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Pharmacology & Toxicology
ISSN 0901-9928

MiniReview

Purinergic Inhibition of Neurotransmitter Release in the Central Nervous System

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(Received November 06, 1994; Accepted April 27, 1995)

Abstract: Neurotransmitter release and the role of adenosine in its regulation has been investigated for more than twenty years, and it is now widely accepted that adenosine tonically inhibits the release of excitatory neurotransmitters. This effect of adenosine is operated by an A_1 adenosine receptor. Since activation of this receptor could inhibit Ca^{2+} conductance, increase K^+ conductance, inhibit adenylate cyclase or phospholipase C, it is not clear if there is only one mechanism or several mechanisms operated by adenosine to inhibit neurotransmitter release, and in that case, what is the relative importance of each mechanism. The mechanism by which adenosine inhibits evoked synchronous transmitter release might be different from that used by the nucleoside to inhibit spontaneous asynchronous release. In some systems adenosine triphosphate *per se* acts like adenosine and inhibits neurotransmitter release. However, in most cases the inhibitory effect of this adenine nucleotide depends upon its hydrolysis into adenosine by a cascade of ectoenzymes, the last step being mediated by ecto-5′-nucleotidase.

Ginsborg & Hirst (1971 & 1972) triggered the interest in the extracellular effects of adenosine on the nervous system. These authors used the innervated rat diaphragm electrically stimulated through the phrenic nerve at relatively low frequencies (0.10–0.7 Hz). They recorded evoked end-plate potentials and miniature end-plate potentials and determined the quantal content of the evoked end-plate potentials as a measure of acetylcholine release. Adenosine decreased the quantal content of the evoked end-plate potentials and the frequency of miniature end-plate potentials without changing their average amplitude. This implied that adenosine caused only prejunctional effects. Adenosine consistently inhibited both the amplitude of evoked end-plate potentials (representing evoked acetylcholine release) and the frequency of miniature end-plate potentials (representing spontaneous acetylcholine release) (Ginsborg & Hirst 1972). Similar results were obtained with adenosine and with adenine nucleotides (adenosine triphosphate and adenosine diphosphate), in both the rat and frog neuromuscular junctions (Ribeiro & Walker 1973 & 1975). These effects of the nucleotides were due to their extracellular degradation into adenosine by the action of ecto adenosine triphosphatases, adenosine diphosphatases and ecto 5′-nucleotidase (Ribeiro & Sebastião 1987; Cunha & Sebastião 1991).

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The prejunctional inhibitory effects of adenosine at the neuromuscular junction have been used during the last twenty years as a point of reference (see e.g. Lupica *et al.* 1992; Prince & Stevens 1992) of the presynaptic effects of adenosine, in particular those related to the adenosine's most extensively studied area of the central nervous system, the hippocampus.

There is a vast consensus that in the central nervous system, adenosine inhibits the release of several neurotransmitters (see e.g. Ribeiro 1991), in particular the excitatory transmitters: acetylcholine, glutamate, noradrenaline, dopamine and 5-hydroxytryptamine. In this Minireview I will discuss the inhibitory action of adenosine and intact adenosine triphosphate on neurotransmitter release.

In the central nervous system, adenosine has pre-, post-, and non-synaptic actions. With the exception of synaptosomal experiments, the finding that adenosine inhibits neurotransmitter release in the central nervous system does not necessarily mean that the nucleotide has a direct effect upon the nerve terminal (Dunwiddie 1985). Postsynaptic and/or non-synaptic effects of adenosine could also result in an apparent decrease in the amount of transmitter released by the affected neurone. Some of the arguments advanced to support the presynaptic effects of adenosine in the central nervous system include: 1) adenosine decreases the amplitude of the excitatory postsynaptic potentials with little or no change in the resting membrane potential and/or in the input resistance of postsynaptic neurones (Okada & Ozawa 1980); 2) adenosine inhibits synaptic transmission without

detectable decreases in sensitivity of postsynaptic neurones to the neurotransmitter (Malenka & Kocsis 1988); 3) adenosine reduces the quantal content of the evoked excitatory postsynaptic potentials without significantly changing the quantal size and decreases the frequency of spontaneous excitatory postsynaptic potentials in the hippocampus (Lupica *et al.* 1992; Prince & Stevens 1992; Scanziani *et al.* 1992; Yamamoto *et al.* 1993). Also in the substantia gelatinosa of the superficial dorsal horn in the mammalian spinal cord, adenosine reduces miniature excitatory postsynaptic currents frequency without significant reduction of their average amplitude (Li & Perl 1994).

