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Topic: AS06 Neural Excitability, Synapses and Plasticity

NERVE-INDUCED MUSCLE CONTRACTION RETROGRADELY REGULATES PRESYNAPTIC M1 AND M2 MUSCARINIC RECEPTORS TO MODULATE NEUROTRANSMISSION VIA PKC-DEPENDENT PHOSPHORYLATION OF MUNC-18 AND SNAP-25

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Acetylcholine (ACh) signalling via muscarinic receptors (mAChR) plays a vital role in regulating various physiological processes, including muscle contraction. One of the crucial signalling pathways regulated by M1 and M2 receptors is the protein kinase C (PKC) pathway, which modulates neurotransmission during synaptic activity. PKC-dependent phosphorylation of Mammalian uncoordinated-18 (Munc-18) and Synaptosome-associated protein of 25kDa (SNAP-25), two key proteins involved in synaptic vesicle fusion and neurotransmitter release, is an essential mechanism for neurotransmission. Although ACh release mechanism is regulated by presynaptic stimulus and retrogradely by the resulting muscle contraction, the M1 and M2 pathways regulation by the pre- and postsynaptic activities had not been studied until now. To separate the effect of presynaptic activity from that of the resulting muscle contraction on M1 and M2 pathways, the rat phrenic nerve was stimulated (1Hz,30min) with and without contraction (abolished by μ-conotoxin GIIIB). M1 and M2 were selectively inhibited (by Pirenzepine and Methoctramine, respectively) to assess the interactions of mAChRs and its PKC-dependent pathways. We used Western blotting and immunohistochemistry techniques. We demonstrate that M2 was downregulated by presynaptic stimulation but during nerve-induced muscle contraction both M1 and M2 were upregulated. Interestingly, activity of M1 in both conditions was downregulated by M2. Regarding PKC signalling, PKCβ1 was downregulated by M1 but PKCξ by M2 during pre- and postsynaptic activities. These changes during presynaptic activity and nerve-induced muscle contraction were extended on the following targets Munc-18 and SNAP-25 which co-ordinately regulate neurotransmission. M1-PKCβ1 pathway controlled phosphorylation of Munc-18 during presynaptic activity and therefore synaptic vesicle fusion. M2-PKCξ signalling, which was modulated by M1 mAChR activity, regulated phosphorylation of SNAP-25 during pre- and postsynaptic activities and therefore neurotransmitter release. These results provide a molecular mechanism of the activity-dependent communication between M1 and M2 mAChR pathways to balance the optimal process of ACh release. Funding:PID2019-106332GB-I00,2017SGR704,PRE2020-092084,2021-FI-B00755.

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SPATIOTEMPORAL ASPECTS OF LIVE CELL NEURONAL SIGNALING USING LOW AND HIGH SPATIAL COHERENCE LATTICE LIGHT-SHEET DETECTION APPROACHES

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In synapses Ca^{2+} entry causes rapid neurotransmitter release, then postsynaptic receptor signaling. These receptors transduce voltage fluctuations, 2nd messengers, and postsynaptic Ca^{2+} entry. These events can be imaged in live cells at physiologically relevant rates and 3D resolutions using light sheet approaches to allow analysis of the biophysics of signaling distributed throughout the neuron's complex structures and within intact tissue. Similarly, in the vestibular periphery, sensory signaling relies on cellular properties of hair cells, their afferents, and the synapse between the two. Since the peripheral afferent arbor has a complex ramification, its biophysical properties can only be adequately analyzed by imaging its complex structure. We used lattice light-sheet (LLS) and incoherent holographic lattice light-sheet (IHLLS) and data obtained in neuron and sensory cell arborizations in intact tissue. We show increases in temporal and spatial resolution obtained using these approaches. The IHLLS detection technique replaces the glass tube lens of the original LLS with a dual diffractive lens system to retrieve the axial depth of the sample without moving the detection objective or the sample stage. In neurons, we have imaged presynaptic evoked Ca^{2+} transients, revealing numbers and kinetic properties of channels responsible for neurotransmission and can resolve Ca^{2+} entry through single channels. We used lipid dye labeling to track subsequent synaptic vesicle fusion. We have also used IHLLS, to reproduce neuronal structures without moving the emission objective focal plane eliminating mechanical artifacts and improving 3D temporal resolution. To address electrical properties of sensory afferents throughout their complex structure, we performed patch recordings from afferents, in combination with simultaneous lattice light sheet phase-locked imaging of voltage-sensitive dyes. We demonstrate high temporal and spatial resolution LLS and IHLLS imaging and the advantages of using these imaging approaches in intact, *ex vivo* tissue, enabling unprecedented spatial and temporal resolution of intact neuronal structures.

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