Chapter 34
Purinergic Signalling in the Cerebellum

Mark J. Wall

Abstract Purinergic signalling is a complex and evolutionarily conserved mechanism of extracellular communication involved in many physiological and pathological functions. The complexity arises from a large number of purine receptor subtypes and multiple endogenous purine receptor ligands (including ATP, ADP, UTP, UDP UDP-glucose and adenosine) which can be directly released or arise from extracellular metabolism. Although much work has defined the distribution of purine receptors in the cerebellum and the cellular effects of purine receptor activation, relatively little is known about how and when purines are released in the cerebellum, the role of purinergic signalling in controlling cerebellar circuit output and the importance of purines in cerebellar motor control.

Keywords ATP • Adenosine • P2 receptors • P1 receptors • Neuromodulation

34.1 Introduction

Purines are important extracellular signalling molecules that mediate diverse physiological and pathological effects via cell-surface receptors (for review see Burnstock 2007). There are several potential endogenous purine receptor ligands but this review will concentrate on ATP and adenosine.

34.2 Mechanisms of ATP Release

All cells contain ATP (as energy currency) and hence they are all potential sources of ATP for extracellular signalling. But how does ATP get from the intracellular compartment into the extracellular space? ATP can be co-released from neurons by vesicular exocytosis, together with *classical* neurotransmitters such as glutamate

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D.L. Gruol et al. (eds.), *Essentials of Cerebellum and Cerebellar Disorders*,
DOI 10.1007/978-3-319-24551-5_34

and GABA. ATP can also be released by exocytosis from glial cells, via hemi-channels (pannexins and connexions, the latter of which combine together to form gap junctions between cells) and by a subset of ATP receptors (PX7), which undergo pore dilatation.

34.3 ATP (P2) Receptors

Following release, ATP activates two types of receptor, termed P2X and P2Y, which are both expressed by neurons and glia throughout the brain (Fig. 34.1). P2X receptors are ligand-gated ion channels. Seven P2X receptor subunits have been cloned (P2X1-7) and can combine to produce either homomeric or heteromeric receptors with distinct properties and pharmacology. P2X receptors are permeable to monovalent cations such as Na^+ and K^+ and divalent cations such as Ca^{2+}. For full review of the molecular physiology of P2X receptors see (Burnstock and Kennedy 2011). Since the synaptic responses to P2X receptor activation are often very small, when compared to GABA and glutamate, this has led to the idea that their role is to modulate other neurotransmitter systems rather than produce postsynaptic depolarisation.

P2Y receptors are G protein-coupled receptors with seven transmembrane domains. At least eight P2Y receptor subtypes have been cloned and modulate synaptic transmission, regulate ion channels and release Ca^{2+} from intracellular stores. For a comprehensive review of P2Y receptors see (von Kugelgen 2006).

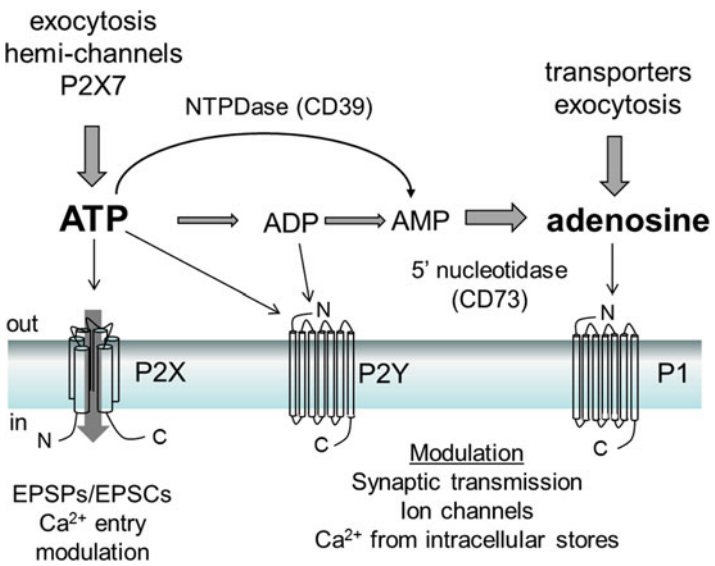

Fig. 34.1 Actions of ATP and adenosine. ATP activates P2 receptors and is then metabolised to adenosine which activates P1 receptors. Adenosine can also be directly released

