

Adenosine – A Physiological Regulator and a Distress Signal

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Abstract: The present brief review argues the case that adenosine can be both a distress signal and a physiological regulator. A key factor in determining which of these possibilities pertain is related to the number of receptors expressed. As the signaling from the adenosine receptor to the functional response generally involves amplification, we have a situation involving so called spare receptors. This has the consequence that alterations in the receptor number lead to shifts in the potency of the endogenous agonist rather than a shift in the maximum response elicited. The roles of adenosine are studied by antagonists and/or animals (mostly mice) with targeted deletions of receptors or enzymes involved in adenosine metabolism. Whereas, adaptive changes in the genetically modified mice can occur for the physiologically important effects, such adaptive changes are less likely to occur for the situations when adenosine acts as a distress signal.

Keywords: Adenosine receptors, ATP, genetically modified mice, receptor reserve.

There are four evolutionarily well conserved receptors for adenosine denoted A_1 , A_{2A} , A_{2NB} and A_3 [1]. They form a distinct group among the so called G protein-coupled receptors. The A_1 and A_3 receptors are predominantly coupling to members of the G_i family of G proteins; the A_{2A} and A_{2B} receptors predominantly couple to members of the G_s family of G proteins. Under some circumstances, especially when over expressed, these receptors can also couple to members of the G_q and G_{12} families of proteins [2].

The potency of adenosine at these receptors is obviously determined by the affinity of the endogenous ligand (adenosine) to the different receptors. Unfortunately, it proves very difficult to determine this affinity. The reason is that adenosine is rapidly metabolized and formed in biological preparations including membrane preparations. Therefore, if metabolism of adenosine is prevented, endogenous adenosine accumulates to confound the measurements. Especially to receptors coupling to G_i proteins this endogenous adenosine can be cryptically bound and also influence the apparent B_{max} values [3, 4]. For this reason, we do not have reliable data on the comparative affinity of the endogenous agonist at the four adenosine receptors.

We must therefore, rely on the determination of the potency of adenosine in functional assays. This introduces another important confounding factor: potency of the agonist is markedly influenced by the receptor number [5-8]. The reason for this is that adenosine receptors are generally coupled *via* several amplification steps to the final response, and they, therefore exhibit the behavior described by pharmacologists as "spare receptors". In such systems, alterations in the receptor number are manifested by parallel shifts in the dose response curve, not as alterations in the maximal response. Therefore, it is important to compare potencies between receptors at comparative receptor densities. When this

is done it is observed that adenosine is equipotent at A_1 , A_{2A} and A_3 receptors, but is some 50 times less potent at A_{2B} receptors if alterations in cAMP are recorded [9]. If, by contrast, we instead examine the ability to activate MAP kinase (which all the receptors do), adenosine is equipotent at all of them [10, 11]. Thus, the potency of endogenous adenosine depends on the receptor number, and on the type of response measured. Furthermore, there is no really good reason to divide the receptors into high affinity and low affinity receptors as is sometimes done.

REGULATION OF ADENOSINE LEVELS

Adenosine is at a crossroad between different metabolic pathways. Hence, there will always be a finite intracellular concentration of adenosine. Furthermore, most, if not all, cells possess equilibrative adenosine transporters [12, 13]. Therefore, there will, by necessity, be also finite levels of adenosine in the extracellular space, even under the most basal conditions. This basal level has been estimated to be in the range of 30-200 nM [14]. From this baseline level, adenosine can increase substantially *via* several mechanisms. The equilibrative transporters (ENT 1-4) are usually sensitive to inhibition by drugs, such as dipyridamole and dilazep, but the ENT4 subtype present in e.g. heart is much less sensitive to such blockade [15]. The blockers can raise levels of adenosine in such cells that are net producers, and at the same time raise extracellular adenosine concentrations, which explains much of their therapeutic interest.

There are two principally different ways in which adenosine levels may be increased – formation intracellularly and export *via* transporters, and formation in the extracellular space from adenine nucleotides released from cells. The earlier literature on adenosine emphasized the former possibility [16, 17]. Adenosine was formed intracellularly whenever there was a discrepancy between the rates of ATP synthesis and ATP utilization. Thus, adenosine would be formed when work load was markedly enhanced or when the supply of metabolizable energy (*viz.* oxygen and glucose) is limiting as would be the case *inter alia* in ischemia.

