Low Incubation Temperature Favors Detection of Depolarization-Induced Coated Vesicles in Motor Axon Endings in Frog Nerve-Muscle Preparations

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KADOTA, K. and KADOTA, T. *Low Incubation Temperature Favors Detection of Depolarization-Induced Coated Vesicles in Motor Axon Endings in Frog Nerve-Muscle Preparations.* Tohoku J. exp. Med., 1978, 126 (4), 399-400 — Depolarization-induced coated vesicles were detected in motor axon endings in frog nerve-muscle preparations stimulated at 10°C but hardly in those activated at 25°C. — frog neuromuscular synapse; low temperature; coated vesicles

While studying morphological changes in motor axon terminals following repetitive excitation, Heuser and Reese (1973) observed formation of coated vesicles (CV). They interpreted CV as an intermediate to re-form synaptic vesicles (SV) that were depleted during excitation. Ceccarelli et al. (1972), however, have a different view; since they did not observe CV, they maintained that SV should be re-formed directly from the axon terminal membrane at the point of exocytosis. Above-mentioned two groups of workers conducted their experiments with different temperatures; Heuser and Reese (1973) incubated preparations at 10°C, whereas Ceccarelli et al. (1972) did so at a room temperature of 22-23°C. From this we hypothesized that low temperature would be favorable for the detection of depolarization-induced CV. We tested it with the cat superior cervical sympathetic ganglion and found it to be true (Kadota and Kadota 1978). A further test of the hypothesis was made in the present experiment with nerve-muscle preparations.

Nerve-muscle preparations were obtained from cutaneous pectoris and sartorius muscles of *Rana nigromaculata*. They were incubated in Ringer solution containing d-tubocurarine chloride (3-10 µg/ml) and glucose (3 mM) at 10°C or 25°C. Motor nerves were stimulated at 10 Hz for 15 min with rectangular pulses of 0.1 msec and 5 V. After stimulation the materials were treated for electron microscopy as described in our previous paper.

Fig. 1 shows photographs obtained from cutaneous pectoris muscle preparations. Fig. 1a demonstrates a non-stimulated control material incubated at 25°C. SV are seen scattered throughout cytoplasm together with mitochondria. Essentially similar findings were obtained with a control material maintained at 10°C. Effects of stimulation of motor nerves are shown in Fig. 1 b and c, for which preparations were incubated at 10°C and 25°C, respectively. In both cases, SV were reduced in number, especially at active zones where the presynaptic membrane faces junctional folds. Mitochondria were swollen. The difference between the two cases is that coated pits and CV are seen in materials stimulated at 10°C (Fig. 1b), but they are hardly seen at 25°C (Fig. 1c).

The present results indicate that as shown with the cat superior cervical ganglion previously, low incubation temperature is an important factor for the appearance of depolarization-induced CV in frog motor nerve terminals as well. Heuser and Reese (1973)

Fig. 1. Neuromuscular junctions of frog cutaneous pectoris muscle. a: Non-stimulated control at 25°C. b: Stimulated at 10°C. c: Activated at 25°C. Arrows, coated pits; Double-headed arrows, coated vesicles (CV); Cis, intraterminal cistern. Bars equal 1 μ m.

succeeded in observing depolarization-induced CV by maintaining preparations at low temperature, but simultaneously they used a long-time block stain with uranyl acetate. The latter procedure may also be favorable for detecting depolarization-induced CV.

References

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