ADENOSINE AND LOW AFFINITY P1 RECEPTOR-MEDIATED MODULATION OF NACHR CHANNEL ACTIVITY AT THE ENDPLATE REGION OF ADULT MOUSE SKELETAL MUSCLE FIBRES

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Adenosine is a powerful modulator of skeletal neuromuscular transmission, operating via inhibitory or facilitatory purinergic-type P1 receptors. 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 ectoenzymes. Adenosine itself is also released into the extracellular compartment from muscle cells. 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 µM range. 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. Besides this, the presence of postsynaptic P1Rs, and specifically R_{A2B} and R_{A3} subtypes, has also been recently reported by immunocytochemistry and Western Blotting analysis at the postsynaptic side of the mouse NMJ 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 µM) 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 A2B 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.

SENSITIVITY OF HIPPOCAMPAL SLICES OF NEWBORN RATS WITH PRENATAL HYPERHOMOCYSTEINEMIA TO 4-AMINOPYRIDINE-INDUCED SEIZURE-LIKES EVENTS

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Homocysteine, a thiol-containing amino acid derived from dietary methionine through demethylation. The breakage of metabolism due to genetic alteration in metabolic enzymes or deficiency in cofactors may lead to hyperhomocysteinemia. Abnormal accumulation of homocysteine during pregnancy induces learning deficits in offspring at early postnatal development. It was shown, that HHCy can contribute to seizures in patients with Down syndrome, depression and in alcohol withdrawal. The goal of this study was to estimate the sensitivity of hippocampal slices of newborn rats with prenatal hyperhomocysteinemia (pHHCy) to 4-aminopyridine–induced seizure-likes events (SLE). Experiments were performed on slices of Wistar rats during second and third postnatal weeks (P9-19) using extracellular field electrodes in the CA3 pyramidal cell layer of hippocampus. To determinate the threshold of 4-aminopiridine for generation of SLE the convulsant was added by increasing doses. Pups with pHHCy were born from females received daily methionine with food. In control group application of 15-35 μM 4-aminopyridine induced a gradual increase of the frequency of multiunit activity of hippocampus neurons. In concentration of 50-75 μM 4-aminopyridine induced SLE in 75% cases (n=15) after 4.3±0.9 min of perfusion. In slices prepared from the hippocampus of rats with pHHCy the elevation of background neuronal firing was observed and application of 15-35 μM 4-aminopyridine induced SLE in 88% of the cases with 6.0±0.6 min of seizure onset (n=26). Our findings indicate that pHHCy significantly lowers the threshold of 4-aminopyridine–induced SLE. It is known that homocysteine and its metabolites are potent agonists of NMDA-receptor, which are linked with epileptogenesis. It is possible that pHHCy can induce the hyperexcitability of neuronal network of immature hippocampus by stimulating NMDA-receptors and changing the electrophysiology property of neurons.

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