34.4 ATP (P2) Receptors in the Cerebellum

Many of the possible P2 receptor subtypes appear to be expressed in the cerebellum, although the functional relevance of individual receptor subtypes is often not clear. Several studies have shown that activation of P2 receptors increases intracellular Ca^{2+} concentration in Purkinje cells, granule cells and glial cells, with P2 receptor activation initiating Ca^{2+} waves that can spread between coupled glial cells in vitro and in vivo. A recent study has shown that increases in Ca^{2+} concentration in Bergmann glial cells enhances the removal of extracellular K^+ leading to changes in Purkinje cell firing. The rise in intracellular Ca^{2+} concentration probably stems from P2Y receptor activation (Ca^{2+} coming from intracellular stores) although whole cell and single channel P2X receptor currents have also been recorded in Purkinje cells, granule cells and astrocytes.

Activation of presynaptic P2 receptors (probably both P2X and P2Y) on basket cells, parallel fibres and Lugaro cells modulates excitatory and inhibitory synaptic transmission to Purkinje cells (reviewed in Deitmer et al. 2006). The P2Y receptors present on Purkinje cells can also enhance $GABA_A$ receptor sensitivity. The mixed effects of P2 receptor activation on excitatory and inhibitory cells makes it difficult to predict the overall effect on cerebellar circuit output.

34.5 Extracellular Metabolism of ATP

Once released into the extracellular space, ATP is rapidly metabolised to adenosine by enzymes called ectonucleotidases (reviewed in Zimmermann et al. 2012). These enzymes include ecto-ATPase (CD39, converts ATP directly to AMP without liberating ADP) which is expressed in the soma and dendrites of Purkinje cells and weakly in the granule cell layer and ecto-5′-nucleotidase CD73 (converts AMP to adenosine) which is localized in glial cells and parallel and climbing fibre synapses.

34.6 Adenosine Release in the Brain

Extracellular adenosine arises from ATP metabolism (as outlined above) but adenosine can also be directly released into the extracellular space via specific nucleoside transporters (equilibrative and concentrative) and by exocytosis (see below). The extracellular concentration of adenosine in the brain can be increased by a number of stimuli including hypoxia, ischemia, hypoglycemia, epileptic seizures and prolonged wakefulness. More recent studies have shown that adenosine can also be released by brief trains of action potentials and thus is potentially important in controlling physiological network activity.

34.7 Adenosine (P1) Receptors in the Cerebellum

Once produced, extracellular adenosine activates G protein-coupled receptors (P1) that are divided into four subtypes: A_1, A_{2A}, A_{2B}, and A_3 (Fredholm et al. 2001). The A_1 receptor is the most widely expressed adenosine receptor in the brain and is inhibitory, as upon activation it opens K^+ channels and closes voltage-gated Ca^{2+} channels, leading to hyperpolarisation of the membrane potential and inhibition of transmitter release. Activation of A_{2A} and A_{2B} receptors can facilitate transmitter release and modulate synaptic plasticity.

The cerebellum contains high levels of the A_1 adenosine receptor, possibly the A_3 receptor (diffuse expression), but not A_{2A} or A_{2B} receptors. Activation of A_1 receptors reduces glutamate release at parallel fibre-Purkinje cell synapses with a similar but smaller effect at climbing fibre synapses. Adenosine also inhibits GABA release at Golgi cell-granule cell synapses via A_1 receptor activation.

34.8 Adenosine Release in Cerebellum

Adenosine is released in the molecular layer of the cerebellum by focal electrical stimulation and can be directly measured using adenosine biosensors. This adenosine release is both action potential and Ca^{2+}-dependent with at least a proportion directly released from parallel fibres by exocytosis (Klyuch et al. 2012). Enough adenosine is released to activate A_1 receptors and inhibit transmitter release at the parallel fibre synapse. This potentially represents an important feedback mechanism for controlling neural activity in the cerebellum. Adenosine is also released by hypoxia and inhibits parallel fibre-Purkinje cell transmission via A_1 receptor activation which is probably neuroprotective.

