ACETYLCHOLINE CHANGES DURING TRANSMISSION OF A SINGLE NERVE IMPULSE, ANALYSIS WITH A QUICK FREEZING DEVICE

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The origin of neuromuscular disorders having a presynaptic origin are poorly understood, since the mechanism of acetylcholine (ACh) release is far from being elucidated. In this direction, we are presently investigating ACh release in the electrical organ of *Torpedo*. The amount of transmitter released by a single or a few nerve impulses is measured in the absence of anticholinesterase drug, using a sensitive radiochemical method.[1] In addition, vesicular and extravesicular ACh are measured by using a stimulator coupled to a rapid tissue freezer, on which a number of tissue samples can be simultaneously quenched at different time intervals during the course of synaptic transmission. Stimulation consists of either a single impulse in the presence of 4-AP, or of a short burst of 20 impulses at 100 Hz. In both cases very significant changes in the total ACh are observed within 100 to 200 ms. In contrast, the amount of vesicular ACh remains constant and no rapid transfer from extravesicular ACh to the vesicles can be detected. These results are, of course, difficult to conciliate with the vesicle hypothesis and confirm previous work showing that the ACh contained in vesicles is a very stable pool which is not immediately available for release. Besides this function of retaining an important reserve of transmitter, synaptic vesicles have recently been reported to exhibit interesting and unexpected properties. For example, cholinergic vesicles from the *Torpedo* electric organ have been shown to have an extremely efficient Ca^{2+}—sequestrating mechanism.[2] It is proposed that Ca^{2+} entry into the axon terminal during the action potential first triggers the mechanism of ACh release. Then Ca^{2+} is promptly taken up by the vesicles situated near the membrane. After exchange with vesicular ACh, Ca^{2+} is concentrated in the vesicles and then expelled by exocytosis. This hypothesis is presently being tested and may provide clues for understanding presynaptic cholinergic disorders.

References

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2. Israël, M. *et al.* 1980. J. Membrane Biol. **54:** 115.