The central nervous system possesses a variety of ways for dynamically adjusting synaptic strength, i.e. the efficacy with which a synapse transmits information. Adenosine seems to be an excellent candidate to adjust synaptic strength to the needs of the central nervous system. To cause inhibition of neurotransmitter release, adenosine acts on inhibitory membrane receptors (A_1 family), located on nerve terminals. Through A1 actions which are linked to G proteins, adenosine inhibits adenylate cyclase and thereby intracellular cyclic AMP accumulation, activates potassium channels, inhibits calcium channels, inhibits and activates phospholipase C, and inhibits protein kinase C (see e.g. Fredholm & Dunwiddie 1988).

On the Inhibitory Adenosine Receptor

In the rat motor nerve terminals and in the hippocampus it is clear now that the presynaptic adenosine receptor that mediates the inhibitory action of adenosine on transmitter release is an A_1 subtype with Ki values obtained for the selective adenosine A_1 receptor antagonist, 1,3-dipropyl,8-cyclopentylxanthine below 1 nM (see Sebastião *et al.* 1990). In the frog motor nerve terminals, the Ki value obtained for 1,3-dipropyl,8-cyclopentylxanthine was 35 nM (Sebastião & Ribeiro 1989). This was determined as in the rat (Sebastião *et al.* 1990), from indirect measurements of neuromuscular transmission, i.e. by recording muscle twitches from innervated muscle preparations, in which the nerves were electrically stimulated. Redman & Silinsky (1993), using end-plate microelectrophysiological recordings from frog innervated muscle preparations, obtained a Ki value for 1,3-dipropyl,8-cyclopentylxanthine of 0.2 nM, which is similar to that found by Sebastião *et al.* (1990) in the rat, but much lower than that previously described for the frog neuromuscular junction by Sebastião & Ribeiro (1989). These differences may be due to: 1) Different manners used to record neuromuscular transmission (electrophysiological versus twitch recordings). Electrophysiological recordings maybe are more accurate to determine Ki values of antagonists than more indirect recordings of neuromuscular transmission. However, in spite of the use of twitch recordings the Ki value for 1,3-dipropyl,8-cyclopentylxanthine obtained in the rat neuromuscular junction (Sebastião *et al.* 1990) is compatible with adenosine A_1 receptor antagonism. 2) The difference, in the case of the frog neuromuscular junction, might also result from the fact that Redman & Silinsky (1994) used an unstable agonist, adenosine. Sebastião & Ribeiro (1989) used 2-chloroadenosine for the determination of the Ki value for 1,3-dipropyl,8-cyclopentylxanthine at the frog neuromuscular junction. 2-Chloroadenosine is a non-degradable adenosine receptor agonist, which is also a poor substrate for the adenosine uptake system. Thus, it is more suitable than nonstable agonists for the determination of Ki values of antagonists (see Kenakin 1987). The idea of the existence of a different entity (neither adenosine A_1 nor A_2 receptor) was advanced on the basis that the agonist profile of this entity was different from the A_1 and the A_2 agonist profiles (see Ribeiro & Sebastião 1986). Later it was also found that the Ki value for 1,3-dipropyl,8-cyclopentylxanthine in the case of this putative A_3 was much higher (35 nM) than that of a typical A_1 (<1 nM), and much lower than that of a typical A_2 (>250 nM). As recently discussed by Linden (1994) these differences might be related to marked species differences in the affinity of xanthines for A_1 receptors, and this putative A_3-receptor might well be part of the adenosine A_1-receptor family.

Adenosine Calcium and Neurotransmitter Release

Under physiological conditions, neurotransmitters can be released from nerve terminals either spontaneously or by action potentials arriving at the nerve terminals. The evoked release is synchronous upon stimulation of the nerve terminals, whereas the spontaneous release is asynchronous. Evoked but asynchronous transmitter release can, however, be induced by several experimental paradigms, such as high extracellular potassium concentration or by calcium ionophores, e.g. ionomycin. Neurotransmitter release can still be separated into calcium-dependent and calcium-independent. Release of neurotransmitters in discrete quanta is assumed to be calcium-dependent, from either extracellular or intracellular calcium sources. Several neurotransmitters (e.g. γ aminobutyric acid and glutamate) can also be released in a calcium-independent manner, by reversal of the neurotransmitter uptake system, but the physiological significance of this type of release is still unclear.

