with molecular weights of 100kDa and 50kDa may be derived from the cleavage of the toxin.

When synaptosomes were treated with neuraminidase, the specific increase in the amount of binding was retained. However, when synaptosomes were treated by trypsin or by heating, the binding ability of toxin to synaptosomes was completely abolished, suggesting the existence of sialic acid and glycoprotein as the toxin receptor.

These results may indicate that (1) D1873 toxin recognizes two receptors with molecular weights of 20kDa or more and 5kDa; probably glycoprotein and gangliosides, respectively, (2) the binding between the toxin and synaptosomes depends on the temperature of incubation, (3) synaptosomal conformation changes at the binding at  $37^\circ\text{C}$  within 5min after the binding, and (4) there occur toxin cleavages to two fragments.