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COMMENTARY

MODULATION OF NEUROTRANSMISSION BY PURINE NUCLEOTIDES AND NUCLEOSIDES*

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This commentary will briefly summarize the growing body of evidence suggesting that extracellular purine compounds, by acting at a postulated cell membrane receptor, cause a diminution of transmitter release and alterations in the responses of the target organ of the released transmitter. The role of cyclic nucleotides or the role of ATPases as effectors in neurotransmission will not be discussed, however.

Release of purine compounds by nerve stimulation

There is abundant evidence that nerve activity is accompanied by release of purines. Holton and Holton [1] first reported evidence for release of ATP from sensory nerve endings. Release from central nervous structures *in vitro* [2–5] and *in in vivo* [6–10] was later reported. There are also reports of release of purines following nerve stimulation in various peripheral organs including the rat phrenic nerve–diaphragm preparation [11], taenia coli [12, 13], stomach [14], subcutaneous adipose tissue [15, 16], kidney [17], heart [18, 19], nictitating membrane [20], vas deferens [21], blood vessels [22], urinary bladder [23], anococcygeus muscle [24], lung [25] and ileum§. Despite probable omissions, the list is sufficiently long to suggest that release of purines following nerve stimulation is a general phenomenon. In all these instances the dominating products recovered were not adenine nucleotides but adenosine and its degradation products, inosine and hypoxanthine.

ATP is rapidly hydrolysed extracellularly to form adenosine, inosine and hypoxanthine. It has been argued, therefore, that the purine nucleosides found after nerve stimulation reflect release of intact ATP (e.g. ref. 12). Indeed, in several of the above-mentioned studies ATP has been detected. The question remains, however, if all the purines released are derived from intact ATP liberated from the cell. Several lines of evidence suggest that this is in fact not the case. For example, in superfused synaptosomes nucleotides accounted for only some 6 per cent of the total radioactive purines released by depolarisation [5], but about 75 per cent of the radioactivity released by hypo-osmotic shock. Pull and McIlwain [26] found that theophylline (0.5 mM) increased the amount of adenosine in the superfusate from electrically stimulated cortical slices but decreased the nucleotide content. Theophylline, in these concentrations, is a potent inhibitor of 5′-nucleotidase [27, 28], the enzyme that catalyses the conversion of 5′-AMP to adenosine. Potentiation of adenosine release by theophylline therefore is not compatible with the opinion that all of the adenosine (and inosine as well as hypoxanthine) derives from extracellularly released 5′-adenine nucleotides. It is possible that some of the adenosine formed during stimulation derives from cyclic AMP [29].

In small non-myelinated nerve fibres, activity is associated with an increase in intracellular phosphate, derived from hydrolysis of high-energy phosphate bonds [30, 31]. The increase in intracellular phosphate was parallelled by an increased efflux of phosphate and adenine compounds. No evidence was found for release of ATP while substantial adenosine liberation was detected [32]. These results demonstrate that nerves may release purines secondarily to intracellular breakdown of ATP. Furthermore, various metabolic inhibitors, or the removal of metabolic substrates, markedly increase the output of adenine derivatives from synaptosomes [5, 33]. Therefore, in the central and peripheral nervous system both nucleotides and nucleosides are released. The proportions may vary with the type of stimulation.

While the release of adenine derivatives has been extensively investigated, there are few studies of the possible release of guanine derivatives. In the entorhinal–hippocampal system, where adenine derivatives are transferred from nerves to postsynaptic elements, transfer of guanine derivatives was small [34]. In the isolated Langendorf perfused heart there was a slight increase in cardiac guanosine level from 3.2 ± 1.4 nmoles/g to 4.1 ± 1.3 nmoles/g, further increasing to 6.6 ± 0.3 nmoles/g after stimulation in the presence of dipyridamole§. Although these changes are much smaller than those in adenosine under similar circumstances [19], they may be taken as evidence for increased guanosine release during nerve stimulation. There is also circumstantial evidence that guanosine may be released from synaptosomes [5]. Finally, evidence has been presented

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that cyclic GMP may be released from presynaptic nerve endings under some circumstances [35]. In view of these data and the well-known role of GTP as a regulator of receptor function [36], further studies of the influence of nerve activity on guanine and guanosine metabolism could be highly interesting.

