BOTULINUM NEUROTOXIN-BINDING SUBSTANCES IN RAT BRAIN SYNAPTOSOMES

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Botulium neurotoxins bind to presynaptic membranes and inhibit the release of neuro-transmitters, causing botulism. The molecular mechanisms for toxin function, however, have not yet been clarified.

In this study, botulinum neurotoxin-binding substances in rat brain synaptosomes were partially purified and characterized.

Botulinum neurotoxin-binding substances were extracted from rat brain synaptosomes by boiling for 20 min in an incubation medium of 3mM KCl, 2mM $MgSO_4$, 2mM $CaCl_2$ and 180mM Nacl. The extract was fractionated by gel filtration with a column of Toyopearl HW-50S. Two protein peaks were eluted. The first peak fraction (toxin-activating fraction) increased the toxicity of the toxin (56%) being injected together with the fraction. The second peak fraction (toxin-inactivating fraction) decreased the toxicity of the toxin (44%). The toxin-activating fraction contained gangliosides and macromolecular proteins with molecular weights of$\geqq$240 KDa, whereas the toxin-inactivating fraction contained proteins of relatively small molecular weight, $\leqq$40 KDa.

The toxin-activating fraction was further divided into 4 subfractions by chromatography using a CM-Toyopearl 650M column equilibrated with 28mM borax-42mM sodium phosphate buffer containing 0.4M Nacl. Although all subfractions showed toxin-inactivating activity, the mixture of the first and second subfractions showed higher toxin-activating activity than before chromatography.

The toxin-inactivating fraction was further divided into 4 subfractions by chromatography under the same conditions. All subfractions decreased toxin activity, with the first subfraction showing the highest inactivating activity, which was 80 times higher than that before chromatography. The first subfraction contained carbohydrate and had an absorbance spectrum in the ultraviolet region, suggesting that it is a glycoprotein.