



Title	BINDING OF CLOSTRIDIUM BOTULINUM TYPE C NEUROTOXIN TO RAT BRAIN SYNAPTOSOMES
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Hokkaido University granted the degree of Master of Veterinary Medicine to the following 22 graduates of the Graduate School of Veterinary Medicine on 25 March, 1981.

The authors' summaries of their theses are as follows :

**BINDING OF *CLOSTRIDIUM BOTULINUM* TYPE C NEUROTOXIN
TO RAT BRAIN SYNAPTOSOMES**

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The binding of *Clostridium botulinum* type C neurotoxin to the synaptosomes was examined by two methods, by determination of the toxicity remaining in the supernate after incubation of the reaction mixture, and by enzyme-linked immunosorbent assay (ELISA) with the bound toxin in the precipitate.

1) By ELISA in the microtiter plate system, 10-1000 ng of toxin could be determined by the use of rabbit anti-(botulinum toxin type C) IgG and goat anti-(rabbit IgG) IgG- β -galactosidase complex.

2) The rat brain synaptosomes were stored at -80°C in 10 % dimethylsulfoxide for three months. The synaptosomes retained 70 % of the activity of ^{14}C -choline uptake in the fresh preparation and maintained the K^{+} -stimulated release of ^{14}C -acetylcholine and the binding capacity to the toxin. However, the synaptosomes stored at 0°C resulted in an impairment of choline uptake for a period of one week.

3) More than 94 % of the toxin (5.3×10^6 MLD/ml, $220 \mu\text{g}/\text{ml}$) was bound when incubated at 0°C with the synaptosomes (2 mg protein/ml) and then measured by the amount of toxicity remaining in the supernate of the reaction mixture. Although the binding was equilibrated within 5 min, the binding amount of toxin to the synaptosomes determined by ELISA was less than 1 % of that obtained by the former method.

4) The dose dependence of the toxin to the synaptosomes showed the typical curvilinear curve proposed by STECK & WALLACH (1965). This finding suggests that there are two kinds of toxic receptors on the synaptic membrane which show a difference of association constant and that the number of receptors per 1 mg of synaptosomal protein are $4.7 \times 10^{11} \text{ M}^{-1}$ and 3.38×10^{15} , and $5.0 \times 10^8 \text{ M}^{-1}$ and 4.63×10^{16} , respectively.

5) The binding amount of heavy and light chains of the toxin to the synaptosomes was less than that of the parental toxin. However, the bound heavy chain was a thousand times higher than that of the light chain, suggesting that the binding site of the toxin may be located in the heavy chain.