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Research article

Muscarinic cholinoreceptors (M1-, M2-, M3- and M4-type) modulate the acetylcholine secretion in the frog neuromuscular junction

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HIGHLIGHTS

- M1-M5 muscarinic receptor subtypes were detected in the frog neuromuscular junction.
- Electrophysiological studies have demonstrated the functioning of M1-M4 subtypes.
- The depressing action of muscarine is mediated via M3 receptor activation.

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ABSTRACT

Muscarinic cholinoreceptors regulate the neurosecretion process in vertebrate neuromuscular junctions. The diversity of muscarinic effects on acetylcholine (ACh) secretion may be attributed to the different muscarinic subtypes involved in this process. In the present study, the location of five muscarinic receptor subtypes (M1, M2, M3, M4 and M5) on the motor nerve terminals of frog cutaneous pectoris muscle was shown using specific polyclonal antibodies. The modulatory roles of these receptors were investigated via assessment of the effects of muscarine and specific muscarinic antagonists on the quantal content of endplate currents (EPCs) and the time course of secretion, which was estimated from the distribution of "real" synaptic delays of EPCs recorded in a low Ca^{2+}/high Mg^{2+} solution.

The agonist muscarine decreased the EPC quantal content and synchronized the release process. The depressing action of muscarine on the EPC quantal content was abolished only by pretreatment of the preparation with the M3 blockers 4-DAMP (1,1-Dimethyl-4-diphenylacetoxypiperidinium iodide) and J 104129 fumarate ((αR)-α-Cyclopentyl-α-hydroxy-N-[1-(4-methyl-3-pentenyl)-4-piperidinyl]benzeneacetamide fumarate). Moreover, antagonists of the M1, M2, M3 and M4 receptors *per se* diminished the intensity of secretion, which suggests a putative up-regulation of the release by endogenous ACh.

1. Introduction

Muscarinic cholinoreceptors regulate a wide spectrum of processes in the central and peripheral nervous systems [1]. It has been demonstrated that these receptors participate in the autoregulation of synaptic transmission in the neuromuscular junction of vertebrates [2–6]. However, the suggestions on the functional role of muscarinic receptors and the molecular mechanisms triggered by their activation remain contradictory. Arenson [2,7] and Santafe et al. [8] demonstrated that the expressiveness and the direction of muscarinic regulation of secretion depended on the level of extracellular calcium: the most prominent changes in the quantal release evoked by low-frequency nerve stimulation were identified at a subnormal concentration of calcium ions in the bathing solution. At a physiologically relevant level of calcium ions in the medium, the effects of muscarinic agents were evi-

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