Release of adenine nucleotides as co-transmitters and transmitters

It is well known that ATP is stored together with catecholamines in the storage granules of adrenal medulla in a ratio of about 1:4 [37]. It is also known that ATP is located together with acetylcholine in synaptic vesicles from cholinergic nerves supplying the electric organ of *Torpedo* at the ratio of 1:5 to 1:11 [38–40]. In catecholamine storage granules of the splenic nerve trunk ATP is stored together with noradrenaline (NA) at a ratio close to 1:20 [41]. In light storage vesicles in the nerve terminals of the castrated vas deferens, the ratio ATP:NA appears to be still lower, approximately 1:50 (unpublished observations). Evidence that release of ATP occurs in parallel with transmitter or hormone release has been provided for the adrenal medulla [42, 43]. On the other hand, good evidence for exocytotic release of ATP from nerve endings is sparse. For example, in the just-quoted study where Stjärne *et al.* [43] were able to detect release of ^{32}P-label, presumably derived from adrenal medullary vesicles, they could find no release of ^{32}P-label following nerve stimulation in the spleen.

The major difficulty in detecting ATP release from nerve endings in peripheral tissues appears to be the very substantial release that occurs from the postjunctional elements. Already in 1962 Abood and coworkers found that membrane depolarization of excitable tissue caused release of high energy phosphates [44]. Moreover, heart cells and endothelial cells have been shown to release ATP in response to hypoxia and other noxious stimuli [45, 46]. Even in very densely innervated tissues such as the *Torpedo* electroplaque it would seem that effector cells are a much more important source of ATP than the nerves [47]. In synaptosomes from *Torpedo* ACh release may occur without detectable ATP release [48], and ATP may be released without any detectable ACh release [49]. Furthermore, it should be borne in mind that depolarization without transmitter release may cause ATP efflux from nerves [44]. It is therefore possible that part or all of the ATP release that does occur from synaptosomes is due to a mechanism initiating or accompanying exocytotic release of transmitter [50] rather than to exocytotic release of the nucleotide.

Hence, the demonstration that nerve stimulation induces release of adenine derivatives is not proof of an exocytotic release of ATP, not even if ATP rather than a metabolite is found extracellularly after nerve stimulation, or if it were shown that ATP was released from isolated nerve endings. Any attempt to calculate the concentration of ATP released into a synaptic gap, based on published estimates of the concentration of noradrenaline in the neuroeffector gap and assumption of the ratio NA:ATP in the granule, is therefore, a rather futile exercise in arithmetic.

These remarks have all been directed towards the evidence that ATP is released as a co-transmitter together with e.g. NA or ACh [51]. Some of the evidence for the interesting proposal that ATP is a transmitter in its own right in a special subset of neurons, the purinergic nerves [52], can be subjected to similar criticism.

Release of purines from the effector cells

As noted above, there is good evidence that purines, including ATP, may be released from the electric organ of the *Torpedo* following nerve stimulation [47]. Indeed, even a single nerve impulse is capable of increasing ATP release from the postsynaptic membrane [53, 54]. Since the electric organ is homologous with skeletal muscle, it seems probable that stimulation of nicotinic receptors also causes release of adenine compounds from the striated muscle cells. Smooth muscle cells also respond to excitation with enhanced purine release. In guinea pig taenia coli, not only contracting but also relaxing stimuli (noradrenaline, papaverine and nitroglycerin) caused an increased purine release [13]. It is well known that these relaxant drugs decrease the ATP content of intestinal smooth muscle [55]. In the dog adipose tissue, cat nictitating membrane, and rabbit kidney and heart, purine release was directly proportional to the degree of contractile response of the muscle, whether induced by nerve stimulation or other stimuli [15, 17, 19, 20, 56]. Constriction of the vascular smooth muscle seems to be the crucial factor in these perfused tissues. It may either be that the actual contraction of the smooth muscle leads to purine release, also as suggested by the data of Su [22], or that the vasoconstriction leads to a regional hypoxia that causes purine release [15]. A working hypothesis, compatible with the available evidence, is that membrane depolarization and/or local disparity in the balance between energy expenditure and energy production is responsible for the release of adenine compounds during nerve activity.

