

**NEUROSCIENCE****RESEARCH ARTICLE**A. Bernareggi et al. / *Neuroscience* 383 (2018) 1–11**Adenosine Promotes Endplate nAChR Channel Activity in Adult Mouse Skeletal Muscle Fibers via Low Affinity P1 Receptors**Annalisa Bernareggi,<sup>a,b\*</sup> Elisa Ren,<sup>a,b</sup> Arthur Giniatullin,<sup>c</sup> Elisa Luin,<sup>a,b</sup> Marina Sciancalepore,<sup>a,b</sup> Rashid Giniatullin<sup>d,e</sup> and Paola Lorenzon<sup>a,b</sup><sup>a</sup> Department of Life Sciences, University of Trieste, Trieste, Italy<sup>b</sup> B.R.A.I.N., Centre for Neuroscience, Trieste, Italy<sup>c</sup> Department of Physiology, Kazan State Medical University, Kazan, Russia<sup>d</sup> Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia<sup>e</sup> A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

**Abstract**—Adenosine is a powerful modulator of skeletal neuromuscular transmission, operating *via* inhibitory or facilitatory purinergic-type P1 receptors. To date, studies have been focused mainly on the effect of adenosine on presynaptic P1 receptors controlling transmitter release. In this study, using two-microelectrode voltage-clamp and single-channel patch-clamp recording techniques, we have explored potential postsynaptic targets of adenosine and their modulatory effect on nicotinic acetylcholine receptor (nAChR)-mediated synaptic responses in adult mouse skeletal muscle fibers *in vitro*.

In the whole-mount neuromuscular junction (NMJ) preparation, adenosine (100  $\mu$ M) significantly reduced the frequency of the miniature endplate currents (MEPCs) and slowed their rising and decay time. Consistent with a postsynaptic site of action, adenosine and the potent P1 receptor agonist NECA significantly increased the open probability, the frequency and the open time of single nAChR channels, recorded at the endplate region. Using specific ligands for the P1 receptor subtypes, we found that the low-affinity P1 receptor subtype  $A_{2B}$  was responsible for mediating the effects of adenosine on the nAChR channel openings. Our data suggest that at the adult mammalian NMJ, adenosine acts not only presynaptically to modulate acetylcholine transmitter release, but also at the postsynaptic level, to enhance the activity of nAChRs. Our findings open a new scenario in understanding of purinergic regulation of nAChR activity at the mammalian endplate region. © 2018 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words:** adenosine, adult skeletal muscle, MEPC, nAChR, P1 receptor.

**INTRODUCTION**

Adenosine and ATP, acting extracellularly *via* purinergic-type receptors (Burnstock, 1972; Burnstock and Verkhratsky, 2009), are known to play important roles in numerous biological processes. Among them, adenosine represents an important autocrine modulator of nicotinic cholinergic synaptic activity (Cunha and Sebastião, 1993; Ribeiro et al., 1996; Todd and Robitaille, 2006). At the skeletal neuromuscular junction (NMJ), the extracellular concentration of adenosine is mainly controlled by ATP, co-released at the nerve terminals with acetylcholine (ACh) and converted into adenosine by ectoen-

zymes. Adenosine itself is also released into the extracellular compartment from muscle cells (Lyngé et al., 2001). At rest, the extracellular concentration of adenosine at the NMJ is estimated to be around 10 nM, whereas during muscle contraction, it increases up to the  $\mu$ M range (Smith, 1991; Cunha and Sebastião, 1993). It is now widely accepted that adenosine modulates the release of ACh through the activation of P1-type receptors (P1Rs), known to be expressed presynaptically on cholinergic nerve terminals (Giniatullin and Sokolova, 1998; De Lorenzo et al., 2004; Baxter et al., 2005; Tomàs et al., 2014; Nascimento et al., 2014). Besides this, the presence of postsynaptic P1Rs, and specifically  $R_{A_{2B}}$  and  $R_{A_3}$  subtypes, has also been recently reported by immunocytochemistry and Western Blotting analysis at the postsynaptic side of the mouse NMJ (García et al., 2013, 2014). This finding opens a new scenario, with adenosine as a potential endogenous modulator of cholinergic neurotransmission also acting at

\*Correspondence to: A. Bernareggi, Department of Life Sciences, University of Trieste, Via A. Fleming 22, I-34127 Trieste, Italy.

E-mail address: [abernareggi@units.it](mailto:abernareggi@units.it) (A. Bernareggi).

**Abbreviations:** HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MEPCs, miniature endplate currents; nAChR, nicotinic acetylcholine receptor; NMJ, neuromuscular junction; P1Rs, P1-type receptors.