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# Quantitative and Qualitative Changes in the $Ca^{2+}$, ATP-Adenosine Cycle and ROS Metabolisms Depending On the Strength of Danger Signals during Apoptosis and Necrosis Compared to Homeostasis in Various Danger/Stress Situations

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**Abstract**

In earlier publications there are only separately mentioned that the danger/stress signals induce increased intracellular $Ca^{2+}$ levels and concomitant release of adenosine triphosphate (ATP) accompanied with the production of reactive oxygen species (ROS). In such situations extracellular ATP acting on purinergic receptors type 2 (P2) can trigger the release of numerous mediator molecules helping the defence of cells. Additionally, extracellular adenosine (Ado) formed from these ATP molecules are acting on P1 receptors and can moderate and control the release of various mediators preventing the hyperactivity of cells maintaining their survival, the repair of ATP pools or the induction of new pathologic states.

In this study, we show three synthesized models based on earlier articles completed with some new private observations: a.) "Synthesized model of extracellular ATP – adenosine danger cycle"; b.) Model of cellular homeostasis, apoptosis and necrosis in various danger/stress situations related to the levels of $Ca^{2+}$, ATP and adenosine depending on the strength of danger signals"; c.) Model of intracellular, extracellular and systemic elements of stress responses related to the concentrations of molecules of ATP-adenosine danger cycle and ROS in relation to the production of ACTH and cortisol. These models demonstrate that depending on the strength of various danger/stress signals quantitative and qualitative changes are taking place in the cells

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concerning the metabolism of $Ca^{2}$+, ATP-Ado, and ROS involving: a.) "transient loss of ATP with repairable loss of ATP, b.) "apoptosis" (programmed cell death) with irreversible loss of ATP or c.) "necrosis," total loss of ATP and viability inducing a secondary inflammation in the organism. It is supposed that especially high and individually different amounts of extracellular ATP, Ado and ROS metabolites are required to reach the state of "systemic stress" based on increased production of ACTH and cortisol.

## Introduction

"The fight -or- flight response or acute stress response is a physiological reaction that occurs in response to a perceived harmful event, attack or threat to survival". This idea was firstly described by Walter Bradford Cannon [1,2]. Afterwards, it was Hans Selye who created the concept of „stress induced general adaptation syndrome" playing role in the „diseases of adaptation" [3]. The activity of stress system is based upon the cooperation between the corticotrophin-releasing hormone and locus ceruleus-norepinephrine/autonomic systems and their peripheral effectors which improve the ability of the organism to adjust homeostasis and increase its chances for survival [4]. "Danger theory" derives from Polly Matzinger [5] suggesting that molecules of "self-constituents" can trigger an immune response if they are dangerous. Danger/alarm signals are compounds released by the body's own cells: Adenosine Triphosphate (ATP), uric acid, heatshock proteins, etc. forming a „danger associated pattern" (DAMP)" [5]. Later, for natural microbiologic dangers was proposed the concept of „Pathogen Associated Pattern**"** (PAMP) [6]. All these ideas have opened important new perspectives especially for the research of inflammations.

Among the danger/stress signals of DAMP extracellular ATP was mentioned on the first place [5]. The extracellular ATP release in danger/stress situations can be related to various danger signals as follows: autophagy, hypoxia, infection, inflammation, Reactive Oxygen Species (ROS), stress, trauma, necrosis, uric acid, nuclear protein HMGB1 [6,7,8]. It is common that all these markers of danger/stress situations induce the release of ATP acting as a further "signal" molecule. Although, it is a curiosity, in a single-celled organism, intracellular Nitric Oxide (NO) mediates the ATP release as a stress signal [9]. However, the intracellular "calcium overload" was firstly regarded to be the most common starting "stressor" effect [10]. The concomitant release of ATP was recognized later [11]. It is common in these observations that extracellular ATP acts as a "signal" molecule. Therefore, in the present work two functional forms of ATP are differentiated as follows: a) "Intracellular energy ATP" as "i ATP E", b) "Extracellular signal ATP" as "e ATP S". Extracellularly ATP and adenosine (Ado) are the key chemical transmitters of purinergic signalling [12]. Cell surface ectonucleotidases (CD39 and CD73) rapidly degrade ATP to ADP, AMP and adenosine (Ado) [13,14]. The P2X and P2Y receptors mediate the signals from ATP and its derivatives, but Ado acts via A1 receptors [15]. Thus, in this work two forms of Ado are differentiated a.)"extracellular signal Ado" as "e Ado S" and b) "intracellular energy Ado" as "i Ado E".

