Rethinking the purinergic neuron–glia connection

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There is excellent evidence that adenosine plays a role in physiology and pathophysiology to modulate neural activity in the brain. For example, adenosine is known to act on adenosine A_1 receptors to decrease neuronal firing and neurotransmitter release (1). This mechanism is of importance in limiting excessive neuronal activity and thereby, epileptic seizures (2, 3). Although the role of adenosine is rather uncontroversial, the origin of the adenosine mediating this anticonvulsant effect has been somewhat contentious. The work by Lovatt et al. (4) in PNAS not only clarifies that adenosine released from neurons is the important source, but it also raises more fundamental issues regarding the interactions between signaling through adenine nucleotides and adenosine and the role of astrocytes.

The ability of adenosine to limit neurotransmission was first shown in the peripheral nervous system (motor neurons and sympathetic and parasympathetic nerves), and here, it was possible to provide good evidence that the source of adenosine was the effector tissue (5). Thus, the release of adenosine and the inhibition of neurotransmitter release after activation of the nerves could be mimicked by receptor agonists and reduced by receptor antagonists. To pinpoint the source of adenosine in the CNS was obviously more difficult, and it was not possible to determine conclusively if adenosine or adenine nucleotides were originally released. Based on a review of the literature in 1980 (5), it was concluded that

> nerve activity practically always releases adenine compounds and their metabolites. The release occurs both from nerve endings and from postsynaptic structures, but the latter source seems to be the more important. Both adenosine and adenine nucleotides seem to be released—their relative proportions varying with the tissue and the type of stimulus. This raises the possibility that adenosine and/or adenine nucleotides may act as local regulators of transmitter release. Since they are predominantly formed by the postsynaptic structures, the terms 'transsynaptic modulation' and 'retrograde transmission' have been coined to describe the phenomenon. The adenine derivatives are sufficiently potent as presynaptic inhibitors, at least in some tissues, to be active already under basal physiological conditions. In other instances adenine-derivatives may be important only when the local concentrations of adenosine are raised above the normal physiological range, for example by ischemia, or by extensive depolarization or by drugs.

There is good evidence that adenosine can be released from neurons after its concentration is raised intracellularly by an increase in activity or a reduction in energy supply. A general concept is that adenosine is released under conditions of distress and acts to limit the causes of its own release and/or the negative consequences thereof (6). To take but one example of a series of papers from the mid 1970s on, it was shown that, in hippocampal slices subjected to depolarization or substrate lack, there was a substantial release of adenosine, and this release was unaffected by blockade of *ecto*-5′-nucleotidase but essentially eliminated by trapping intracellular adenosine as S-adenosyl homocysteine by the addition of L-homocysteine (7).

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Based on such data, measurements of endogenous adenosine levels, and a comparison with the situation in many peripheral tissues, it was suggested that we could have basal adenosine levels (perhaps 20–200 nM) occurring under physiological conditions, levels occurring under extreme physiological conditions (0.1–1 μM), and pathophysiological levels (1 μM and above) (6). The potency of adenosine as an agonist at the adenosine receptors depends on the number of receptors expressed, because in general, adenosine receptors have so-called spare receptors. This term means that an increased number of receptors causes a leftward shift of the dose–response curve and not to any change in the maximal response (1). The important consequence of this finding is that, in cells where the number of receptors is very high (e.g., adenosine A_{2A} receptors on medium-sized spiny neurons in the so-called indirect output pathway from the striatum), already basal levels of adenosine are sufficient to cause a degree of tonic activation. This finding is the reason why the adenosine receptor antagonist caffeine can exert some effects already physiologically.

It may, however, be reasonably argued that such release of adenosine from an entire slice subjected to field depolarization or generalized metabolic deficiency is not representative of a more physiological situation. Furthermore, there is evidence from both experiments with receptor antagonists and studies on animals with target receptor deletions that endogenous adenosine is playing a physiological role also at sites where receptor number is rather low. This finding is clearly difficult to explain, unless there are one or more mechanisms to produce high local concentrations of adenosine. There is good evidence that ATP can be released from many different types of cells, including neurons and glial cells (8–10). In particular, there is superb evidence that ATP is released by astrocytes, and this occurrence is of critical importance in establishing networks of astrocytic signaling (8, 9). Rapid degradation of such ATP could provide high local concentrations of adenosine. This concept has gained a considerable following.