Inhibition of noradrenaline release by adenosine and adenine nucleotides

The first evidence that adenine compounds affect adrenergic neurotransmission by a prejunctional action seems to be the observation that cyclic AMP enhanced NA release induced by nerve stimulation in spleen and vas deferens [57, 58]. There was no significant effect of dibuturyl cyclic AMP in canine subcutaneous adipose tissue, however [59], and the role of cyclic AMP has later on been considered minimal also in vas deferens [60]. Nevertheless, in none of these studies was inhibition of transmitter release observed, which seems to be the principal effect produced by other adenine compounds. Thus, ATP was found to inhibit NA release in blood perfused canine adipose tissue *in situ* [59], isolated blood vessels from dog, rat and rabbit [61–63], rat vas deferens [64] and rabbit kidney [65]. The two other adenine nucleotides, ADP and AMP, are also effective as inhibitors of nerve-induced NA release, as indicated by observations with rat blood vessels [62] and rabbit kidney [65]. Adenosine was also found to be effective, and of approximately the same potency as its nucleotide congeners, in all the afore-

mentioned tissues, as well as in guinea pig vas deferens [66], rat salivary gland [67], and rabbit and guinea pig heart [67, 68]. It may, however, be noted that adenosine has been tested and found ineffective on nerve-induced release of NA in three different feline tissues: nictitating membrane*, spleen [69] and heart [70]. The reason for the exceptional behaviour of these and possibly other feline tissues is not known, and one can only speculate that the cat lacks purine sensitive receptors on adrenergic nerve terminals.

Some tissues are exquisitely sensitive to presynaptic inhibition by adenosine. Thus, in the rabbit kidney 0.1 μM adenosine sufficed to depress nerve simulation induced release of NA by approximately 20 per cent. The inhibition by adenosine increased dose-dependently and was more than 70 per cent at 10 μM [66]. The degree of presynaptic inhibition by adenosine was inversely related to stimulation frequency and did not exhibit tachyphylaxis. The effect was rapid in onset (manifest within 60 sec) and readily reversible. The finding that 0.1 μM adenosine significantly inhibited NA release is particularly interesting because the concentration of endogenous adenosine, inosine and hypoxanthine in the venous effluent may reach and surpass this value following renal nerve stimulation [65]. In the rabbit heart, the peak rate of efflux of adenosine and its metabolites, inosine and hypoxanthine, following 10 Hz stimulation was 19 nmoles/min [18]. This value is close to the amount of adenosine (25 nmoles/min) required to inhibit by 40 per cent the release of NA in response to 10 Hz stimulation [68]. The similarity between the amounts of adenosine released by nerve stimulation and those required for a significant presynaptic action in the heart is stressed further by the finding that when adenosine was infused in a concentration of 2.5 μmoles/l, more that 50 per cent was inactivated by tissue uptake and a further 25 per cent metabolized to inosine and hypoxanthine [18]. The above-mentioned data strongly suggest adenosine as a candidate for feed-back control of NA release, in particular since its metabolites, inosine and hypoxanthine seem to lack effect [65].

It is possible that also purine nucleotides may participate in such a mechanism. Indeed, nucleotides are released by nerve stimulation (see previous section), and they have documented effect on NA release, being approximately as potent as adenosine [62–65]. It has been suggested that the nucleotides must be hydrolysed to adenosine in order to cause presynaptic inhibition [64]. However, the stable ATP analogue, β,γ-methylene-ATP, seems to be as potent as ATP, ADP, AMP and adenosine in inhibiting NA release in the rabbit kidney [65], implying that the nucleotides may be active by themselves. As pointed out by Su [63], it does not really matter whether adenosine or an adenine nucleotide is the substance actually present in the vicinity of the nerve terminal, since all are virtually equipotent. Dephosphorylation of the adenine nucleotides does not alter the presynaptic inhibitory potency. It is only when adenosine is deaminated to inosine and further metabolized that presynaptic inhibitory potency is lost.