Supposedly, in the strong "cause and effect" relation existing between danger signals and stress responses, "e ATP S" and "e Ado S" can be that stable pair of molecules which mediates the biochemical transmission of "a danger signal" to "a stress response" but both they have also vital metabolic effects for survival and regeneration.

## Results

### The synthesized model of extracellular ATP-adenosine danger cycle

In majority of danger/stress situations the starting processes are as follows: a) The increase in intracellular $Ca^{2+}$ level($Ca^{2+}$ overload) [10], b) The release of ATP and formation of "e ATP S"[15]. The quantitative extent of these response processes is corresponding to the strength of a danger signal [16]. The stronger the danger signal, the higher the ATP release. Inversely, the less the rest of "i ATP E", the stronger the danger signal.

The function of "e Ado S" formed from "e ATP S" [13] is dual: a) In the "early" phase of dangers it can stimulate ATP release [17] and controls the survival of cells preventing hyperactivity, overstimulation [18,19,20,21,22], b) in the "late" decreasing phase of dangers it supports the repair, the regeneration of intracellular ATP pools, [15,23,24,25,26] and determines the perspectives of cellular existence among the following possibilities: *homeostasis (survival),*or *apoptosis or necrosis* [2,27,25]. Necrotic cells do not have sufficient "i ATP E" anymore. However, they may still act as further danger signals provoking new inflammations [28]. The increased mitochondrial $Ca^{2+}$ overload is not only accompanied by decrease in the intracellular ATP concentration, but also by the increase in intracellular ROS generation, occurrence of mitochondrial permeability transition, and activation of caspase-9 leading to cell death [29].

The main role of "e ATP S" is to induce an intensive release of extracellular messenger mediator molecules including enzymes, cytokines, nucleotides, hormones, growth factors, lipid mediators, nitric oxide, Reactive Oxygen Species (ROS) etc. from numerous types of cells, not only from the white blood cells or endothelial cells but possibly from all ones owning P2 receptors. These reactions are serving the "defence" against the danger/stress signals [16]. In the early phase of danger situations still a lot of other mechanisms including ecto-ATP synthase [30] or even "e Ado S" derived from "e ATP S"[17], or "eATP S" itself [31] can take part in the production of large amounts of "e ATP S". The "whip effect" is related to "e ATP S" in this defence phase. In parallel, however, "e Ado S" controls the size and intensity of response processes preventing the overstimulation of cells and supporting their survival and ATP regeneration [26]. In this economic cellular regulation of "defence", "survival" and "regeneration" cycle based on the ATP-Ado crosstalk ("danger cycle"), only those cells can take parts which have P1 and P2 purinergic receptors. Supposedly, the most significant part of human cells participates in this vital cycle, but there can be some rare exceptions, too. (We found such a case among the cells of peripheral blood. K. Pázmándi, data in press).The new "Synthesized Model of Extracellular ATP and Adenosine Danger Cycle" is presented in **Figure 1.**

