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A new cytochemical method for in situ detection of cholinergic synaptic transmission by staining of Cu^{2+} incorporated in frog neuromuscular junction during nerve stimulation

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ABSTRACT

A new cytochemical method was devised in order to visualize Cu^{2+} ions in the synaptic area after their intracellular penetration during nerve stimulation of the frog neuromuscular junction (NMJ). The motor nerves were stimulated in presence of Cu^{2+}. After total blockade of the neuromuscular junction, the tissue was treated by ferrocyanide, a precipitating agent of Cu^{2+}, and fixed for optical and electron microscopic observation. The oxidoreductase-like catalytic activity of the copper ferrocyanide precipitate was used to amplify the cytochemical staining by a treatment with diaminobenzidine and H_2O_2, after permeabilization of cell membranes by Triton X-100. At optical level, an intense staining was observed in the synaptic area. Application of d-tubocurarine (d-TC), a selective inhibitor of nicotinic acetylcholine receptors (nAChRs), markedly reduced the staining. No reaction could be observed in absence of membrane permeabilization. These results suggest that Cu^{2+} was localized in the cytoplasm of muscle cells after its penetration through nAChRs. At electron microscopic level, cytochemical reaction was found in the cytoplasm of muscle cells near the postsynaptic membrane, and in a few synaptic vesicles in the vicinity of the active zone. This method may be used for the identification of cholinergic inputs in central and peripheral nerve systems and, generally speaking, for the detection of synaptic

activity elicited by specific nerve stimulation.

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