Apparently, adenosine is only one out of several naturally-occurring agents that are capable of regulating the release of NA. Theoretically, adenosine could act by interfering with at least two other presumed mechanisms, the NA-α-adrenoceptor-mediated autoinhibition [70, 71] and that operated by E prostaglandins [72]. Adenosine enhances α-adrenoceptor mediated contractions in vascular and non-vascular tissues (see below) and adenine nucleotides may cause increased release of prostaglandins [73]. However, none of these systems seem to be directly involved in the presynaptic action of adenosine. Thus, α-adrenoceptor blockade or blockade of prostaglandin synthetase did not reduce the inhibitory effect of adenosine on transmitter overflow [62, 66, 74].

Theophylline has been extensively used as a blocker of cellular actions of adenosine [52], and evidence has been presented that theophylline may interfere with the transmission effects of adenosine in adrenergically innervated tissues [61, 62, 64, 75, 76]. In the rabbit kidney [76] theophylline dose-dependently and reversibly antagonized the inhibitory effect of adenosine on NA release, as well as its stimulant effect on vasoconstrictor responses to nerve stimulation and administration of NA. The direct vasoconstrictor response to adenosine was also antagonized. Similar results have also been obtained with caffeine (unpublished observations). The antagonistic effects of theophylline were surmounted by an increase in the adenosine concentration, indicating competitive antagonism. According to Burnstock's classification of purine receptors [77], the results suggest the presence of P_1-receptors at both nerve terminal membrane and effector cell. In the canine saphenous vein theophylline antagonizes the inhibitory effect of ATP on neurogenic responses but not its direct contractile effect, indicating the presence of inhibitory prejunctional (P_1) receptors and excitatory postjunctional (P_2) receptors [75]. Theophylline not only antagonizes the effect of exogenous adenosine on adrenergic transmission, but *per se* enhances nerve induced release of NA [76]. This effect is not due to phosphodiesterase inhibition, because it occurs with theophylline concentrations having no effect on kidney cyclic AMP phosphodiesterase. Moreover, other potent phosphodiesterase inhibitors are inactive on NA release. Therefore, the possibility exists that the transmission effects of theophylline, and presumably other methylxanthines, reflect antagonism of endogenous adenosine [78]. This possibility is strengthened by the above mentioned observations that nerve stimulation may cause significant release of adenosine.

Inhibitors of adenosine uptake (dipyridamole and dilazep) and deamination (EHNA) potentiate the presynaptic effect of administered adenosine [65, 79]. These results indicate that adenosine is active *per se* and also that it is active on the outside of the cell. There seems to be two types of adenosine receptors in the cell membrane; one directed outwards and one inwards [80]. The presynaptic effect of adenosine is shared by agents that activate the external receptor

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such as phenyl-isopropyl-adenosine and 2-chloro-adenosine, but not by agents that interact with the internal receptor such as 2-deoxy-adenosine and SQ 22356 (unpublished data). It is also of interest that dipyridamole, dilazep and EHNA by themselves inhibit nerve-induced release of NA in rabbit kidney and rat blood vessels, and more so when dipyridamole or dilazep were given in combination with EHNA [64, 65]. These observations are consistent with a decreased inactivation of endogenous adenosine, and hence increased extracellular concentration of the compound, leading to inhibition of NA release.