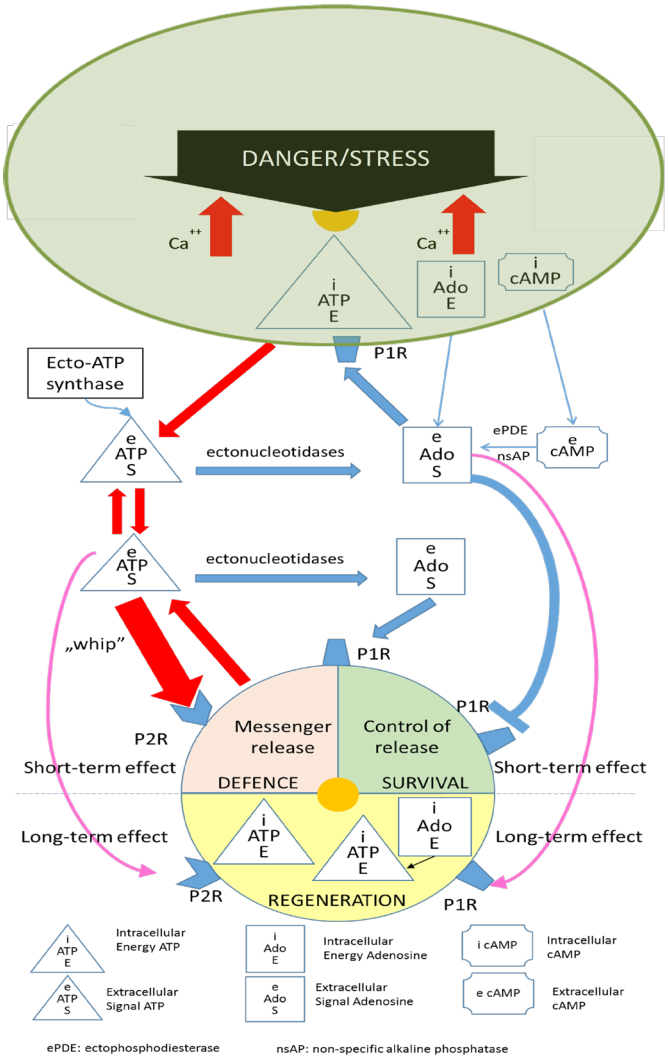


**Figure 1:** Synthesized Model of Extracellular ATP¬- Adenosine Danger Cycle.

**Regulation of cellular homeostasis, apoptosis and necrosis in various danger/stress situations related to $Ca^{2+}$, ATP and adenosine metabolism**

The concept of "homeostasis" derived from Claude Bernard and it was developed further by W. Cannon [2]. In the state of "homeostasis" the cells are free of any significant danger/stress signals. Their "i ATP E" pools are sufficiently filled up. Supposedly, only that minimal change of environment can mean a "danger/stress signal" which is able to induce a certain level of elevation in the intracellular amount $Ca^{2+}$ and causes some release of intracellular ATP. As the level of "i ATP E" decreases, "e ATP S" appears simultaneously. It is metabolized to "e Ado S" very quickly. In parallel, a part of Ado ("i Ado E") can replenish the "i ATP E" pools [24], too. One of our central ideas is that the actual concentration of intracellular ATP is the crucial marker which determines the future state of a cell varying among "survival", "apoptosis" and "necrosis". Depending on the strength of danger/stress/signals there can be some limits in the tolerability of ATP loss and the chance to regenerate the pool of "i ATP E" [27]. Several grades of cellular defence may exist for the control of survival as follows: 1) on a weak danger signal a few amount of "e ATP S" is released with a "transient" and repairable loss of ATP and the chance of survival. The compensable homeostasis remains; 2) on a stronger signal a greater release of "e ATP S" occurs but still a sufficient amount of "I ATP E" is left to avoid necrosis. However, a greater number of cells turns to apoptosis; 3) on a heavy danger/stress signal a large amount of "e ATP S" is released with a fatal loss of "i ATP E" leading to cell death, necrosis. In this phase even the P1 receptors are lost. They do not work anymore [32]. In addition, the necrotic cells can become "danger signals" themselves inducing further inflammations [28]. These processes are illustrated in **Figure 2**.

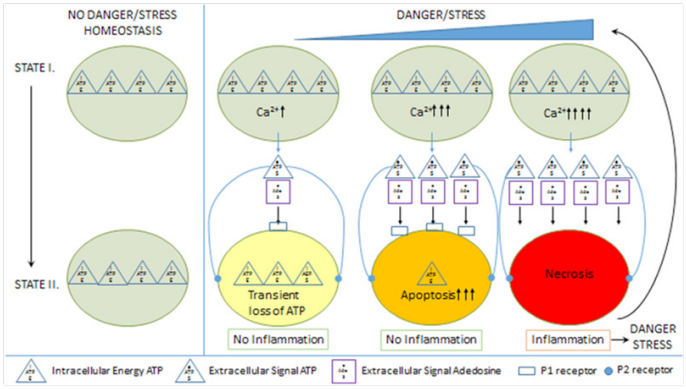


**Figure 2:** Regulation of cellular homeostasis, transient ATP loss, apoptosis and necrosis in various danger/stress situations related to the Ca2+, ATP and adenosine metabolism.

