Exocytosis and modulation

Studies of Neurotransmitter Release at Cholinergic Synapses Formed Between Sympathetic Neurons in Culture: Role of Ca\(^2+\) Channels in Neurotransmitter Release

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1 Introduction

Superior cervical ganglion neurons (SCGNs) of neonatal rats have been known to form cholinergic synapses in culture (O’Lagure et al. 1974). This article illustrates a useful system of mammalian synapse, SCGNs synapse formed in culture, for investigations of molecular mechanisms of neurotransmitter release and their modulation (Mochida et al. 1994ab, 1995, 1996; Mochida 1995). Two features of this preparation are particularly useful for applying electrophysiological studies: 1) the large size of the cell bodies (30-40 µm) of SCGNs and (2) the short diffusion distance from the cell body to release sites at the synaptic terminals. This combination of features makes it possible to introduce molecular probes, such as peptides, proteins, or antibodies into the presynaptic neuron while detecting resultant changes in acetylcholine (ACh) release by measuring postsynaptic electrical responses. With this microinjection approach, we have obtained evidence supporting roles for the interaction of N-type Ca\(^2+\) channels with synaptic core complex of the synaptic vesicle protein VAMP/synaptobrevin (Trimble et al. 1988) and synaptic membrane proteins syntaxin (Bennett et al. 1992; Inoue et al. 1992; Yoshida et al. 1992) and SNAP-25 (Oyler et al. 1989) in regulating exocytosis at presynaptic terminals.
2 Synapses Formed Between SCGNS In Culture

These synapses have been employed in investigations of synapse formation and trophic factors. Isolated SCGNs form synapses with some other ganglionic neurons when cultured in the presence of nerve growth factor (Rees and Bunge 1974; Johnson et al. 1976). The neurite endings form presynaptic varicosities (Ko et al. 1976; Wakshull et al. 1979) that contain small clear synaptic vesicles (Johnson et al. 1976), and these synapses generate postsynaptic responses sensitive to nicotinic receptor blockers (O'Lague et al. 1974; Ko et al. 1976; Wakshull et al. 1979). A variety of externally applied factors and conditions lead to a reduction of synaptic catecholaminergic properties and simultaneously induce cholinergic properties in SCGNs in long-term culture (Landis 1990). Several cholinergic switching factors that might be released by the target tissue have been purified (Weber 1981; Fukuda 1985; Wong and Kessler 1987; Adler et al. 1989; Saadat et al. 1989; Rao and Landis 1990).

2.1 Synapse Preparation

Superior cervical ganglia were dissected from 7-day postnatal rats, desheathed, and incubated with collagenase (0.5 mg/ml; Worthington Biochem. Co.) in L-15 medium (Gibco) at 37°C for about 20 min. Following enzyme treatment, small tissue chunks were triturated gently through a small-pore glass pipette until a cloudy suspension was obtained. After washing by low-speed centrifugation (1200 rpm for 3 min) and resuspension, the collected cells were plated onto cover slips in plastic dishes (35 mm in diameter, approximately one ganglion per dish) containing the growth medium: 84% Eagle's minimum essential medium (Gibco), 10% fetal calf serum (MAB), 5% horse serum (Gibco), 1% penicillin/streptomycin solution (Gibco), and 25 ng/ml nerve growth factor (2.5 S, Collaborative Research). Cells were maintained at 37°C in a water-saturated atmosphere of 95% air-5% CO₂; the medium was changed twice per week. Somata appeared to be round (10-20 μm) when isolated flattened and processes extended and ramified over the cover slip dish within 1 week. The somata enlarged (30-50 μm) and neurites formed complex connections in 2-5 weeks of culture (Mochida et al. 1994a).

2.2 Presynaptic Terminal Proteins Expressed in SCGNs Synapses

Presence of the proteins identified in mature presynaptic terminals such as synaptic vesicle proteins as well as presynaptic plasma membrane proteins in these synapses formed in culture could be confirmed by immunofluorescence staining, indicating that these cultured synapses express many proteins characteristic of mature synapses (Mochida, 1995). Synaptophysin, a protein associated with synaptic vesicles (Jahn et al. 1985; Wiedenmann and Franke