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More recently, interest has centered on the role of regulated release of ATP as an important source of extracellular adenosine. Whereas the focus here was initially on the release of ATP as a neurotransmitter, stored together with other transmitters [18], several other mechanisms have now moved to the foreground. One major reason for this is that, the classical transmitter vesicles are rather small, and since they contain at least an order of magnitude less ATP than classical transmitter the amounts released will be quite limited especially over any distance [19]. Another reason is that, ATP is released from many cells that do not release transmitters. Among the mechanisms to be considered are: 1) release from cells with damage to the cell membrane e.g. in necrotic cell death, 2) release from large storage vesicles containing hormones, 3) *via* connexin/pannexin “hemichannels”, 4) from transport vesicles delivering proteins to the cell membrane, and 5) from a subset of lysosomes. It is well known that ATP is released from many cells, where cell membranes subjected to stretch [20], perhaps *via* one of the above mentioned mechanisms.

Release from cells with damaged cell membranes could provide large increases in extracellular purine levels since ATP levels in cells are typically 3-5 mM and extracellular adenosine levels 30-200 nM. Release from large storage vesicles that also contain hormones or enzymes is potentially a more important source than the transmitter storing vesicles, since they are typically much larger and hence the total amount of ATP is higher. For example, the evidence that takes place in pancreatic islets is quite convincing [21]. By contrast, the evidence for an important kiss-and-run release from synapses is more controversial [22], even though stronger evidence is now emerging [23].

Connexins form gap junctions. Clearly that much cell-cell contact is mediated *via* gap-junctions formed by connexins (or connexin like proteins). The cells that express connexins will sometimes express them on such a position of the cell membrane, where they cannot contact a connexins on another cell. This constitutes a connexins “hemichannel” [24]. Such hemichannels have been proposed as the channel that causes the release of ATP e.g. in astrocytes [24], and an important role in early development has been postulated [25]. It is attractive to consider the same molecule as mediator of two separate modes of cell-cell communication: gap junction contact and ATP-mediated communication [24]. Within the context of the present review, a role of these channels in endothelial cells, cardiac cells [26], smooth muscle cells and cells of the immune system [27] is particularly interesting to consider. It is potentially important that connexins have been suggested to play a critically important role in preconditioning [28], as has adenosine (see below).

The process whereby newly synthesized membrane proteins are inserted in the cell membrane involves fusion of intracellular vesicles with the plasma membrane, and in the process nucleotides can be released [29]. It has also been shown, that lysosomes contain abundant ATP, (perhaps because of their low pH) and that they release ATP *via* partial and full exocytosis, and that this process can be increased by relevant stimuli, such as metabolic deprivation or extracellular ATP [30]. The relative importance of these different mechanisms probably differs depending on the cell type and the stimulus.

Once ATP (or ADP) is released, the phosphate groups of extracellular ATP are rapidly split off by ecto-enzymes working in concert, first *via* nucleoside triphosphate diphosphohydrolases (NTDPases) similar to CD39 [31], followed by hydrolysis *via* ecto-5'-nucleotidase, CD73 [32]. Knock-outs of these enzymes have revealed their importance in different organs and situations.

PHYSIOLOGICAL VERSUS PATHOPHYSIOLOGICAL ROLES

Several studies estimate the resting extracellular levels of adenosine to be in the range 20 – 300 nM [14, 33-38], and the levels can rise to the low micromolar range in extreme physiological conditions, such as strenuous exercise or subsistence at high altitude, and hence low ambient oxygen [14, 33, 34, 38]. In ischemic areas or after massive tissue trauma leading to cell death by necrosis levels can increase to perhaps 30 μ M [36, 38, 39].

If these data are related to the estimated potency of adenosine receptors we arrive at a picture like that illustrated in Fig. (1). One can see that, in places where the receptors are very abundant, there will be a physiological role of adenosine and in places where receptors are fewer may only be activated under extreme or pathological circumstances.

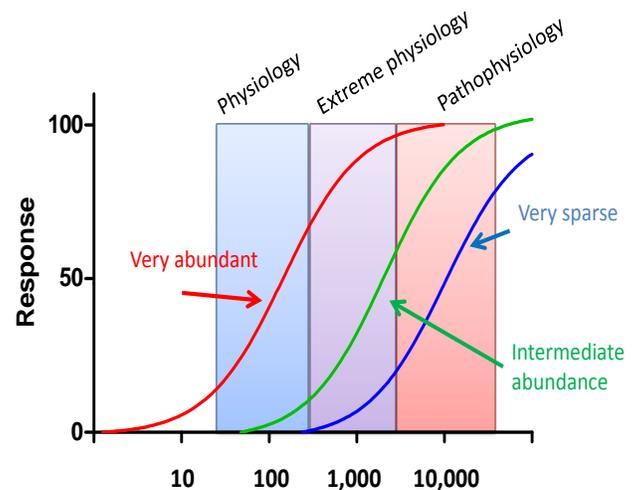


Fig. (1). Schematic illustration of the relationship between adenosine concentration and the effect mediated by adenosine receptors when the receptors are very abundant (as for example the A_{2A} receptors on striatopallidal neurons where levels are well above 100,000 receptors per cell), or when they are less abundant (down to a 100 receptors or less per cell). Typical experimental data showing the relationship between receptor number and position of the dose-response curve are found in references [5-8]. This relationship is then superimposed on data on the levels of adenosine (in nM) in tissue fluids under different circumstances. This is also based on actual measurements referred to in one of the several studies using microdialysis referred to in this review.

