

# Adenosine, glutamate and pH: interactions and implications

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*Adenosine's role in the nervous system is multifaceted. As the core molecule of adenosine triphosphate (ATP), adenosine exists in equilibrium with the adenine nucleotide pool and contributes to cellular energy charge, a quantification of relative amounts of available ATP, ADP, AMP and adenosine. Beyond participating in overall energy balance and thus in maintaining cellular homeostasis, adenosine critically influences dynamic signaling in the nervous system. In particular, adenosine has an effect on and is affected by excitatory synaptic transmission. This report describes the ubiquitous nature of adenosine's influence, outlines specific scenarios of clinical import and highlights emerging knowledge about the regulation of adenosine. [Neurol Res 2005; 27: 000–000]*

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## ADENOSINE AND SYNAPTIC TRANSMISSION

Although not a classical neurotransmitter, adenosine is a critically important neuromodulator, which regulates synaptic transmission in a number of brain regions<sup>1</sup>. Acting at several membrane bound G-protein coupled receptor subtypes, adenosine can profoundly influence neuronal cell function both pre- and post-synaptically. Adenosine's combined actions on membrane potential<sup>2</sup>, transmitter release<sup>3</sup> and second messenger systems<sup>4</sup> result in interactions and co-operation with a wide range of other neurotransmitters and signaling molecules in the nervous system<sup>5</sup>. The presence of adenosine and its receptors throughout the central nervous system<sup>6</sup> render this nucleoside an integral part of any *in vivo* or *in vitro* system under investigation.

Neuromodulation by adenosine is particularly important in the forebrain, the substrate for cognition and higher functioning in the mammalian central nervous system. Endogenous levels of adenosine are sufficient to tonically activate two adenosine receptor subtypes, the adenosine A<sub>1</sub> receptor and the adenosine A<sub>2A</sub> receptor. The adenosine A<sub>1</sub> receptor is highly abundant in both the cerebral cortex and the hippocampus, and the adenosine A<sub>2A</sub> receptor is highly localized to the striatum<sup>6</sup>. A number of studies outline the functionally opposing interaction between the adenosine A<sub>2A</sub> receptors and dopamine D<sub>2</sub> receptors in the striatum<sup>7,8</sup>, and highlight the recently recognized clinical importance of this interaction<sup>9–11</sup>. Of particular interest is the role of the adenosine A<sub>2A</sub> receptor as a

potential target in the prevention and treatment of Parkinson's disease<sup>12–16</sup>.

In a number of brain regions, adenosine's presence sets the level of neuronal excitability via its actions at the adenosine A<sub>1</sub> receptor. The 'tone' of excitability serves as the baseline for all ongoing activity—synaptic transmission, drug responses, injury responses, learning and the formation of memories. This receptor subtype plays an inhibitory role both pre- and post-synaptically<sup>2</sup>, and the capacity for the inhibitory influence of adenosine A<sub>1</sub> receptors to regulate dynamically, or even abolish synaptic transmission has important implications for a number of physiological and pathophysiological conditions. During pathophysiological conditions where excessive excitation and excitotoxicity can occur, adenosine is released in large quantities and is known to play a neuroprotective role<sup>1</sup>. Under these same conditions large amounts of glutamate are also released, either due directly to excessive neuronal activity or to a reversal of the glutamate transporter<sup>17,18</sup>. Especially under these conditions, adenosine acting at adenosine A<sub>1</sub> receptors has a profound ability to decrease glutamate release at excitatory nerve terminals<sup>19</sup>, as well as hyperpolarize neuronal membranes, thus decreasing both the occurrence and efficacy of excitatory events.

## THE CLINICAL RELEVANCE OF ADENOSINE IN THE HIPPOCAMPUS

The remainder of this report will focus on the role of the adenosine A<sub>1</sub> receptor in regulating excitability in the hippocampus, a particularly 'plastic' structure in the brain known to be involved in learning and memory, and exceptionally vulnerable to neuronal injury.

