70. Blockade of Transmitter Release by a Synthetic Venom Peptide, ω -Conotoxin

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Conotoxin GVIA (ω -conotoxin) is a venom peptide purified from the fish-eating marine mollusc *Conus geographus*,^{9),10)} and its amino acid sequence has already been described.⁹⁾ The effect of ω -conotoxin has been shown to be due to its inhibition of the Ca^{2+} influx of the presynaptic nerve terminal.⁵⁾ Recently, this polypeptide toxin was synthesized by Peptide Institute, Protein Research Foundation (Minoo, Japan). Since there is a possibility that the tertiary structures of synthetic and natural ω -conotoxin are different, it is worth to check the potency of the synthetic ω -conotoxin. In the present investigation, we studied the effects of synthetic ω -conotoxin on the frog neuromuscular junction, and observed that the synthetic toxin also has strong inhibitory effects on the transmitter release as does purified natural toxin.

Cutaneus pectoris and *sartorius* nerve-muscle preparations isolated from the frogs, *Rana catesbeiana* and *Rana nigromaculata* were used. The conventional intracellular recording technique was employed for measurements of the endplate potential (EPP). A digital memory/laboratory microcomputer was routinely used for analysis of the mean quantal content (m) and quantal height (q), by means of the variance method.³⁾ For analysis of m and q , the transmitter release was reduced by adding 2 mM MgCl_2 . Alternatively, d -tubocurarine was used for measurement of the time course of ω -conotoxin block. The concentrations of d -tubocurarine were 0.8–1.6, 3.8–6.4, 6.4 and 8.0–9.9 μM for 0.9, 2.7, 6.0 and 10 mM Ca^{2+} Ringer's solution, respectively.

For measurements of directly evoked EPP, muscle preparations were exposed to 1 μM tetrodotoxin. The EPP was triggered by application of an electrical pulse with an extracellular electrode located close to the junction.⁴⁾ The diameter of the extracellular electrode was approximately 50 μm . The directly evoked EPP was recorded using the intracellular electrode.

The Ringer's solution used consisted of (mM): NaCl 115, KCl 2.5 and CaCl_2 0.9, unless otherwise noted. pH was adjusted to 7.4 using 2 mM HEPES/NaOH buffer. Phenol red ($5 \times 10^{-4}\%$) was used as an aid for adjusting the pH of the Ringer's solution. Synthetic ω -conotoxin was supplied by Peptide Institute, Protein Research Foundation (Minoo, Japan). Other drugs were obtained from commercial suppliers.

Within a Ca^{2+} concentration range from 0.9 to 2.7 mM, the amplitude of

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the EPP was dramatically reduced by 40 nM ω -conotoxin (Fig. 1A). The reaction was completely irreversible, since there was no sign of recovery even after 5 hr of washout. However, the effects of ω -conotoxin changed with the extracellular Ca^{2+} concentration. The rate of reduction of the EPP decreased in 6 mM Ca^{2+} Ringer's solution until finally, at 10 mM Ca^{2+} , there was no apparent reduction of EPP (Fig. 1A).

Fig. 1. A, B: Effects of ω -conotoxin on the peak height of the endplate potential (EPP), mean quantal content (m) and quantal height (q). Values were normalized by control. The vertical bar shows standard error. The exchange of bathing solution was completed within 2–3 min. A: Averages of the time course of inhibition of the EPP by ω -conotoxin. V , amplitude of the EPP. The concentration of ω -conotoxin was 40 nM. The external Ca^{2+} concentrations were 0.9, 2.7, 6 and 10 mM for triangles, crosses, filled circles and open circles, respectively. Values are averages of 4 experiments for each concentrations of Ca^{2+} . B: Dose-dependent inhibition of mean quantal content (m) by ω -conotoxin. m and q were measured 30 min after perfusion of a given concentration of ω -conotoxin. Values are averages of 7–10 experiments. Open circles, m ; open triangles, q . The slight increase of q by 20 nM ω -conotoxin was due to a decrease in S/N ratio.

Statistical analysis revealed that the decrease in the peak height of the EPP was due to the reduction of m ; the quantal height remained unaffected (Fig. 1B). ω -Conotoxin lacked any postsynaptic effects, since the muscle membrane potential and the peak height of the miniature endplate potential unaltered by ω -conotoxin (data not shown). In addition, ω -conotoxin had no effect on the wave form of the focally recorded presynaptic action potential⁵⁾ (and our unpublished data).

All of the above findings and the data reported by Kerr and Yoshikami⁵⁾ indicate that synthesized ω -conotoxin has completely the same effects as the natural toxin, and is a blocker of the presynaptic Ca^{2+} channel. To confirm this possibility, the effects of ω -conotoxin on the directly evoked EPP were studied. In the presence of tetrodotoxin, it is possible to stimulate the voltage-dependent Ca^{2+} channels in the membrane of the presynaptic nerve terminal without generating the action potential.⁴⁾ The finding that 40 nM ω -conotoxin decreased and finally abolished the directly evoked EPP (Fig. 2), supported the idea that ω -conotoxin blocks the voltage-dependent Ca^{2+} channel in the presynaptic nerve membrane.

Fig. 2. Effects of ω -conotoxin on the directly evoked endplate potential (EPP). The nerve action potential was blocked with $1\ \mu\text{M}$ tetrodotoxin. A: Control. B: 30 min after perfusion of $40\ \text{nM}$ ω -conotoxin. a, evoked EPP depressed with the toxin; b, stimulus artifact only. Stimulation was $2\ \mu\text{A}$, $0.5\ \text{msec}$. Records A and B are from the same endplate. Resting membrane potentials were -71 and $-88\ \text{mV}$ for A and B, respectively. Similar results were obtained in 4 other experiments. Calibrations, $2\ \text{msec}$ and $0.5\ \text{mV}$.

The ω -conotoxin blockade resembled the condition in which the extracellular Ca^{2+} concentration was reduced. The recovery of ω -conotoxin inhibition through elevation of the extracellular Ca^{2+} concentration (Fig. 1A) is consistent with the idea that the target of ω -conotoxin is the Ca^{2+} channel. In fact, reduction of transmitter release by various inorganic Ca^{2+} antagonists such as Mg^{2+} and Mn^{2+} , and several aminoglycoside antibiotics such as streptomycin and neomycin, recovers following elevation of extracellular Ca^{2+} ^{11),12)}

Recent investigations of the effects of various organic Ca^{2+} antagonists, e.g. verapamil, nifedipine and diltiazem, appear that the pharmacological effects of such drugs on Ca^{2+} channels have species and tissue selectivity.¹³⁾ Indeed, D600 and diltiazem block the open state of the ACh-receptor ion channel complex of frog endplate^{6),7)} but the effect of D600 on the transmitter release is weak.¹⁾ ω -Conotoxin also has high species specificity, blocking neuromuscular transmission in frog and fish, but apparently exerting no effect when intraperitoneally injected into mice.⁹⁾

There are accumulating evidences that at least 3 types of Ca^{2+} channels are present in the neuron and other various cells.⁸⁾ Since binding of ω -conotoxin does not compete with any of the Ca^{2+} antagonists, Cruz and Olivera²⁾ proposed that ω -conotoxin defines a new receptor site which affects voltage-dependent Ca^{2+} channels. In this regard, it is highly probable that ω -conotoxin and the other Ca^{2+} antagonists bind different types of voltage-dependent Ca^{2+} channels and this toxin could become a useful tool for the study of differences existing in the voltage-dependent Ca^{2+} channel in various tissues.

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