Adenosine inhibits evoked synchronous release and spontaneous asynchronous release of neurotransmitters differently, i.e. adenosine decreases more efficiently the amplitude of evoked end-plate potentials than the frequency of spontaneous end-plate potentials (Ribeiro & Sebastião 1986). Adenosine also decreases the amplitude of the excitatory postsynaptic currents more effectively than the frequency of miniature excitatory postsynaptic currents recorded from cultured rat hyppocampal pyramidal neurones (Scholz & Miller 1991).

Evoked release. Two main hypotheses have been put forward to explain how adenosine decreases evoked release. One is that adenosine decreases release by decreasing calcium entry, and another hypothesis is in favour of adenosine acting on a mechanism downstream of calcium entry, i.e. related

to the site where calcium acts to cause neurotransmitter release (see Silinsky 1984). The main arguments in favour of the hypothesis that adenosine acts on a mechanism downstream of calcium entry are that adenosine inhibits quantal acetylcholine release from frog motor nerve terminals regardless of whether the trigger calcium emanated from voltage-dependent calcium channels (evoked synchronous release), calcium-filled synaptosomes (Silinsky 1984) or calcium ionophores (Hunt & Silinsky 1993), which evoke neurotransmitter release in an asynchronic manner. Furthermore, calcium currents responsible for initiating evoked acetylcholine release at frog motor nerve endings do not appear to be blocked by adenosine (Silinsky & Solsona 1992). However, there is not yet evidence that for example the synaptic vesicle proteins or other intracellular systems involved in exocytosis could be affected by activation of presynaptic adenosine inhibitory receptors. Ginsborg & Hirst (1972) were the first to suggest that adenosine does not inhibit evoked acetylcholine release by competing with calcium in the manner of magnesium. However, these authors did not exclude that adenosine could affect calcium entry. It is not possible to make experiments to study the effect of adenosine on evoked synchronous neurotransmitter release in the absence of external calcium On the other hand, there is evidence that adenosine decreases calcium entry into synaptosomes (e.g. Ribeiro *et al.* 1979; Wu *et al.* 1982; Shinozuka *et al.* 1985; Gonçalves *et al.* 1991), and decreases calcium currents in neuronal cells (e.g. Zhu & Ikeda 1993). Therefore, one should not exclude that, in the case of evoked synchronous neurotransmitter release, the inhibitory action of adenosine is, at least in part, due to an inhibition of calcium entry needed to trigger neurotransmitter release. Adenosine decreases ω-conotoxin sensitive presynaptic calcium channels in the chick ciliary ganglion. (Yawo & Chuhma 1993). Adenosine reduces the electrically stimulation-evoked increase in intracellular calcium concentration to the same extent (by approximately 80%) as ω-conotoxin does. The remaining 20% of intracellular calcium increase, is insensitive to ω-conotoxin and to adenosine (Yawo & Chuhma 1993). These authors suggest that ω-conotoxin sensitive calcium channels and the adenosine A_1 receptors are closely located in the presynaptic terminal. In the guinea-pig hippocampal slices, adenosine, in a supramaximal concentration (500 μM), reduces the transient calcium channels (ω-conotoxin sensitive) by a mean value of 40%, and inhibits unidentified calcium channels, probably Q-type calcium channels, by 73%. So, it seems that in some systems adenosine inhibits both N-type and Q-type calcium channels but not ω-agatoxin-IVA-sensitive calcium channels (Wu & Saggau 1994). The results of Wu & Saggau (1994) suggest that the reduction in these calcium currents by adenosine is sufficient to account for the majority of the inhibition of evoked synaptic transmission caused by this nucleoside. They consider that a presynaptic mechanism downstream to calcium influx should play a minor role in the inhibition of evoked (synchronous) neurotransmitter release. Although there is evidence that adenosine is able to inhibit N-type calcium channels it is not yet clear how this effect of adenosine could be responsible for the inhibitory action of the nucleoside on neurotransmitter release in some preparations. Mynlieff & Beam (1994) investigated the adenosine A_1 receptor inhibitory effects on the N-type calcium current in mouse motorneurones. They found that adenosine decreases N-type current, but they could not relate this inhibitory effect to adenosine inhibition of acetylcholine release. In fact, since transmission is not blocked by ω-conotoxin at the mammalian neuromuscular junction (Sano *et al.* 1987), the N-type channels, should not play a primary role in eliciting evoked transmitter release from mammalian motor nerve terminals.