Effects on non-adrenergic transmission

Adenosine and adenine nucleotides have been shown to depress acetylcholine release from motor nerve endings [81–83]. The nicotinic transmission in the parasympathetic ganglia of the cat urinary bladder was inhibited by AMP, ADP and ATP [84], presumably by a presynaptic mechanism of action. Also in the *Torpedo* electroplaque, adenine derivatives are capable of inhibiting acetylcholine release [54, 85]. Based on a statistical analysis of neurotransmission in the soleus muscle it was concluded that adenosine reduced the mean number of quanta released by depressing the store of quanta available for release rather than the probability of quantal release [86]. A mechanistic interpretation of these statistical terms is, however, difficult.

Adenosine and related adenine nucleotides dose-dependently inhibit release of acetylcholine (ACh) and ensuing contraction responses to transmural nerve stimulation in the guinea pig ileum [87–91]. On the other hand, they have little or no effect on contraction responses induced by ACh [88, 90–92]. Theophylline and other methylxanthines antagonized inhibition of the cholinergic transmission by purines [88, 92]. By itself, theophylline, in concentrations having no effect on cyclic AMP phosphodiesterase activities [93], enhanced ACh release and contraction responses to nerve stimulation in the guinea pig ileum [88, 94]. However, higher concentrations of theophylline inhibited neurogenic responses in parallel with inhibition of phosphodiesterase [93]. This latter effect of theophylline is in agreement with earlier studies linking together smooth muscle relaxation and cyclic AMP accumulation [55]. The biphasic effect of theophylline on the transmission may thus be explained in terms of low doses antagonizing the inhibitory effect of endogenous purines on ACh release, and high doses seemingly leading to inhibition of the transmission by causing a progressive increase in cyclic AMP accumulation and smooth muscle relaxation.

It has been shown that inhibitors of adenosine uptake (dipyridamole, hexobendine and dilazep) enhance the inhibitory effect of purines on cholinergic transmission and that they decrease the threshold dose of adenosine by approximately one order of magnitude (to 0.1 μM) [91, 93, 95]. In the same doses, dipyridamole markedly enhanced the release of purines induced by nerve stimulation, and caused the level of purine nucleosides to reach 0.1 μM in the surrounding medium [96, 97]. Dipyridamole and dilazep by themselves inhibit cholinergic transmission in guinea pig ileum by a mechanism which is antagonized by theophylline [90, 92, 95]. These observations are consistent with the concept that adenosine, or a related purine nucleotide, acts as a negative feed-back modulator of ACh release. Acetyl-CoA has also been considered an endogenous modulator of cholinergic neurotransmission [98], although to date no conclusive data have been presented.

It has been proposed that there are autonomic efferent nerves that are neither adrenergic nor cholinergic and which release ATP or a related purine compound as principal transmitter, for example in the urinary bladder [52]. It may be noted, however, that adenine nucleotides and adenosine dose-dependently and reversibly inhibited non-adrenergic non-cholinergic transmission in the urinary bladder, and that the inhibition showed the same susceptibility to antagonism by theophylline and enhancement by dipyridamole and EHNA as that in adrenergic and cholinergic transmission [99]. However, in this case the inhibition seems to be largely postjunctional. Thus, the proposed excitatory transmitter in the purinergic nerves would seem capable of inhibiting its own actions.

Actions of adenosine in the CNS

A role for adenosine in the regulation of cyclic AMP generating systems in the CNS has been firmly established [100, 101]. The effect of adenosine on cyclic AMP levels is independent of adrenergic α- or β-receptors and of histamine receptors [102]. On the other hand, adenosine appears to potentiate the actions of noradrenaline and histamine on cyclic AMP [100, 102].

Adenosine probably acts on a specific 'adenosine-receptor' located on the outer surface of the cell membrane [101]. Specific adenosine binding sites in the CNS with several characteristics of the proposed receptor were recently demonstrated [103]. Methylxanthines act as competitive antagonists of adenosine actions and the structural requirements for an action on these 'adenosine-receptors' appear to differ from those for inhibition of phosphodiesterase(s) [104]. Adenosine present in brain slices under basal conditions and following depolarization contributes significantly to the level of cyclic AMP [100]. The same appears to be true in cultured cells of nervous origin [105] and in homogenates of brain tissue [106–107]. This probably explains the repeated finding that theophylline may decrease cyclic AMP accumulation in slices as well as in brain homogenates (see refs. 101 and 106). Non-methylxanthine phosphodiesterase inhibitors may also influence adenosine mechanisms. For example, papaverine and Ro 20-1724 increase in the formation of adenosine; dipyridamole and papaverine inhibit its inactivation [101, 108]. The choice of phosphodiesterase inhibitor in experiments regarding central cyclic nucleotide mechanisms is therefore of crucial importance, and the possibility that interactions with adenosine mechanisms contribute significantly to the overall effects of several phosphodiesterase inhibitors must be seriously considered [78, 101, 109].

