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Review article

Role of the purinergic signaling in epilepsy

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ABSTRACT

Adenine nucleotides and adenosine are signaling molecules that activate purinergic receptors P1 and P2. Activation of A1 adenosine receptors has an anticonvulsant action, whereas activation of A2A receptors might initiate seizures. Therefore, a significant limitation to the use of A1 receptor agonists as drugs in the CNS might be their peripheral side effects. The anti-epileptic activity of adenosine is related to its increased concentration outside the cell. This increase might result from the inhibition of the equilibrative nucleoside transporters (ENTs). Moreover, the implantation of implants or stem cells into the brain might cause slow and persistent increases in adenosine concentrations in the extracellular spaces of the brain. The role of adenosine in seizure inhibition has been confirmed by results demonstrating that in patients with epilepsy, the adenosine kinase (ADK) present in astrocytes is the only purine-metabolizing enzyme that exhibits increased expression. Increased ADK activity causes intensified phosphorylation of adenosine to 5'-AMP, which therefore lowers the adenosine level in the extracellular spaces. These changes might initiate astrogliosis and epileptogenesis, which are the manifestations of epilepsy. Seizures might induce inflammatory processes and vice versa. Activation of P2X7 receptors causes intensified release of pro-inflammatory cytokines (IL-1β and TNF-α) and activates metabolic pathways that induce inflammatory processes in the CNS. Therefore, antagonists of P2X7 and the interleukin 1β receptor might be efficient drugs for recurring seizures and prolonged status epilepticus. Inhibitors of ADK would simultaneously inhibit the seizures, prevent the astrogliosis and epileptogenesis processes and prevent the formation of new epileptogenic foci. Therefore, these drugs might become beneficial seizure-suppressing drugs.

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Introduction: relations between purinergic signaling in the central nervous system (CNS) and epilepsy

The aim of this review is to focus the reader's attention to the possible prospects underlying the relations between P1 and P2

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receptors and their agonists for the development of new therapies for epilepsy. Specifically, there is a sequence of biochemical processes that ranges from elevated activity of adenosine kinase (ADK) through the induction of astrogliosis and epileptogenesis and finally to the manifestation of epilepsy (Fig. 1).

Epilepsy is a common neurological disease that affects approximately 0.5%–1.0% of the general population. Hart and Sander [1] cited a definition of epilepsy according to the International Seizure Classification and put forward by the International League Against Epilepsy (ILAE) that is based on the clinical and electroencephalographic manifestations of seizures [2]. This definition is as follows: "the occurrence of transient paroxysms of excessive or uncontrolled discharges of neurons, which may be caused by a number of different etiologies, leading to epileptic seizures". Generally, the classification of epileptic seizures divides partial seizures into simple and complex partial seizures. In general, seizures are divided into generalized tonic-clonic seizures, absence seizures, myoclonic seizures, atonic and tonic seizures [1]. The essence of seizure is hyperexcitability of the neurons of the cerebral cortex caused by an imbalance of synaptic excitation and inhibition [3,4]. Recently, results of research on adenine nucleosides and nucleotides have suggested that these molecules are related to epilepsy. Therefore, these molecules could potentially be used in the treatment of epilepsy.

Nucleotides, such as ATP, ADP, UTP and UDP, and the nucleoside adenosine activate the following two classes of P-type receptors: adenosine P1 receptors and nucleotide P2 receptors [5]. The P1 receptors (metabotropic, i.e., cooperating with G proteins) are divided into sub-types A1, A2A, A2B and A3 [6].

In the CNS, adenosine receptors are located in nerve cells and glial cells (astrocytes and microglia). There are some controversies regarding the presence of A2B receptors on microglial cells. In recent years, the only reliable report regarding their presence is a paper by Koscsó et al. who proved that adenosine increases the release of IL-10 via the activation of A2B receptors on the microglial cells [7]. Adenosine is generated inside and outside the cells by the hydrolysis of AMP by 5'-nucleotidase [8]. Adenosine might also be released directly *via* exocytosis from neurons and astrocytes. Adenosine is metabolized into AMP by adenosine kinase (ADK) or into inosine by adenosine deaminase [8] (Fig. 2).

Based on the mechanism of signal transmission, P2 receptors are divided into the following two sub-types: ionotropic P2X receptors and metabotropic P2Y receptors. Both types of receptors and the enzymes that participate in ecto-nucleotide conversions, such as NTPDases and 5′-nucleotidase (CD73), are present in neurons, astrocytes, oligodendrocytes, both types of glial cells and arterial endothelial cells. In addition to P2X7, P2X4, P2Y6 and P2Y12 receptors are also present on microglial cells and participate in inflammatory processes [9,10].