Spontaneous release. Scanziani *et al.* (1992) discussed the possibility that the mechanism mediating adenosine inhibition of spontaneous quantal glutamate release in the hippocampus is the same as that mediating inhibition of evoked synchronous glutamate release. According to these authors the arguments in support are: 1) The magnitude of the inhibitory effects of adenosine on both evoked and spontaneous glutamate release are comparable; and 2) The time course of onset and recovery for these inhibitory effects are similar. The authors conclude that it is difficult to conceive a mechanism operated by adenosine that would be critical in spontaneous release, but not involved in evoked release. However, the reverse might occur, i.e., one could imagine a mechanism that affects evoked synchronous transmitter release (e.g. by a decrease in calcium entry through voltage-sensitive calcium channels) without affecting asynchronous spontaneous release. At the neuromuscular junction adenosine inhibits the amplitude of evoked end-plate potentials, the frequency of miniature end-plate potentials in the presence of external calcium (Ginsborg & Hirst 1972; Ribeiro & Walker 1975), and the frequency of miniature end-plate potentials recorded in the absence of external calcium in the medium, and in the presence of the calcium chelating agent, ethylenediaminotetraacetic acid (EDTA) (Ribeiro & Dominguez 1978). Comparing the effect of adenosine on the amplitude of evoked end-plate potentials with the frequency of miniature end-plate potentials it emerges that the inhibitory effect of adenosine is more intense on the amplitude of evoked end-plate potentials than on the frequency of miniature end-plate potentials (Ribeiro & Sebastião 1986). Furthermore, there is no clear-cut correlation between the effects of adenosine on evoked end-plate potential amplitude and on the frequency of miniature end-plate potentials (Ribeiro & Sebastião 1986). Since there are differences in the inhibitory efficiency of adenosine in regard to evoked and spontaneous release, maybe sequential mechanisms also exist, which regulate these two forms of neurotransmitter release. One mechanism may affect only evoked synchronous release related to a decrease in calcium entry, and another mechanism may be independent of calcium entry and related to some intracellular mechanism downstream to calcium entry, which would affect both evoked and spontaneous release.

G-protein coupled inhibition. The presynaptic inhibitory effect of adenosine in the hippocampus is sensitive to pertussis toxin (e.g. Stratton *et al.* 1989). Pertussis toxin reverses adenosine receptor-mediated inhibition of neuronal glutamate release in cerebellar primary cultures (Dolphin & Prestwich 1985) and blocks the inhibitory effect of the A_1 agonist, N^6-cyclopentyladenosine, on the frequency of spontaneous miniature excitatory postsynaptic currents recorded from rat hippocampal pyramidal neurones in culture (Scholz & Miller 1991). This means that the effect of adenosine should result from activation of a G-protein coupled receptor but does not indicate if it is through adenylate cyclase/cyclic AMP or through other G protein (Gi/Go subtypes) coupled transducing systems. For example adenosine via a pertussis toxin-sensitive G-protein inhibits N-type calcium channels and unidentified calcium currents in the rat superior cervical ganglion (Zhu & Ikeda 1993), and it inhibits N-type calcium channels in motorneurones (Mynlieff & Beam 1994). Pertussis toxin-insensitivity of the presynaptic inhibitory effect of adenosine has also been reported (Fredholm *et al.* 1989; Thompson *et al.* 1992). Fredholm (1995) speculated about potential interactions between adenosine and the pertussis toxin-insensitive synaptic protein, rab 3A, known to be involved in transmitter release. Evidence that adenosine A_1 receptor activation will operate such protein(s) will give support to those who claim that adenosine is decreasing evoked transmitter release through a mechanism downstream to the calcium entry (see e.g. Silinsky & Solsona 1992).

Adenylate cyclase. Adenosine inhibits adenylate cyclase in brain tissue (Ebersolt *et al.* 1983; Fredholm *et al.* 1986). Synapsin I contains cyclic AMP-dependent phosphorylation sites (Huttner & Greengaard 1979). The phosphorylation state of synapsin I can alter the availability of neurotransmitter to be released (Llinás *et al.* 1991). Whether this or other similar mechanisms are involved in the adenosine inhibitory effect on transmitter release needs to be investigated. Adenosine decreases evoked acetylcholine release from the rat motor nerve terminals through inhibition of adenylate cyclase/cyclic AMP, since in the presence of an adenylate cyclase inhibitor, adenosine does not inhibit acetylcholine release (Correia-de-Sá & Ribeiro 1994).