Adenosine increases not only cyclic AMP but also

cyclic GMP levels in brain slices [108, 110, 111]. These effects are shared by adenine nucleotides, but not by guanosine, adenine, cytidine or uridine [111]. The accumulation of cyclic GMP is entirely dependent upon the presence of extracellular calcium ions [110, 111], while the accumulation of cyclic AMP induced by adenosine was unaltered in the absence of extracellular calcium [110, 111]. However, removal of calcium may induce an increased release of adenosine leading to enhanced 'basal' cyclic AMP [108, 110]. Theophylline inhibits adenosine induced cyclic AMP accumulation much more effectively than cyclic GMP accumulation [111]. Thus, the action of adenosine on the two cyclic nucleotides may be effected through different mechanisms. The stable adenosine analogue 2-chloroadenosine increases glycogenolysis in the rat caudate nucleus, but this effect is probably not mediated via cyclic AMP dependent phosphorylation of phosphorylase kinase and phosphorylase [112, 113].

Several studies demonstrate a depressant action of adenosine and adenosine derivatives on the discharge of central neurons, both *in vivo* and *in vitro*. Thus, under *in vivo* conditions, a depressant action of adenosine is found in the rat caudate nucleus [114], toad spinal cord [115], and rat cerebral and cerebral cortex [116–118]. Moreover, drugs that potentiated the effect of electrophoretically applied adenosine, such as EHNA and papaverine, had a depressant action of their own, suggesting an effect of endogenous adenosine on firing rate [117]. Electrophysiological studies of brain slices *in vitro* have similarly shown a depressant action of adenosine in olfactory cortex [119–121], as well as hippocampus [10, 122–124], but not in the superior colliculus [124]. At least in the hippocampus, the actions of various drugs are compatible with a role for endogenous adenosine.

The depressant action of adenosine on cortical neurons is shared by a number of adenine nucleotides, including cyclic AMP, but not by adenosine breakdown products such as inosine, hypoxanthine, xanthine and adenine [122]. The inhibitory effect of adenosine and the adenine nucleotides was antagonized by methylxanthines given locally by iontophoresis or by the systemic route [122]. Guanosine and guanosine nucleotides had a much weaker effect on corticospinal neurons [122]. In the toad spinal cord guanosine either had no effect or depolarized the neurons, while adenosine caused hyperpolarization [115].

There is good electrophysiological evidence that adenosine (and 5'-AMP) depresses synaptic mechanisms [10, 121–123], presumably by inhibiting the release of an excitatory transmitter. Indeed, inhibition of the release of acetylcholine [125], dopamine [125, 126], noradrenaline [127], serotonin [125] and GABA [125, 128] has been reported. In all these instances release of labelled transmitter was induced by potassium-depolarization or by ouabain and the effect was generally small. In our own studies (unpublished) we have been unable to detect significant inhibition by adenosine on veratridine-induced transmitter release. The apparent contradiction between these *in vitro* studies on transmitter release and the electrophysiological studies could indicate that transmitter release induced by action potentials is much more sensitive to inhibition by adenosine than is transmitter release induced by depolarization or by ouabain. Another possibility is that only some of the sites at which transmitter release may be evoked by depolarizing slices *in vitro* are markedly affected by adenosine. It is interesting to note that in both the electrophysiological [10, 122] and biochemical studies [125–128] theophylline and other methylxanthines were potent antagonists of the adenosine effects.