The paper by Lovatt et al. (4) critically examines the possible role of astrocytic ATP release as a source of adenosine to limit neuronal activity. First, it is shown that, whereas the degradation of ATP to AMP can be catalyzed by a variety of different enzymes, the additional degradation of extracellular AMP to adenosine occurs only with the help of CD73. Furthermore, the amounts of CD73 are quite low in most areas of the brain (11), indicating that it is definitely rate-limiting. Therefore, if one wishes to examine the role of ATP release, elimination or blockade of CD73 (also called Nt5e) is a very attractive approach. Also, the work by Lovatt et al. (4) shows that elimination of *ecto*-5′-nucleotidase does not alter seizure latency or heterosynaptic depression after high-frequency stimulation, whereas elimination of adenosine A_1 receptor activity does. Thus, the well-known role of activation of endogenous A_1

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receptors to limit excessive nerve activity apparently does not depend on ATP release. Finally, the work by Lovatt et al. (4) shows that, even if they selectively activate ATP release from astrocytes by photolysis of caged Ca^{2+}, there is no inhibition of neurotransmission. Thus, the ATP that is released from astrocytes during high-frequency stimulation does not produce sufficient amounts of adenosine at the appropriate location to influence neuronal activity. It is also important in this regard to remember that the ability of exogenous ATP to limit neurotransmission is entirely dependent on adenosine A_1 receptors (12). This finding also emphasizes that, although incubation of a brain slice with high concentrations of adenine nucleotides can generate sufficient adenosine to exert clear adenosine receptor-dependent effects, this finding does not mean that biologically relevant, highly localized, and quantitatively limited endogenous ATP release can do it.

Although a role for ATP release was difficult to show, the work by Lovatt et al. (4) shows that selective activation of neurons by means of a patch pipette induces release of adenosine. This release of adenosine inhibited the EPSP induced by electrical activation of the synaptic input. This adenosine release and presynaptic inhibition is not influenced by elimination of *ecto*-5′-nucleotidase, but it is blocked by competitively antagonizing the equilibrative adenosine transport by administration of inosine through the patch pipette. This elegant experiment confirms and extends earlier findings (13–15). It is not surprising that a depolarization of dendrites, with their large surface to volume ratio, will necessitate massive ATP consumption to restore energy balance and consequently, raise cytosolic adenosine levels substantially. Indeed, this finding provides a conceptually very satisfactory explanation for large local adenosine release in response to localized activity.

What could be the role of the astrocytes? Do they contribute to adenosine release? It has been proposed that astrocytes may release adenosine during energy deprivation, but recent evidence suggests that this release does not occur during mild hypoxia, which is consistent with the general resistance to hypoxia (16). Instead, it seems likely that they play a more important role by being the most important eliminator of adenosine in the environment of the synapse. The postsynaptic neuron clearly cannot remove adenosine, because the equilibrative transporters are working in the opposite direction. The presynaptic elements might contribute to elimination but are rather tiny, and therefore, they have a limited capacity. More important is probably the astrocytes and their processes that surround the synapse. To eliminate extracellular adenosine, it is necessary that the intracellular adenosine concentration is kept low, because otherwise, the equilibrative transporters will not do much of a mopping-up job. This job is mostly achieved by adenosine kinase, an enzyme with a predominantly astrocytic location (17). Even rather minor changes in astrocyte adenosine kinase can produce major changes in adenosine levels and seizure activity. The enzyme activity can be regulated by various means, including diet, and this finding seems to be a valuable way to approach otherwise therapy-resistant epilepsy (18). Thus, astrocytes are far from unimportant; it is just that our focus should shift from looking at them only as a source of adenosine to regarding them perhaps more as cells that ensure that the local increases in adenosine are indeed kept local. Therefore, the biologically meaningful local adenosine feedback is not producing untoward depressant effects in unrelated neuronal circuitry.

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