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Failure of Type A Botulinum Toxin to Inhibit Acetylcholinesterase

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Recently, Marshall and Quinn reported in this journal on the in vitro inhibition of electric eel acetylcholinesterase by crystalline Type A botulinum toxin (R. Marshall and L. Y. Quinn, J. Bacteriol. **94**:812, 1967). To the best of our knowledge, this was the first time that such experimental evidence had been offered. Since only partial inhibition was demonstrated with a noncholine substrate, we have attempted to reproduce this finding by using acetylcholine as well as the chromogenic substrate employed by Marshall and Quinn.

The acetylcholinesterase (type III, electric eel, lot 115B-8551) used in this study was obtained from Sigma Chemical Co., St. Louis, Mo. Indophenyl acetate (IPA) was purchased from Mann Research Laboratories, Inc., New York, N.Y., and acetylcholine chloride (ACh) from Sigma Chemical Co. Crystalline Type A botulinum toxin was kindly supplied by E. J. Schantz, U.S. Army Biological Laboratories, Fort Detrick, Md. The spectrophotometric assay of acetylcholinesterase was done by the method of D. N. Kramer and R. M. Gamson (Anal Chem. **30**:251, 1958) except for the substitution of 0.05 M phosphate buffer, *p*H 8.0, for the *p*H 8.0 Clark and Lubs buffer. The titrimetric enzyme assay was performed at 25 C in 0.02 M Veronal buffer, *p*H 7.4, which was made 0.04 M in $MgCl_2$; 0.02 N NaOH was used as the titrant. A Beckman DK-2A recording spectrophotometer and a Radiometer Type TTTla titrator were employed for spectrophotometric and titrimetric measurements, respectively. The final substrate concentrations were 1.3×10^{-6} M IPA and 6.6×10^{-4} M ACh. Between 0.64 and 0.72 units of enzyme were used per assay, 1 unit of acetylcholinesterase activity being defined as that amount of enzyme capable of hydrolyzing 1 μmole of ACh per min, determined by the titrimetric assay procedure. The crystalline botulinum toxin was dialyzed against 0.9% NaCl or diluted with the same solution just before use. Bioassay of toxin solutions were performed with 20- to 25-g Swiss albino mice. Two assays were run at six different dose levels, with 10 animals per level. The mice were injected intraperitoneally with 0.25 ml of toxin solution and the deaths were recorded 72 hr postadministration. The LD_{50} value was determined by probit analysis. The potency of toxin was found to be 1.5×10^{10} LD_{50}/g, or half the value reported by Marshall and Quinn (J. Bacteriol. **94**:812, 1967).

TABLE 1. *Activity of electric eel acetylcholinesterase in the presence of crystalline botulinum toxin, Type A*

Toxin dose level (LD_{50})	Acetylcholinesterase activity	
	Titration, units (±SD)	Spectrophotometry (OD_{625}± SD)
0	0.649 ± 0.010	—
20[a]	0.642 ± 0.015	—
0	0.649 ± 0.010	0.403 ± 0.037
100[a]	0.644 ± 0.007	0.395 ± 0.018
0	0.698 ± 0.014	0.403 ± 0.037
1,000[a]	0.702 ± 0.011	0.383 ± 0.015
0	0.712 ± 0.030	—
100[b]	0.709 ± 0.011	—
0	0.712 ± 0.030	0.400 ± 0.019
1,000[b]	0.715 ± 0.032	0.394 ± 0.005

[a] Dialyzed toxin.
[b] Diluted toxin.

For inhibition studies, the enzyme solutions were incubated for 10 min at 25 C with 20, 100, and 1,000 LD_{50} doses of botulinum toxin prior to assay. Triplicate determinations were run at each toxin dose level and duplicate control assays were performed intermittently. The results are summarized in Table 1.

No enzyme inhibition occurred at any toxin dose with either IPA or ACh as the substrate. The enzyme used by Marshall and Quinn was reported to be Sigma Chemical Co. lot 1158-8551, instead of the lot 115B-8551 employed in this investiga-

tion. According to the distributor, lot 1158-8551 has not been marketed. Therefore, it appears that the same enzyme preparation was used in both studies, and the difference in lot designation must be attributed to a typographical error. In view of the fact that no inhibition could be achieved with high toxin-enzyme ratio, exceeding by the factor of 10^4 that used by Marshall and Quinn, it is difficult to account for the reported findings of these investigators.