Protein kinase C and phosphoinositide metabolism. Activation of protein kinase C markedly reduces the ability of A_1 agonists to inhibit evoked release of glutamate (Barrie & Nichols 1993) and of acetylcholine (Sebastião & Ribeiro 1990). This is probably not related to an ability of protein kinase C to affect (through phosphorylation) the adenosine receptor, since the affinity of adenosine receptor antagonists is not modified by protein kinase C activation (Sebastião & Ribeiro 1990). According to Barrie & Nichols (1993) activation of protein kinase C can cause a decoupling of adenosine inhibition of glutamate exocytosis, probably by a protein kinase C-mediated phosphorylation and inactivation of an inhibitory G-protein.

Evidence that adenosine inhibits phosphoinositide metabolism in nerve cells has been produced (Petcoff & Cooper 1987; Kendall & Hill 1988; Rubio *et al.* 1989). These authors also showed that the adenosine receptor involved in the inhibition of phosphoinositide metabolism has an agonist profile more similar to the agonist profile of the adenosine receptor mediating inhibition of neurotransmitter release at the frog neuromuscular junction (Ribeiro & Sebastião 1986), than the agonist profiles of the adenosine receptors involved in inhibition or in stimulation of adenylate cyclase (Daly *et al.* 1981). This receptor present in the frog motor nerve terminals also appears to cause inhibition of neurotransmitter release through inhibition of phosphoinositides turnover (Sebastião & Ribeiro 1990).

Adenosine Triphosphate and Neurotransmitter Release

In the rat cerebral cortex adenosine triphosphate as such presynaptically inhibits acetylcholine release, whereas in the hippocampus adenine nucleotides are a source of endogenous extracellular adenosine, which tonically inhibits acetylcholine release (Cunha *et al.* 1992). Adenosine triphosphate and stable adenine nucleotides also inhibit noradrenaline release in rabbit brain cortical slices (von Kügelgen *et al.* 1992). These effects of adenosine triphosphate as such are not modified by adenosine deaminase, by blockade of ecto-5′-nucleotidase, or by blockade of P_2-purinoceptors with suramin, but are blocked by the adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (Cunha *et al.* 1994; von Kügelgen *et al.* 1992). Whether these effects of adenosine triphosphate are due to an interaction with a nucleotide-sensitive A_1 adenosine receptor or to an interaction with a putative P_3 purinoceptor (Westfall *et al.* 1991) awaits further investigation.

Adenosine triphosphate as such also increases glutamate release in cultured hippocampal neurones perhaps through activation of P_2 purinoceptor operated channels (Inoue *et al.* 1992).

Role of 5′-Nucleotidases

Cholinergic nerve terminals immunopurified from the rat cerebral cortex do not seem to possess functional enzymatic activities to metabolize adenosine triphosphate into adenosine (Richardson *et al.* 1987). Further evidence that this is the case was provided by experiments studying the metabolism of adenosine monophosphate (Cunha *et al.* 1992). Adenosine monophosphate is degraded into adenosine at immunopurified cholinergic motor nerve terminals from the hippocampus but not at immunopurified cholinergic motor nerve terminals from the cerebral cortex. However, ecto-5′-nucleotidase activity appears to be present in the cerebral cortical slices, which suggests that ecto-5′-nucleotidase activity is associated with non-cholinergic nerve terminals and/or located outside nerve terminals. Since in the cerebral cortex adenosine triphosphate as such inhibits acetylcholine release (Cunha *et al.* 1994), it appears that in the absence of

an efficient system to form adenosine from released adenine nucleotides, adenosine triphosphate itself subserves a role that, in most cases is exclusive to adenosine, i.e. negative modulation of neurotransmitter release. It would be interesting to know whether or not cerebral cortical noradrenergic nerve terminals, where adenosine triphosphate as such also inhibits noradrenaline release (von Kügelgen *et al.* 1992), lack ecto-5′-nucleotidase.