In the extracellular space, ecto-nucleotidases (E-NTPDases) lower the nucleotide concentration by hydrolyzing extracellular ATP and ADP, which results in the release of agonists from the P2 receptors; in this manner, the signals mediated by nucleotides are terminated [11]. In turn, ecto-5'-nucleotidase hydrolyzes AMP into adenosine, which is an another signaling molecule and a ligand of the P1 receptors. The cellular membranes of the brain core and hippocampal neurons exhibit high levels of NTPDase1 and NTPDase2 activity, whereas low activity levels of these enzymes are found in the cerebellum and medulla oblongata [11]. High levels of activity of ecto-5'-nucleotidase and ecto-adenosine deaminase are found in the majority of brain regions [11]. Adenosine kinase (ADK) participates in the conversion of adenosine to 5'-AMP. High levels of expression of this enzyme have been proven in astrocytes.

Pro-convulsive and pro-inflammatory action of ATP

Results of the recent research on purinergic signaling are already being applied in the treatment of the Parkinson's disease (i.e., istradefylline) and cerebral ischemic stroke (i.e., clopidogrel, ticlopidine, and dipyridamole) and might be useful in the treatment of multiple sclerosis, migraine and neuropathic pain [12–18]. One example of a pathological process that results from a disturbed balance between ATP and adenosine is the inflammatory processes that constitute the basis of many CNS diseases. Activations of P1 receptors by adenosine and P2 receptors by ATP play opposite roles in the induction of inflammatory processes. The dominant role in inflammatory processes is played by P2X7 receptors, whereas the activation of adenosine receptors leads to the suppression of these processes. Under physiological conditions, the processes activated by ATP are suppressed by the adenosine formed during ATP degradation [19]. Therefore, a balance exists between the stimulation of these processes by ATP and their extinction by adenosine. Consequently, disturbances in this balance might cause many neurological diseases.

ATP concentrations vary across brain structures. The highest concentrations occur in the hippocampus and pulvinar cells [20]. ATP is not only a signaling molecule but also affects the signaling mediated by other neurotransmitters. There is a correlation between the amounts of ATP and catecholamines released in the locus coeruleus and the hippocampus [21–23]. In the hippocampus, a correlation has been demonstrated between the concentrations of ATP and glutamate [24]. In convulsive seizures, ATP is released from astrocytes in response to an increase in neuronal activity. Extracellular ATP stimulates inflammatory processes via the activation of P2 receptors, primarily the P2X7 receptor. This stimulation results in increased secretion of proinflammatory cytokines, such as the matured form of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) and in the proliferation of microglia and lymphocytes [25]. Outside the cell, ATP is degraded by ecto-enzymes (E-NTPDase, E-NPP, alkaline phosphatases, ecto-5' nucleotidase) into adenosine.

During an epileptic seizure, the extracellular ATP concentration increases, which might be connected to the decrease in ATPase activity [26,27]. Outside the cell, ATP exhibits pro-convulsive action, which has been proven to result in increased motor convulsive seizures based on microinjections of ATP analogs into the rat brain core [28]. ATP released into the intercellular space acts during the initial few minutes of an epileptic seizure as an excitotoxic molecule, whereas in the prolonged period, ATP causes neuroinflammation [27]. Therefore, ATP induces necrosis/apoptosis in CNS but also induces microglia proliferation [27]. ATP acts in these processes, particularly astrogliosis, *via* the activation of P2X7, P2Y1 and P2Y2 receptors [27]. Therefore, in the future, antagonists of P2 receptors might become efficient anti-epileptic drugs.

The P2X7 receptor of the rat brain was cloned for the first time in 1996 [29]. In the brain this receptor most frequently occurs in the homomeric form, although the presence of its heteromeric

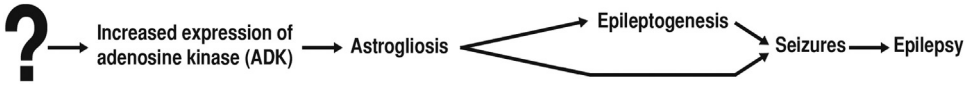

Fig. 1. Sequence of the metabolic processes that lead to the manifestation of epilepsy. The reason for the increase in ADK activity in the initial step of this chain of events is currently unknown.