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Ongoing synaptic transmission in the hippocampus has been shown to be sensitive to and regulated dynamically by activity at the adenosine A<sub>1</sub> receptor. Adenosine's tonic presence exerts a significant inhibitory influence on synaptic transmission as revealed by adenosine A<sub>1</sub> receptor antagonists both in CA1<sup>20</sup> and CA3<sup>21</sup>, and the ongoing influence of adenosine in the latter region is particularly striking. Applying large amounts of exogenous adenosine abolishes excitatory synaptic transmission in hippocampal slices from normal rats and mice<sup>22</sup>. The same or even higher concentrations of exogenous adenosine do not significantly influence synaptic transmission in slices from adenosine A<sub>1</sub> receptor knockout mice, underscoring the dominance of adenosine A<sub>1</sub> receptor subtype effects in the hippocampus<sup>23</sup>.

Neurons in the hippocampus are particularly sensitive to any type of metabolic stress. For example, after hypoxia or seizures, neurons in the hippocampus undergo more necrosis and apoptosis than cells in any other region of the brain<sup>24,25</sup>. Given the critical role of the hippocampus in memory formation, and its differential vulnerability to these stressful conditions, it is important to try to identify strategies to limit hippocampal damage. As noted, the adenosine A<sub>1</sub> receptor is highly expressed in the hippocampus, and has been shown to play both a neuroprotective and an anticonvulsant role<sup>26</sup>. Thus, inhibiting excitatory synaptic transmission to diminish metabolic stress and reduce potential excitotoxicity may be particularly important in this vulnerable brain region<sup>25</sup>.

Therapeutic strategies, which activate adenosine A<sub>1</sub> receptors directly, have profound side effects, primarily due to the presence of identical adenosine A<sub>1</sub> receptors in heart tissue<sup>26</sup>. Thus, alternate strategies must be adopted. Local and precise regulation of adenosine concentrations could focus therapeutic benefits, while simultaneously minimizing peripheral side effects. For example, augmenting adenosine release in areas under metabolic or excitotoxic stress is one strategy that has been considered. Potential targets include inhibiting either adenosine kinase, the enzyme that converts adenosine into AMP and phosphorylates it back into the adenine nucleotide pool or adenosine deaminase, which deaminates adenosine into inosine and thus renders it largely inactive at the adenosine A<sub>1</sub> receptors. Inhibiting either of these enzymes enhances the inhibitory influence of adenosine, a result that has been shown clearly in hippocampal slice preparations<sup>27</sup>. Beyond targeting these regulatory enzymes, the adenosine transporter, which normally serves to uptake adenosine from the extracellular space, presents another therapeutic prospect. Reducing the activity of the adenosine transporter could prolong and augment the effects of endogenous adenosine<sup>28</sup>.

An alternate evolving and promising strategy is cell therapy. Adenosine effectively suppresses seizures even in drug-resistant models of epilepsy<sup>29</sup>, so there are significant potential benefits of adenosine as an anticonvulsant<sup>30</sup>. Transplanting cells engineered to release adenosine locally can almost completely suppress

seizures without producing peripheral side effects in a kindling model of epilepsy, but thus far these cells have limited viability<sup>31</sup>. To date, no clinically useful approach is available to harness the potential of adenosine under such pathophysiological conditions.

## REGULATION OF ADENOSINE IN THE EXTRACELLULAR SPACE

Given the critical neuromodulatory role of adenosine and its therapeutic potential as a neuroprotective molecule, it is important to understand how adenosine levels are regulated under normal conditions. As a basic science approach, we have focused on understanding proximal cellular events, which may modulate and regulate adenosine. A wide range of stimuli have been shown to enhance the adenosine concentration in the extracellular space. For some time it has been recognized that large amounts of adenosine are released into the synaptic space during metabolically stressful events like hypoxia<sup>32,33</sup>, stroke<sup>34</sup> and seizures<sup>35,36</sup>. Under these conditions intracellular consumption of ATP likely outstrips its rephosphorylation to produce a large increase in free intracellular adenosine. When intracellular levels of adenosine rise above extracellular levels, transport through the equilibrative nucleoside transporter reverses, resulting in increased extracellular adenosine<sup>37</sup>. Additionally, there is evidence that adenosine A<sub>1</sub> receptor activation can promote adenosine efflux<sup>38</sup> and that various purines can regulate each other by enhancing outflow through the transporters<sup>39</sup>.