The most common localization of ecto-5′-nucleotidase in the brain is in myelin and glial cells (Kreutzberg *et al.* 1986). However, in the hippocampus, ecto-5′-nucleotidase was observed at the surface of neuronal membranes (see Kreutzberg *et al.* 1986). Since the specific activity of ecto-5′-nucleotidase is about fifty times higher in the cholinergic nerve terminals of the hippocampus than in the hippocampal slices or whole hippocampal synaptosomal preparations (Cunha *et al.* 1992), and since the immunopurified nerve terminals have only very low amounts of myelin as the main contaminant, it appears that this enzyme in the hippocampus is highly associated with cholinergic nerve terminals. Whether or not this association also applies to non-cholinergic nerve terminals is unknown. It is worth noting that the specific activity of ecto-5′-nucleotidase in slices from the hippocampus is higher than in slices from the cerebral cortex (Cunha *et al.* 1992) and that this difference correlates with differences in the potency of adenosine as a modulator of acetylcholine release in these brain areas. This supports the suggestion (Phillis & Wu 1981) that the activity of ecto-5′-nucleotidase determines the synapses where adenosine is active as a neuromodulator.

Concluding Remarks

In the synaptic transmission there is a sequence of events that can be affected by adenosine: 1) The action potential that invades the presynaptic nerve terminal could be decreased by adenosine. Adenosine decreases the amplitude of compound action potentials in the frog sciatic nerve (Ribeiro & Sebastião 1984), and adenosine analogues decrease $^{22}Na^+$ uptake by rat brain synaptosomes stimulated by veratridine (Simões *et al.* 1988; Lobo & Ribeiro 1992). In the rat locus coeruleus neurones, adenosine decreases action potential duration (Pan *et al.* 1994); 2) Depolarization of nerve terminals causes changes in conductances of sodium, potassium and calcium ions, and adenosine can decrease sodium and calcium conductances, and increase potassium conductance; 3) The change in intracellular calcium ion concentration (and indirectly intracellular sodium ions) triggers exocytosis. Adenosine by decreasing calcium entry would inhibit evoked neurotransmitter release; and 4) The intracellular second messengers (cyclic AMP, protein kinase C, phosphoinositides) can be modified by A_1 adenosine receptor activation. There is still the interregulatory aspects: glutamate increases adenosine release, which then decreases glutamate release (e.g. Poli *et al.* 1991; White *et al.* 1993). This could occur in the hippocampus as a chain of events where glutamate activates NMDA receptors, which in turn release adenosine from interneurones, and this nucleoside will act at a distance to inhibit presynaptically the release of glutamate from excitatory synapses (see e.g. Manzoni *et al.* 1994).

In conclusion, adenosine inhibits both evoked and spontaneous excitatory neurotransmitter release. This can be demonstrated *in vitro* and *in vivo*. By activating presynaptic inhibitory receptors, adenosine inhibits several transducing systems (adenylate cyclase/cyclic AMP; phospholipase C/diacyl glycerol-phosphoinositides), opens potassium channels and inhibits calcium channels. It is not clear as yet if adenosine acts independently on each of these mechanisms, sequentially, in series or in parallel, some of these mechanisms only being activated in very precise conditions. For example, adenosine can decrease transmitter release by decreasing calcium entry into the nerve terminals. However, in the absence of external calcium, adenosine may inhibit transmitter release by depressing the intracellular systems that regulate transmitter release (e.g. protein kinase C, cyclic AMP, phosphoinositides). Each of these systems will be activated according to the needs of the cell in compliance with the role of adenosine as a 'retaliatory' metabolite. On the basis of this concept, the presynaptic inhibitory action of adenosine will allow the nerve terminals, that release neurotransmitters, to adjust their energy supply, and to 'retaliate' against their stimulation, which would cause excessive ATP breakdown (see Newby 1984). Then the life-preserving function of adenosine might use all means at disposal, e.g. if protein kinase C is maximally inhibited by other inhibitor of transmitter release, cyclic AMP could in part compensate this inhibition, facilitating transmitter release, and in this case adenosine would inhibit cyclic AMP accumulation, and in consequence neurotransmitter release. This or any other sequence involving the transducing systems that regulate transmitter release might or might not be hierarchy-dependent or representing some form of hegemonic function (or preference by adenosine) of one system in relation to the next one.

Since our knowledge of the detailed mechanisms involved in neurotransmitter release is limited, adenosine's regulatory mechanisms of neurotransmitter release appear complex. Indeed, an enormous 'plasticity' is needed to understand the subtle meanders used by the 'omnipresent' adenosine in its role as a neuromodulator.

Acknowledgements

I thank Dr. A. M. Sebastião for critically reading the manuscript. The research in the author's laboratory has been supported by the Gulbenkian Institute of Science, Junta Nacional de Investigação Científica e Tecnológica (JNICT), and European Union. The author is involved in an European Union concerted action (Biomed/ADEURO).

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