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REDOX-SENSITIVE SYNCHRONIZING ACTION OF ADENOSINE ON TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION

A. TSENTSEVITSKY,[a] I. KOVYAZINA,[a] E. NIKOLSKY,[a,b] E. BUKHARAEVA[a,b]* AND R. GINIATULLIN[c]

[a] *Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, P.O. Box 30, Kazan 420111, Russia*

[b] *Kazan State Medical University, Butlerov Street 49, Kazan 420012, Russia*

[c] *Department Neurobiology, A. I. Virtanen Institute, University of Eastern Finland, P.O. Box 1627, Neulaniementie 2, 70211 Kuopio, Finland*

Abstract—The kinetics of neurotransmitter release was recognized recently as an important contributor to synaptic efficiency. Since adenosine is the ubiquitous modulator of presynaptic release in peripheral and central synapses, in the current project we studied the action of this purine on the timing of acetylcholine quantal release from motor nerve terminals in the skeletal muscle. Using extracellular recording from frog neuromuscular junction we tested the action of adenosine on the latencies of single quantal events in the pro-oxidant and antioxidant conditions. We found that adenosine, in addition to previously known inhibitory action on release probability, also synchronized release by removing quantal events with long latencies. This action of adenosine on release timing was abolished by oxidants whereas in the presence of the antioxidant the synchronizing action of adenosine was further enhanced. Interestingly, unlike the timing of release, the inhibitory action of adenosine on release probability was redox-independent. Modulation of release timing by adenosine was mediated by purinergic A1 receptors as it was eliminated by the specific A1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and mimicked by the specific A1 agonist N(6)-cyclopentyl-adenosine. Consistent with data obtained from dispersion of single quantal events, adenosine also reduced the rise-time of multiquantal synaptic currents. The latter effect was reproduced in the model based on synchronizing effect of adenosine on release timing. Thus, adenosine which is generated at the neuromuscular junction from the breakdown of the co-transmitter ATP induces the synchronization of quantal events. The effect of adenosine on release timing should preserve the fidelity of synaptic transmission via ''cost-effective'' use of less transmitter quanta. Our findings also revealed important crosstalk between purinergic and redox modulation of synaptic processes which could take place in the elderly or in neuromuscular diseases associated with oxidative stress like lateral amyotrophic sclerosis.

Key words: neuromuscular junction, kinetics of transmitter release, release timing, adenosine, reactive oxygen species, redox modulation.

INTRODUCTION

Accumulating evidences suggest that the synaptic transmission in central and peripheral nervous system could be modulated via changes in the probability of transmitter release or through regulation of release timing (Van der Kloot, 1988; Bukharaeva et al., 2002, 2007; Lin and Faber, 2002). At the neuromuscular junction extracellular ATP and its degradation product adenosine are well known modulators of synaptic transmission (Ginsborg and Hirst, 1972; Cunha and Sebastião, 1993; Redman and Silinsky 1993; Giniatullin and Sokolova, 1998). Adenosine could be released per se (Dunwiddie, 1980; Cunha and Sebastião, 1993; Lovatt et al., 2012) or generated from the extracellular hydrolysis of ATP co-released together with acetylcholine in Ca^{2+}-dependent manner (Ribeiro and Sebastião, 1987; Redman and Silinsky, 1994; Ribeiro et al., 1996). At the neuromuscular junction adenosine inhibits transmitter release via A1 receptors (Redman and Silinsky, 1993; Mei et al., 1996; Giniatullin and Sokolova, 1998; Perissinotti and Uchitel, 2010). The functional role of adenosine-mediated inhibition is considered to be 'neuroprotective' and directed to minimize the excessive synaptic and neuronal activity (Ribeiro and Sebastião, 1987; Meriney and Grinell, 1991; Boison, 2008; Sperlágh and Vizi, 2011).

Unlike the well-known action of adenosine on the probability of transmitter release its action on the kinetics of transmitter release from motor terminals remains unknown. Importantly, synchronization of transmitter release could enhance the efficiency of synaptic transmission using less quanta comparing with asynchronous release (Wood and Slater, 2001; Bukharaeva et al., 2002, 2007; Lin and Faber, 2002; Gilmanov et al., 2008). Previously we showed that the action of parent nucleotide, extracellular ATP, on transmitter release at the neuromuscular junction is redox-dependent (Giniatullin et al., 2006). Recently we also found that the inhibitory action of the mild oxidant H_2O_2 is associated with changed timing of quantal

*Correspondence to: E. Bukharaeva, Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, P.O. Box 30, Kazan, 420111, Russia. Tel: +7843-2927647; fax: +7843-2927347.
E-mail addresses: elbukhara@gmail.com, ellyab@mail.ru (E. Bukharaeva), Rashid.Giniatullin@uef.fi (R. Giniatulli.

Abbreviations: CPA, N(6)-cyclopentyl-adenosine; DPCPX, 8-cyclopentyl dipropyixanthine; EPCs, end-plate currents; *m*, quantal content; MEPCs, miniature end-plate currents; NAC, *N*-acetyl-cysteine; ROS, reactive oxygen species.