Ongoing stimuli that are not necessarily pathological also regulate adenosine. For instance, NMDA receptor activity<sup>40</sup>, small temperature changes<sup>41</sup>, and increases in adenosine-metabolizing enzyme activity and expression<sup>42</sup> all can alter extracellular adenosine levels. An additional source of extracellular adenosine, occurring during both normal and even more profoundly during heightened neuronal activity, is ATP released into the synaptic cleft<sup>43</sup>. This ATP is rapidly broken down into adenosine by a family of enzymes known as ecto-ATPases and this extracellular adenosine likely contributes to the tonic inhibition of synaptic transmission<sup>44,45</sup>. This adenosine is then taken back into cells via the equilibrative nucleoside transporter<sup>37</sup>. Taken together, evidence suggests that adenosine and its inhibitory influence undergo constant physiological modulation, resulting in a dynamic influence on transmitter release and neuronal excitability, as well as pathological modulation, such as the massive release of adenosine that occurs during hypoxia and stroke.

Common to many stimuli that result in altered extracellular adenosine are changes in intracellular calcium levels and intracellular pH. These changes may be part of a signaling pathway that causes adenosine release and further research could help reveal specific mechanisms responsible for regulating adenosine. pH is known to modulate both adenosine kinase<sup>46</sup>, and 5'-nucleotidase<sup>47</sup>, which together control the intracellular levels of free adenosine. Adenosine deaminase also shows some pH-dependence, which

could effect extracellular degradation of adenosine<sup>48</sup>. Intracellular pH gradients help drive both the production of ATP<sup>49</sup> and the loading of glutamate in synaptic vesicles<sup>50</sup>. Intracellular acidification can decrease ATP production via oxidative phosphorylation<sup>51</sup>, but its effects on the loading of synaptic vesicles is less clear<sup>52</sup>.

In addition to pH, changes in calcium could also regulate many aspects of adenosine metabolism. Calcium appears to be required for NMDA receptor-dependent release of adenosine<sup>53</sup>, as well as the activity dependent release of ATP, although calcium withdrawal can cause activity-independent ATP release<sup>54</sup>. These two intracellular ions, protons and calcium, are intimately linked to adenosine metabolism and release, but further investigation is required to determine where and when they act.

Of particular interest to many neuroscientists is the importance of microdomains within the cell, especially in the synaptic compartments. Neuronal activity, particularly excitatory activity, results in dramatic changes in calcium<sup>55</sup> and pH<sup>56</sup> in both the pre- and post-synaptic compartments. Localized calcium signaling has been well documented in both the pre- and post-synaptic terminals<sup>57</sup> and calcium transients have been shown to be spatially restricted along specific branches of dendrites<sup>58</sup>. These localized calcium transients control cellular events such as neurotransmitter release<sup>59</sup>, protein kinase activation<sup>60,61</sup>, gene transcription<sup>62</sup> and many other cell functions. While calcium has long been recognized in neuronal cell signaling, more recently recognized are local pH changes in the synaptic region<sup>63</sup>. pH deviations, which are often thought to occur only globally and pathologically<sup>64</sup>, could be relevant to adenosine release and thus play a critical role in neuromodulation. Recent findings show that large pH transients can be seen isolated microdomains similar to calcium transients and may have similar properties for controlling local events<sup>65</sup>. These localized pH changes create pH gradients, which are maintained for prolonged periods due to restricted diffusion of protons and may contribute to more multifaceted signaling mechanisms than have previously been considered. When local calcium and pH transients are combined with different expression and localization of purinergic metabolism, and transport molecules distinct regions of adenosine modulation could occur.

## EMERGING KNOWLEDGE

The presence of adenosine in any system under investigation, and its dynamic influence on neuronal activity, make this fundamental nucleoside impossible to ignore. Adenosine has long been recognized as part of cellular energy cycles, and as such was labeled a 'retaliatory metabolite'<sup>66</sup>, serving to inhibit synaptic transmission during times of metabolic stress or excessive activity. The therapeutic potential of adenosine as an anticonvulsant and neuroprotective agent is clear, albeit hampered currently by peripheral side effects. Ultimately, strategies that control adenosine's

regulation locally and target the proximal cellular mechanisms controlling adenosine in the extracellular space are likely to yield more viable options. Finally, the tonic and significant influence of adenosine on any *in vivo* or *in vitro* phenomenon, including all forms of synaptic plasticity and adaptation, are just now being fully appreciated.

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