INHIBITION OF ACETYLCHOLINE RELEASE FROM RAT CEREBRAL SYNAPTOSOMES BY *CLOSTRIDIUM BOTULINUM* TYPE C NEUROTOXIN

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Inhibition mechanism of acetylcholine release from synaptosomes *Clostridium botulinum* type C neurotoxin was studied.

To obtain synaptosomes with constant activities in [^{14}C]-choline uptake and [^{14}C] acetylcholine release, the method of preservation was examined first. When stored at $-80°C$ for 6 months in the presence of 10% dimethyl sulfoxide, rat cerebral P_2 fraction maintained 70% of the choline uptake-activity of the fresh P^2 fraction.

At 37°C for 60 min there was [^{14}C] choline uptake by synaptosomes, and [^{14}C] acetylcholine was accumulated in the synaptosomes. The acetylcholine accumulated in the synaptosomes was preincubated at 0°C for 10 min with toxin (toxin-binding synaptosomes). After being re-incubated at 37°C for 20 min, both K^+-stimulated and spontaneous release of [^{14}C] acetylcholine from the synaptosomes were inhibited. The inhibition was toxin-concentration dependent, and there was a time lag before maximum inhibition was reached.

When toxin-binding synaptosomes were incubated with an excess amount of anti-toxin IgG at 0°C and further incubated at 37°C for 20 min, the inhibition of [^{14}C] acetylcholine release from synaptosomes was not observed. However, when an excess amount of anti-toxin IgG was added to toxin-binding synaptosomes at 37°C at time intervals, anti-toxin IgG had an insignificant effect on the inhibition after the elapse of 1 min.

These results may suggest that there are at at least three steps in the inhibition mechanism of acetylcholine release from synaptosomes by toxin. The probable steps are binding, temperature dependent lysis formation, and occurrence of some conformational changes in both the toxin molecule and the synaptosomal membrane.