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release (Tsentsevitsky et al., 2011). One potential mechanism for the redox-dependent depression of transmitter release is the inactivation of the key presynaptic SNARE protein Snap25 (Giniatullin et al., 2006). Interestingly, Snap25 likely determines the kinetics of transmitter release in certain synapses (Sakaba et al., 2005). Some data indicate that SNARE proteins could also be involved in the presynaptic action of adenosine (Searl and Silinsky, 2006).

In the current project we tested potential redox-dependent action of adenosine on quantal release from the frog motor nerve terminals, a classic model to study transmitter release and its timing (Katz and Miledi, 1965). By analyzing synaptic delays of uni-quantal synaptic events we show here that adenosine induces synchronization of transmitter release via inhibitory A1 receptors. Unlike the action of adenosine on the probability of release the effect of this endogenous neuromodulator on release timing was redox-dependent since it was eliminated by pro-oxidants whereas in the presence of the antioxidant the synchronization action of adenosine was further increased.

EXPERIMENTAL PROCEDURES

Preparation

The *cutaneous pectoris* muscle with preserved motor nerve was isolated from anesthetized with ether frogs *Rana ridibunda*. The neuromuscular preparation pinned to the bottom of the recording chamber was continuously rinsed with the Ringer solution. All experiments were performed according with European Communities Council Directive (24th November 1986; 86/609/EEC). All efforts were made to minimize the number of animals used for these studies.

Electrophysiological recordings

Neuromuscular preparation was superfused (3 ml/min) with solution containing (all in mM): NaCl 113.0; KCl 2.5; $NaHCO_3$ 3.0; $MgCl_2$ 4.0; $CaCl_2$ 0.3; pH = 7.3; temperature = 20.0 ± 0.3 °C. The motor nerve was stimulated by the supramaximal rectangular impulses with duration of 0.1 ms and stimulating frequency of 0.5 s^{-1}. The motor nerve action potentials and end-plate currents were registered extracellularly with the Ringer filled glass micropipettes (2.0–4.0 MΩ resistance) from the proximal part of the nerve terminal (placed 5–10 μm from the end of the last myelinated nerve segment). The time-course of quantal release was reconstructed from the delays of uni-quantal synaptic events as previously described (Bukcharaeva et al., 1999; Nikolsky et al., 2004; Kovyazina et al., 2010). From 250 to 400 uni-quantal events were recorded, digitized at the sampling frequency of 5 μs and stored at the hard disc of the personal computer. Special attention was paid to the invariance of the amplitude and temporal parameters of nerve ending currents to provide reliable long-lasting extracellular recording of uni-quantal end-plate currents. Only those recordings were taken into consideration that did not demonstrate any changes in the nerve ending current parameters. Multiquantal extracellular end-plate currents (EPC) and spontaneous miniature EPCs (MEPCs) were recorded when the Ringer's solution contained 0.9 mM $CaCl_2$ and 6.0 mM $MgCl_2$.

Analysis of data

Signals were analyzed off-line using the software developed in our laboratory. Synaptic delays were estimated from the time interval between the nerve sodium spike to the early (20% of maximum amplitude) phase of EPC. For quantitative analysis of the time-course of evoked release of neurotransmitter quanta we plotted the delay histograms of the uni-quantal EPCs and corresponding cumulative curves (Bukcharaeva et al., 1999; Nikolsky et al., 2004). Cumulative curves were used to estimate the parameter P_{90} – the interval including 90% of all synaptic delays – which characterizes the degree of synchronicity of transmitter release. The mean value of the shortest 5% of delays was taken as the minimal synaptic delay. The following parameters of spontaneous MEPCs and evoked multiquantal EPCs signals were analyzed: amplitude, 20–80% rise-time, and e-fold decay time (τ). The mean quantal content of end-plate currents (m) was determined using the ''method of failures'' (Del Castillo and Katz, 1954).

Drugs

The following drugs were used (all from Sigma–Aldrich, St. Luis, MO, USA): adenosine, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), $FeSO_4$, *N*-acetyl-cysteine (NAC), N(6)-cyclopentyl-adenosine (CPA), H_2O_2. The drugs were added to the superfusing solution, and the measurements started 20 min after the drug application.

Statistics

Data are presented as mean ± SEM for the experiment. n is the number of animals. Comparison between data from individual synapses before and after drug application was performed using paired Student's t-test. The statistical significance of the differences between two cumulative curves was determined by the Kolmogorov–Smirnov statistic (Bronstein and Semendjaev, 1986). Mann–Whitney test was used to compare the non-parametric data. Statistical analysis was performed by means of the Microcal Origin software. $P < 0.05$ was taken as significant.

RESULTS

The action of adenosine on the timing and probability of release

In control the timing of transmitter release was highly dispersed as follows from the very variable distribution of the latencies of uni-quantal EPCs (Fig. 1A). Adenosine (100 μM) applied for 20 min essentially shortened the timing of transmitter release predominantly removing quanta with long latencies (Fig. 1B). This result is presented as the reduced