**ATP-adenosine and ROS related intracellular, extracellular and systemic basic elements of stress responses**

The molecules of "Reactive Oxygen Species (ROS)" which are produced similarly to ATP in the mitochondria play also crucial roles in stress responses [33]. Their actions can be "good", "bad" and "ugly". The last two effects are realized in the phase of "oxidative stress" provoked by extraordinarily strong danger signals [34]. The important and elegant experimental proof is relatively new [29] which demonstrates the series of events starting with the step that the increased mitochondrial $Ca^{2+}$ overload is accompanied by a decrease in the intracellular ATP concentration, a more intensive generation of ROS, an increased mitochondrial permeability transition (for ATP and ROS), and activation of caspase-9 ending with cell death. ROS molecules can result in an increase in the amount of extracellular adenosine [35] metabolized from ATP by NADPH oxidase dependent CD39 (ectonucleotidase) [36]. Thus, a generalized scheme, a model can be created synthesizing the basic elements (factors) and processes of intracellular, extracellular and systemic stress responses as follows: a) *intracellular factors:* $Ca^{2+}$ overload, decrease in "i ATP E" level, release of "e ATP S", production of ROS, release of ROS; b) *extracellular factors:* "e ATP S – "e Ado S" reactions catalysed by ROS dependent ectonucleotidase (CD39) in association with a great number of other ROS effects are resulting in an abundance of" **e Ado S;** c) the individually different but functionally sufficient high amounts of "e Ado S" can induce the release of ACTH and cortisol [37,38,39] which are the basic hormones, *factors of systemic stress* reactions. Afterwards, in the lack of physiological ceasing and moderating mechanisms, the various forms of "adaptation diseases" may come into being during severe and long lasting systemic stress situations [3]. These data are presented in **Table 1**. The high extracellular concentrations of Ado can also contribute to the symptoms of various diseases, e.g. bronchial asthma [40] or "abdominal compartment syndrome [41]. The first observations on the physiological roles of Ado derived from Drury and Szentgyörgyi [42]. However, it should be emphasized that our approach concentrates only on the ATP-Ado cycle and ROS production. There can be also other considerations analysing the complex mechanism of stress [43].

**Table 2:** $Ca^{2+}$ ATP – Adenosine and ROS related intracellular, extracellular and systemic basic elements of stress responses.

| | INTRACELLULAR RESPONSE | | | EXTRACELLULAR RESPONSE | SYSTEMIC RESPONSE |
|---|---|---|---|---|---|
| **Danger Signal** → | **$Ca^{2+}$↑** | **$Ca^{2+}$↑↑** | **$Ca^{2+}$↑↑↑** | | |
| | Transient loss of ATP | Apoptosis | Necrosis | | |
| | **i ATP E↓** | **i ATP E↓↓** | **i ATP E↓↓↓** | **→ e ATP S→CD39 → e Ado S →ACTH/Cortisol ↑** | |
| | Transient loss of ATP | Apoptosis | Necrosis | ↑ | |
| | **ROS ↑** | **ROS↑↑** | **ROS↑↑↑** | → **ROS** | |
| | Transient loss of ATP | Apoptosis | Necrosis | | |

## Discussion

The biology of purinergic signalling has ancient roots. ATP is very reactively participating in more chemical reactions than any other compound on the Earth's surface, except water. The purinergic signalling system is expressed in virtually all types of tissues and cells. It mediates numerous physiological reactions, and contributes to pathological responses in a variety of diseases. There are both short and long-term forms of purinergic signalling in the biological adaptation "where evolution brings $Ca^{2+}$ and ATP together to control life and death" [12,44]. Based on our presented hypothesis on cellular homeostasis and induction of apoptosis and necrosis, perhaps we also can make a generalizing remark as follows: "The life or death of a cell depend on the compensable or not compensable loss of its "i ATP E" content in a danger/stress situation".

From the present work it can be concluded that in every situation where the state of "homeostasis" has changed in consequence of several danger/stress effects [45] the following phenomena are common and organically coupled to each other: a) Increase in intracellular $Ca^{2+}$ level, b.) concomitant release of intracellular "energy ATP ("i ATP E") which acts further as extracellular signal ATP "e ATP S", c) production and release of ROS, d) appearance and persistence of "e Ado S" produced from "e ATP S" which takes part in the prevention of overstimulation of cells during the early phase of stress, then in the late phase, in the regeneration of "i ATP E" pools. These factors can determine the future state of a cell varying among "Survival", "Apoptosis" and "Necrosis" reflecting its "adaptation energy" [46]. For a "systemic stress state" especially and individually different high amounts of "eAdoS" should be required to sustain the increased production of ACTH and cortisol. These processes related to the "extracellular ATP-adenosine danger cycle" are summarized in the three new models of this work.

### Disclosure statement

The authors have no conflicts of interest to declare.

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