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THE INVOLVEMENT OF P2Y12 RECEPTORS, NADPH OXIDASE, AND LIPID RAFTS IN THE ACTION OF EXTRACELLULAR ATP ON SYNAPTIC TRANSMISSION AT THE FROG NEUROMUSCULAR JUNCTION

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Abstract—Adenosine 5'-triphosphate (ATP) is the main co-transmitter accompanying the release of acetylcholine from motor nerve terminals. Previously, we revealed the direct inhibitory action of extracellular ATP on transmitter release via redox-dependent mechanism. However, the receptor mechanism of ATP action and ATP-induced sources of reactive oxygen sources (ROS) remained not fully understood. In the current study, using microelectrode recordings of synaptic currents from the frog neuromuscular junction, we analyzed the receptor subtype involved in synaptic action of ATP, receptor coupling to NADPH oxidase and potential location of ATP receptors within the lipid rafts. Using subtype-specific antagonists, we found that the P2Y13 blocker 2-[(2-chloro-5-nitrophenyl)azo]-5-hydroxy-6-methyl-3-[(phosphonoxy)methyl]-4-pyridinecarboxaldehyde did not prevent the depressant action of ATP. In contrast, the P2Y12 antagonist 2-methylthioadenosine 5'-monophosphate abolished the inhibitory action of ATP, suggesting the key role of P2Y12 receptors in ATP action. As the action of ATP is redox-dependent, we also tested potential involvement of the NADPH oxidase, known as a common inducer of ROS. The depressant action of extracellular ATP was significantly reduced by diphenyleneiodonium chloride and 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, two structurally different inhibitors of NADPH oxidase, indicating that this enzyme indeed mediates the action of ATP. Since the location and activity of various receptors are often associated with lipid rafts, we next tested whether ATP-driven inhibition depends on lipid rafts. We found that the disruption of lipid rafts with

methyl-beta-cyclodextrin reduced and largely delayed the action of ATP. Taken together, these data revealed key steps in the purinergic control of synaptic transmission via P2Y12 receptors associated with lipid rafts, and identified NADPH oxidase as the main source of ATP-induced inhibitory ROS at the neuromuscular junction. Our data suggest that the location of P2Y receptors in lipid rafts speeds up the modulatory effect of ATP. Uncovered mechanisms may contribute to motor dysfunctions and neuromuscular diseases associated with oxidative stress. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: neuromuscular junction, synaptic transmission, ATP, P2Y receptor, NADPH oxidase, lipid rafts.

INTRODUCTION

Over 30 years ago, it was demonstrated that adenosine 5'-triphosphate (ATP) is released from the motor nerve endings in the neuromuscular junction along with the major transmitter acetylcholine (ACh) (Silinsky, 1975). The extracellular pool of ATP, currently accepted as an important signaling molecule for intercellular communications, may be further boosted by ATP release from perisynaptic Schwann cells (Liu et al., 2005) and from the muscles (Smith, 1991; Santos et al., 2003). The latter was first reported in a seminal paper by Forrester and Lind (1969). The main functional role of extracellular ATP at the neuromuscular junction is supposed to be the modulation of transmitter release during motor activity (Silinsky et al., 1999) through ATP-sensitive P2 receptors (Giniatullin and Sokolova, 1998) or via degradation to adenosine-operating P1 receptors (Ribeiro and Sebastião, 1987, for review, Cunha and Ribeiro, 2000).

In our previous studies, we showed the direct inhibitory action of ATP on transmitter release at the neuromuscular junction (Giniatullin and Sokolova, 1998) and that this effect was mediated by metabotropic P2Y presynaptic receptors coupled to specific second messenger cascades (Sokolova et al., 2003; Giniatullin et al., 2005; Grishin et al., 2005). Similarity in the action of ATP and adenosine 5'-diphosphate (ADP) (Ribeiro and Walker, 1975) and its stable analog 2-MeSADP (Giniatullin et al., 2005) suggested involvement of ADP-sensitive P2Y1, P2Y12, or P2Y13 receptor subtypes as targets for extracellular ATP in neuromuscular junction. However, the inhibitory effect of 2-MeSADP was not

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Abbreviations: 2-MeSAMP, 2-methylthioadenosine 5'-monophosphate; ACh, acetylcholine; ATP, adenosine 5'-triphosphate; ADP, adenosine 5'-diphosphate; AEBSF, 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride; CholOx, cholesterol oxidase; DPI, diphenyleneiodonium chloride; EPCs, end-plate currents; MCD, methyl-beta-cyclodextrin; MEPCs, miniature end-plate currents; NOX, NADPH oxidase; ROS, reactive oxygen species.