Adenosine as a postsynaptic modulator

Adenosine may not only influence the release of transmitter but also the response to the transmitter. There are several studies suggesting that effects mediated by α-adrenoceptors are potentiated by adenosine. This is true, for example, as regards cyclic AMP stimulation in brain slices [100, 101]. α-Adrenoceptor-mediated vasoconstrictor responses may also be enhanced by adenosine in some circumstances [66]. In the guinea pig vas deferens adenosine causes a dose-dependent potentiation of noradrenaline induced concentrations; guanosine and inosine being much less potent [129]. In this tissue adenosine produces a dose-dependent sustained depolarization [130], which may explain the potentiation.

In contrast, β-adrenoceptor mediated effects are often inhibited by adenosine. This effect has been extensively characterized in the fat cells [131–133] and appears to be due to inhibition of cyclic AMP formation. There are two types of adenosine receptors, one intra- and one extracellular [134]. The extracellular site, on which various N^6-substituted adenosine derivatives are the most potent agonists, appears to be the physiologically important [131–135]. The internal type of receptor, where deoxyribose derivatives are particularly active, seems to be of considerable pharmacological interest, but may be physiologically irrelevant in view of the low affinity for adenosine at this site [134, 135]. Inhibition of β-responses by adenosine is also evident in the CNS [135, 136] and heart [137]. Therefore, increasing levels of adenosine may shift the balance between α- and β-effects.

It may seem paradoxical that adenosine, which stimulates adenylate cyclase for example in brain and heart, is capable of inhibiting in a competitive manner the effect of β-adrenoceptor agonists, which also stimulates adenylate cyclase. The reasons for this has been worked out by Braun and Levitzki [135]. The adenosine receptor is permanently coupled to the adenylate cyclase. By contrast, the β-receptor activates adenylate cyclase by a bimolecular collision reaction. The two receptors operate on a common adenylate cyclase through a common GTP-regulatory site. The catecholamine-induced adenylate cyclase will be inhibited by adenosine in the present of GTP, when the GTP'ase turn-off reaction takes place, because adenosine is a less potent agonist than are the β-agonists [135]. In the cholinergic system it seems that adenosine does not influence the responses to acetylcholine acting on the muscarinic receptor [89]. By contrast, there is some evidence that adenine derivatives may potentiate responses on the nicotinic receptor. Thus,

adenine derivatives seem to enhance depolarization induced by acetylcholine in frog muscle [138].

Finally, it should be pointed out that adenosine and other adenine derivatives may have direct postjunctional actions. These direct effects could influence the net response induced by nerve stimulation. For example, the well known vasodilatory action of adenosine tends to antagonize the vasoconstrictor action of the catecholamines (except in the kidney). There are often considerable differences between the direct actions of ATP and those of adenosine, possibly because they have different affinities for the postjunctional purine receptors, denoted P_1 and P_2 receptors by Burnstock [77].

Mechanisms of inhibition of transmitter release

Very little is known about the mechanism by which adenosine (or for that matter any presynaptically active drug) inhibits transmitter release. Despite the pronounced effects of adenosine on cyclic AMP in nervous tissue it seems clear that the presynaptic effects of adenosine are not mediated over cyclic AMP [10, 139, 140]. In particular, it should be mentioned that if cyclic AMP has any definite effect on transmitter release it stimulates it (see ref. 141).

It is well known that calcium plays an important role in excitation–secretion coupling, and adenosine may somehow interfere with the calcium disposition in the nerve terminae. Adenosine does not influence tyramine-induced release of noradrenaline [137, 142], which occurs by a calcium-independent process. In a recent paper, Ribeiro and coworkers [143] report that adenosine inhibits K^+-induced calcium uptake by synaptosomes. However, a closer inspection of the data indicates that in submillimolar concentrations, adenosine, if anything, increases the initial rate of calcium accumulation. Thus, a direct effect on calcium transport is not proven. The data of Ribeiro *et al.* [143] may, however, indicate that adenosine reduces the level of free intracellular Ca^{2+}. Such an effect was also suggested by Branisteanu *et al.* [86] from their results of experiments on frog neuromuscular transmission. Vizi and Knoll [88] were unable to antagonize the presynaptic inhibitory effect of adenosine by enhanced extracellular calcium. Others have, however, demonstrated a dependency on extracellular Ca^{2+} but concluded that 'classical' competition with Ca^{2+} (in the manner of Mg^{2+}) could not explain the actions of adenosine on transmitter release [81, 86].