34.9 Breakdown and Uptake of Adenosine

The extracellular concentration of adenosine is controlled by several mechanisms including specific equilibrative and concentrative transporters which transport adenosine into neurons and glia (Fig. 34.2). Adenosine can then be inactivated, by metabolism to inosine (by adenosine deaminase, ADA) or phosphorylated to AMP (by adenosine kinase, ADK), maintaining low concentrations of intracellular adenosine. ADK is exclusively expressed in glial cells with ADA present in glia and neurons and to a smaller extent in the extracellular space. In common with many brain regions, ADK activity is the major determinant of the basal extracellular concentration of adenosine in the cerebellum, with adenosine deaminase playing only a minor role.

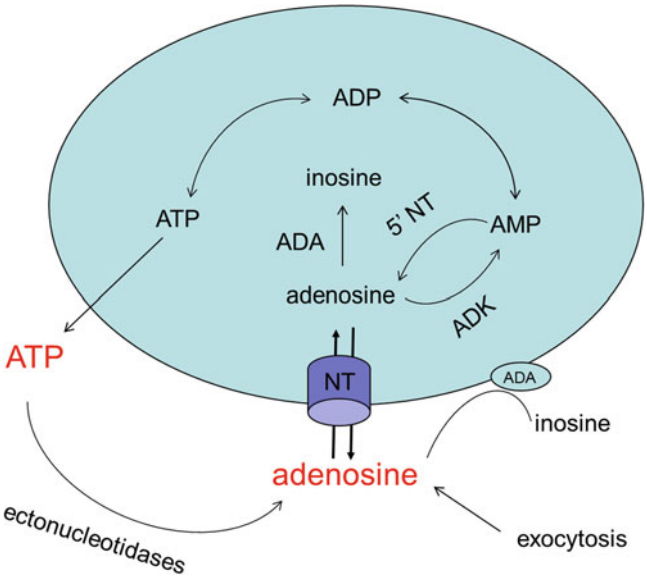

Fig. 34.2 Control of extracellular adenosine concentration. Adenosine is removed from the extracellular space by nucleoside transporters (NT) and can then be converted to AMP by adenosine kinase (ADK) or metabolised to inosine by adenosine deaminase (ADA). There is a small amount of extracellular ADA. ADK dominates adenosine clearance during basal conditions, leading to replenishment of intracellular ATP

34.10 The Role of Purinergic Signalling in the Cerebellum and in Motor Control

The role that purinergic signalling plays in cerebellar function remains unclear. Experiments in which Bergmann glia (which utilise ATP signalling to produce Ca^{2+} waves) were transgenically removed, showed defects in long term depression and eye blink conditioning although motor co-ordination was unaffected. It is tempting to suggest that glial ATP signalling is important for correct cerebellar function, but it could be that other roles of glia (such as glutamate uptake) underlie the deficits. There are several P2Y receptor knockout mice but they show no obvious cerebellar phenotype. Knockout of the adenosine A_1 receptor also has little effect on co-ordination and locomotion. There is however evidence that adenosine signalling in the cerebellum is involved in the ataxia produced by alcohol and cannabinoid intoxication and is impaired in the neurodegenerative disease Niemann–Pick Type C.

34.11 Conclusions and Future Work

A great deal of work has defined the distribution of purine receptors and their effects on cellular function within the cerebellum but many questions remain unanswered. What effect does purine signalling have on cerebellar neural network activity,

cerebellar output and motor control? Mixed excitatory effects (P2X, P2Y) and inhibitory effects (A1 and P2Y) on both excitatory (glutamatergic) and inhibitory (GABAergic) neurons and on glial cells makes this difficult to predict. What is the source of ATP and adenosine and what form of cerebellar activity results in their release? ATP is probably released from Bergmann glia, astrocytes and maybe from molecular layer interneurons. Adenosine appears to be released from parallel fibres although there are probably other sources. Currently neither ATP nor adenosine release has been directly linked to a motor behaviour.

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