There have been many studies examining the roles of the adenosine receptors in different biological processes. Some of the key results are summarized in Table 1.

Table 1. Selected Effects Mediated by Adenosine via the Four Adenosine Receptors. For this Table I have Predominantly Used Data from Genetically Modified Organisms. The Column Labelled “Adaptation” Refers to Known (or Presumed/Possible?) adaptive Reactions Obvious in the Phenotype of Knock-Out Animals

Receptor	Effect	Physiology/Pathophysiology	Adaptation	Reference
A ₁	Decreased renal blood flow, Tubuloglomerular feedback	Phys	No	[51, 54]
	Inhibition of lipolysis	Phys	No	[55]
	Inhibition of neurotransmitter release	Extreme phys	No?	[7]
	Inhibition of insulin/glucagon release	Phys	No	[56]
	Reduced heart rate	Phys	No?	[57]
	Sleep	Phys	Yes	[58, 59]
	Analgesia	Extreme phys	No	[7, 60]
	Preconditioning	Path	No	[61]
A _{2A}	Wakefulness, locomotion	Phys	No	[49, 62-64]
	Neurodegeneration (including Parkinson’s disease and Alzheimer’s disease)	Path	No	[65-67]
	Immunosuppression	Extreme/Path	No?	[68-70]
	Vasodilatation	Phys/Extreme	No?	[49]
	Inhibition of platelet aggregation	Extreme	No?	[49]
	Angiogenesis	Extreme	No?	[71]
A _{2B}	Vascular integrity	Phys/Extreme	No	[72, 73]
	Preconditioning	Extreme	No	[74]
A ₃	Increased mast cell activation	Extreme/Path	No?	[50, 75]
	Airway contraction	Path	No?	[76]
	Inflammatory pain	Extreme/Path	No?	[77]
	White cell chemotaxis	Extreme/Path	No?	[78]

HOW TO STUDY THE ROLES OF ADENOSINE RECEPTORS

The roles of the receptors can be studied either by pharmacological means or altering the expression of the receptor protein. For a long time, the only available approach was the pharmacological one: Using agonists one could determine which type of responses might be elicited and using antagonists which of these, in fact, did occur and under which circumstances. Over the years, several very useful pharmacological tools have been developed as presented in several reviews [1, 40-47]. Nevertheless, their specificity is rarely complete and most of the selective compounds have physicochemical properties that limit their usefulness, especially for *in vivo* studies. Of particular interest is of course caffeine, the most widely used of all psychoactive drugs, that in habitually used doses blocks the three first adenosine receptors, and this accounts for many of its effects [48].

A more recent alternative is the use of genetically modified organisms [7, 49-52] and/or targeting with siRNA [53]. In many instances, the results obtained with drugs and those observed in the genetically modified animals are entirely

consistent. However, this is not always the case. One reason is that the drugs may not be as selective as was thought, and indeed the use of genetically modified animals to test the specificity of drugs is becoming a more and more established practice. Another reason for the discrepancy is that drugs rarely achieve a complete blockade of an adenosine receptor for any length of time and complete elimination of a response and partial blockade may have different consequences. A third possibility is that the genetic modification has resulted in major adaptive changes.

There is a common misconception that genetic elimination of one of the adenosine receptors should lead to up regulations of one or more of the other adenosine receptors. There are really few, if any, examples of this type of adaptation. Alternatively, completely different processes may show adaptive changes. However, this also appears to occur only rarely [e.g. 7, 8]. Perhaps one should expect only such processes that are physiologically regulated by an adenosine receptor to be compensated for in a targeted receptor deletion. There is little pressure to induce adaptations of processes that occurs rarely in a life time. Furthermore, not all physiological processes need to or can be compensated for. It is

therefore, of some interest that I have found very few examples of adaptive compensations in receptor knock-out mice, but several examples of where possible adaptations have been insufficiently studied (Table 1).

In summary, adenosine receptors are involved in many different processes, both physiological and pathophysiological. The fact that they are doing so many things, not only offering therapeutic opportunities, but also providing limitations for future drug development.

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