In the cholinergic, as well as the noradrenergic system, the presynaptic effect of adenosine is strongly frequency dependent [61, 65, 67, 88, 90]. At high stimulation frequencies the inhibitory effect is small or non-existent. This indicates that adenosine does not primarily act by preventing the spread of impulses into the terminal area [88]. Studies by Stone [144] on the spinal cord also argue against an action of adenosine on nerve terminal excitability. It has been pointed out that at high stimulation frequencies more calcium may be left at the active releasing sites [145]. It is an old finding that adenine nucleotides and adenosine markedly shorten the action potential in atrial muscle [146, 147]. If this occurs also in the nerve ending it would offer a good explanation for the presynaptic effect of adenosine and its high frequency dependence, as suggested by Wakade and Wakade [67]. A theoretical possibility is that adenosine increases chloride influx. It has indeed been found that adenosine does promote chloride influx in brain slices and in primary astrocyte cultures [148]. Another possibility is that adenosine acts by accelerating repolarization secondarily to increased K^+-efflux [146]. Further studies of these possibilities will be of considerable interest.

Finally, it should be mentioned that a 'vesigate', a structure apposed to the inner face of the presynaptic membrane and which binds transmitter to saturation, has been suggested to be the structure actually involved in acetylcholine release [149]. Calcium is the likely trigger of release from this postulated structure. A presynaptic modulator such as adenosine could act on such a membrane associated 'vesigate' by altering binding of transmitter to it or release of transmitter from it. It is readily apparent from the above considerations of possible mechanisms that the precise mode of action of adenosine cannot be defined, and is unlikely to be so, until our knowledge about both the mechanisms of transmitter release and the actions of adenosine on the ion transport across nerve terminal membranes and on levels of ions in the nerve cell cytoplasm are considerably larger than at present.

Functional significance

Firstly, it should be pointed out that inhibition of transmitter release by adenosine and adenine nucleotides seems to be a very general phenomenon even though it is not demonstrable everywhere.

Secondly, these compounds seem to act on the outside of the cell. The inhibitory potency is essentially equal between the adenosine nucleotides and adenosine. On the other hand, the guanine series of compounds and the adenosine breakdown products are virtually inactive. Consequently, it does not matter from a functional point of view which compound is present at the terminal as long as it is adenosine or an adenine nucleotide. When present in a sufficient concentration these compounds will all depress neurotransmission to a similar extent. In particular, dephosphorylation of ATP to ADP, to AMP or to adenosine does not inactivate it as a modulatory signal for transmitter release. It is only when the compound is internalized into a cell and/or deaminated to inosine that the inhibitory potency vanishes.

The third point to make is 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' [150] and 'retrograde transmission' [85] 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.

Fourthly, since adenosine and adenine nucleotides are of actual or potential significance in the regulation of neurotransmission, drugs may act by increasing or decreasing the influence of these purines. A particularly interesting possibility is that some of the actions of methylxanthines in the central and peripheral nervous systems may be due to their ability to inhibit the actions of adenosine and adenine nucleotides.

Finally, it is important to note that these compounds are active not only on nerve endings but they modify contractility of muscle cells and metabolic activity, as well as blood flow. The importance of this is illustrated in the heart and the adipose tissue [132, 137]. If the stimulation is very strong then there is a severe discrepancy between oxygen demand and supply. The adenosine that accumulates under these circumstances serves to decrease the release of stimulatory transmittor and inhibit metabolic activity, as well as to increase blood flow. Adenosine, therefore, tends to limit its own formation in several ways, acting as a true feed back signal.

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