On the history of ecto-ATPases: The role of W. A. Engelhardt

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The concept of purinergic signalling is based on three key observations. (1) In 1929, Drury and Szent-Györgyi crystallized the physiologically active substance and concluded that it was adenylic acid. They also reported that this substance, nowadays referred to as AMP, affects contraction of the mammalian heart and blood vessels [1]. After more than a 20-year lag phase, the actions of extracellular purines were confirmed in all types of cells studied so far (for more details, see [2]). (2) In the early 1970s, Burnstock and co-workers demonstrated that “non-adrenergic, non-cholinergic” regulation of smooth muscle contraction was mediated by ATP as a co-neurotransmitter [3–5]. (3) Since the early 1990s, four P1, seven P2X and eight P2Y receptors have been identified and functionally characterized [6]. At the same time, more than ten ectonucleotidases, including ecto-ATPases, were cloned from the mammalian genome [7, 8]. These enzymes protect purinoceptors from desensitization and downregulation via the rapid normalization of extracellular nucleotide concentration and may be considered as analogues of acetylcholine esterase and Na⁺-coupled noradrenaline transporter in cholinergic and adrenergic signalling, respectively.

To the best of my knowledge, the presence of ectonucleotidases was initially demonstrated by Wladimir A. Engelhardt, who was Professor in the Department of Biochemistry at M. V. Lomonosov Moscow State University at the time when I was a graduate student in the Department of Biophysics of the same University. In 1930, he observed almost instant degradation of intracellular nucleosides to inorganic phosphate after haemolysis induced by freezing and thawing of avian erythrocytes [9]. It should be underlined that this and his following studies focused on the negative correlation between respiration and the accumulation of inorganic phosphate [10, 11]. Because of this, nucleoside breakdown by extracellular enzyme(s) did not attract attention to the background of this internationally well-recognized discovery of oxidative phosphorylation. In 1939, Dr. Engelhardt and Militza Lyubimova, his wife and former postgraduate student, reported data on the ATPase activity of skeletal muscle myosin [12]. This second outstanding discovery led to the concept of ATP as a universal intracellular source of energy used for diverse cellular functions. Later on, Dr. Engelhardt studied the nature of the Pasteur effect that governs interrelations between respiratory and anaerobic metabolism. These experiments were completed in the spring of 1941, i.e. a few months before invasion by the Nazis and 2 years before his postgraduate student and co-author Nickolai Sakov perished on the battlefields of Stalingrad. At that time, it was not possible to send the paper to a Western journal. Thus, data on the key role of redox regulation of phosphofructokinase in the Pasteur phenomenon were published in a Russian journal [13] and remained almost completely unknown.

After the Second World War, Dr. Engelhardt continued to study the origin of rapid degradation of intracellular nucleotides evoked by cell rupture by comparing the kinetics

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of ATP degradation in intact and haemolysed pigeon erythrocytes. In collaboration with his postgraduate student Tatyana Wenkstern, he found that ~98% of total ATPase is firmly localized on the exterior surface of the cells, and its catalytic centre is exposed to the extracellular milieu. In a paper submitted to the Proceedings of the Academy of Sciences of the USSR on 4 January 1955, they called this highly active enzyme “ecto-ATPase” to underline its difference from exoenzymes secreted by cells [14]. In contrast to nucleated avian erythrocytes, they failed to register any ecto-ATPase activity in rabbit erythrocytes, which was consistent with the negative results obtained in mammalian erythrocytes by Garzo and co-workers [15]. Adenylpyrophosphatases were also detected on the surface of ascites tumour cells [16] as well as in human erythrocytes [17], but its activity was ~100-fold lower than in nucleated avian erythrocytes and below detection limits of methods employed in previous experiments with mammalian erythrocytes [14, 15]. Later on, Wenkstern and Engelhardt found that ecto-ATPase in avian, amphibian and fish erythrocytes was activated by extracellular Mg^{2+} and completely blocked by ethylenediaminetetraacetate (EDTA) [18, 19]. They suggested that ectonucleotidases may be involved in the regulation of extracellular adenylyl nucleosides, known to be potent physiologically active compounds [14]. It should be noted, however, that the role of intracellular ATP as a source of intracellular energy was dominated by the discussion of possible ecto-ATPase functions. Even in 1982, when the concept of purinergic signalling had already been formulated, Dr. Engelhardt wrote [20]: “The biological role of the ecto-ATPase represents an intriguing mystery. We have to suppose that the enzyme never meets its substrate, for there is no measurable amount of ATP in the blood plasma. Perhaps, it is a peculiar ontogenetic relic from the process of erythropoiesis”.

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