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Author(s)	SHIBATA, Chikako
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ANALYSIS OF BOTULINUM C-ST TOXIN STRUCTURE  
BY MONOCLONAL ANTIBODIES

Chikako SHIBATA

*Department of Biochemistry  
Faculty of Veterinary Medicine  
Hokkaido University, Sapporo 060, Japan*

Monoclonal antibodies (Mabs) against C-ST toxin, which is produced by *Clostridium botulinum* type C strain Stockholm (C-ST) were prepared. The structure of C-ST, especially the binding site of C-ST toxin, was analyzed by means of the Mabs.

One Mab, N-C-1, was newly established by immunizing the active fragment of C-ST toxin; N-C-1 reacts with the light chain of C-ST toxin and C-6813 but not with D-1873 and D-SA toxins, and had neutralizing activity for C-ST and C-6813 toxins. These facts suggest that N-C-1 recognizes a common antigenic site, and that common site participates in some stage of the expression of toxin function.

Three Mabs, CA-12, N-CA-137, C-9, reacted with the binding fragment of C-ST toxin and one Mab, N-C-1, reacted with the light chain to inhibit the binding of  $^{125}\text{I}$ -C-ST toxin to rat brain synaptosomes. CA-12, especially, showed strong inhibition.

These four Mabs were examined to determine their effects on the dissociation of  $^{125}\text{I}$ -C-ST toxin from  $^{125}\text{I}$ -C-ST toxin-binding synaptosomes. CA-12 dissociated the  $^{125}\text{I}$ -C-ST toxin from the synaptosomes as the native toxin. N-CA-137 showed weak dissociation only in high concentration. CA-12 also showed high neutralizing activity *in vivo*.

These results suggest that the binding site of C-ST toxin to synaptosomes or nerve endings is identical to or very near the site which is recognized by CA-12. Since N-CA-137 inhibited the toxin binding but weakly dissociated the bound toxin, N-CA-137 may recognize a site near the CA-12 epitope. C-9 inhibited the binding of toxin to synaptosomes but did not dissociate the bound toxin. This suggests that N-CA-137 and C-9 recognize sites near the CA-12 epitope, and that the N-CA-137 epitope is located nearer to the CA-12 epitope than C-9 epitope is. The competition between N-C-1 and CA-12 for binding to C-ST toxin molecules was examined by electrophoresis. No competition was observed. N-C-1 seems to change the tertiary structure of the toxin molecule by an allosteric effect and cause the binding site to lose its binding function indirectly. Five Mabs did not inhibit binding, but had neutralizing activity. This suggests that the sites which the 5 Mabs recognize participate in the expression of toxin function after binding. Because 3 of these Mabs reacted with binding fragment of the C-ST toxin molecule, the information concerning internalization may also be located on the binding fragment.