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PRELIMINARY COMMUNICATIONS

THE EFFECT OF β-BUNGAROTOXIN ON THE RELEASE OF ACETYLCHOLINE FROM BRAIN SYNAPTOSOMAL PREPARATIONS

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The venom of Bungarus Multicinctus has a curare-like action which has been studied in detail recently. Change and Lee[1] separated the venom into four different fractions, the α-, β- and γ-bungarotoxins and a nontoxic cholinesterase. α-Bungarotoxin acts on the nicotinic acetylcholine receptor[1,2] producing a postsynaptic neuromuscular blockade, whereas β- and γ-bungarotoxins, which also block neuromuscular transmission, appear to act exclusively on the presynaptic side of the neuromuscular junction[1,3,4].

The blocking effect of β-bungarotoxin has been studied on rat phrenic nerve-diaphragm preparations where it has been shown to initially increase the frequency of miniature end-plate potentials (MEPP) and quantal content of end-plate potential, but subsequently to decrease the output of acetylcholine ultimately leading to a neuromuscular block[5]. The present communication describes neurochemical studies on the direct effect of β-bungarotoxin on the release of acetylcholine (ACh) from rat cortical synaptosomal enriched fractions.

Methods and Materials. The β-bungarotoxin used in this study, kindly provided by Dr. Jean Rosenthal, was purified from the crude venom according to the procedure of Lee et al.[6]. Fraction V was used in all studies.

The cerebral cortices of male, 21-30 day old rats were dissected out rapidly after decapitation, as much white matter as possible was removed, and the remaining grey matter homogenized in 0.32 M sucrose containing 2 mM Tris-HCl pH 7.4 and 40 μM EDTA. The 'P_2' fraction was prepared according to the method of Cotman[7] and washed twice before use. Under the electron microscope this fraction was judged to be approximately 70-80% synaptosomal.

The P_2 fraction was loaded with labeled ACh by a preincubation for 30 min at 37° with [methyl-^{3}H]choline, specific activity 10.1 Ci/mmol, final concentration 9.4 x 10^{-6}M, in Krebs-Ringer bicarbonate medium (KR), gassed with 95% O_2 - 5% CO_2. After preincubation, the

suspension was kept in ice for 5 min and centrifuged at 15,000 g for 10 min. The supernatant was discarded and the pellet was washed once with an equal volume of KR containing 0.2 mM eserine sulphate. The P_2 fraction was resuspended in KR containing eserine and incubated at 37° in normal KR (5.3 mM K^+) or in KR containing a high KCl concentration (53 mM) with or without various concentration of β-bungarotoxin. After 20 min, the tubes were transferred to ice for 5 min, centrifuged rapidly, and the supernatant was diluted 1:1 with 1 M formic acid-acetone ($1:3_{V/V}$) and centrifuged. An aliquot of the supernatant, to which cold carrier ACh was added, was subjected to electrophoresis to separate ACh from choline[8]. The ACh spot was cut out, eluted, and the radioactivity determined using Aquasol scintillation liquid. Protein was measured by the procedure of Lowry et al.[9] using bovine serum albumin as standard.

In experiments without calcium, P_2 was washed once after preincubation with calcium-free KR containing 5 mM EGTA and resuspended in this modified KR. Incubation experiments to study the release were also carried out in this modified KR.

Results and Discussion. As shown in Fig. 1, the basal release of ACh, i.e. release in KR containing 5.3 mM KCl, increased linearly as the concentration of β-bungarotoxin was increased from 10^{-9} M to 10^{-6} M and leveled off at 10^{-5} M. The release of ACh at high KCl concentration (53 mM) also increased with increasing concentration of β-bungarotoxin but the percentage increase was much less than at 5.3 mM KCl. Hence, although the actual amount of labeled ACh released is increased, K^+-stimulated release of the transmitter is actually decreased. This is represented in Fig. 2, which shows that the K^+ stimulated release of acetylcholine is decreased as the toxin concentration increases and reaches a minimum at 10^{-7} M.

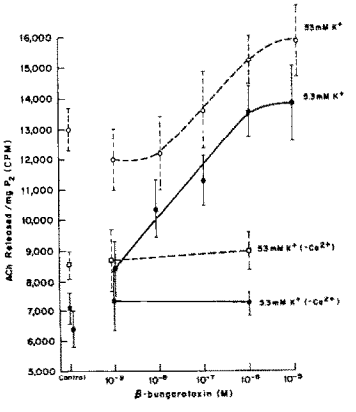

Figure 1.

Effect of β-bungarotoxin on ACh release. Conditions are described in the text. ACh released in normal KR at 5.3 mM K^+ ——●——, at 53 mM K^+ ----O----; ACh released in Ca^{++}-free KR at 5.3 mM K^+ ——■——, at 53 mM K^+ ----□----. Results are expressed as standard error of mean.

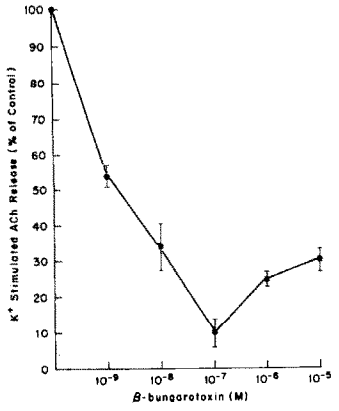

Figure 2.
Effect of β-bungarotoxin on K^+-stimulated ACh release. Release of ACh is expressed as percentage of control.

It is also shown in Fig. 1 that the effect of β-bungarotoxin requires Ca^{2+} regardless of the K^+ concentration in the medium.

When P_2 was incubated for 30 min with labeled choline in KR in the presence of 10^{-7} M toxin, the labeled ACh content of the tissue was decreased by 50% compared to a control preparation. Preliminary results suggest that this finding may be due to the inhibitory effect of β-bungarotoxin on the high affinity choline uptake system.

As stated earlier, Chang et al.[5] noted in their studies that β-bungarotoxin first increased MEPP and secondarily decreased ACh output leading to a block in transmission. These authors also showed a dependency of this effect on Ca^{2+}. Thus, our biochemical studies appear to correlate exactly with these electrophysiological experiments. Experiments are in progress to further define the mechanism of action of β-bungarotoxin.

References

1. C. C. Chang and C. Y. Lee, Arch. Int. Pharmacodyn., 144, 241 (1963).
2. C. Y. Lee and C. C. Chang, Mem. Inst. Butantan Sao Paulo, 33, 555 (1966).
3. C. Y. Lee, Clin. Toxicol., 3, 457 (1970).
4. C. Y. Lee, Annu. Rev. Pharmacol., 12, 265 (1972).
5. C. C. Chang, T. F. Chen and C. Y. Lee, J. Pharm. Exp. Ther., 184, 339 (1973).
6. C. Y. Lee, S. L. Chang, S. T. Kau and Shing-Hui Luh, J. Chromatog., 72, 71 (1972).
7. C. W. Cotman, Methods in Enzymol., 31, 445 (1974).
8. G. P. Sgaragli and J. R. Cooper, Biochem. Pharmacol., 23, 911 (1974).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. Biol. Chem., 193, 265 (1951).