CHAPTER

19

Purinergic Signaling

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OUTLINE

NOMENCLATURE OF PURINES AND PYRIMIDINES

Purines such as ATP and adenosine play a central role in the energy metabolism of all life forms. This fact probably delayed recognition of other roles for purines as autocrine and paracrine substances and neurotransmitters. Today it is recognized that purines are released from neurons and glial cells and that they produce widespread effects on multiple organ systems by binding to purinergic receptors located on the cell surface. The principal ligands for purinergic receptors are adenosine, ATP and UTP.

A nucleoside consists of a purine or pyrimidine base linked to a pentose, either d-ribose to form a ribonucleoside or 2-deoxy-d-ribose to form a deoxyribonucleoside. Three major purine bases and their corresponding ribonucleosides are adenine/adenosine, guanine/guanosine and hypoxanthine/inosine. The three major pyrimidines and their corresponding ribonucleosides are cytosine/cytidine, uracil/uridine and thymine/thymidine. A nucleotide such as ATP (Fig. 19-1) is a phosphate or polyphosphate ester of a nucleoside.

PURINE RELEASE

Interstitial nucleotides are derived from intracellular sources. In addition to its central role in cellular energy metabolism, ATP is a classical neurotransmitter that is packaged into secretory granules of neurons and can be released in quanta in response to action potentials, as illustrated in

Fig. 19-2. This source of ATP is responsible for rapid phasic changes in local extracellular concentrations of this purine. Often ATP is co-released with other transmitters.

Chemical transmitters must be transported into synaptic vesicles against a concentration gradient (Ch. 3). A recent discovery has identified a specific vesicular nucleotide transporter (VNUT) that is responsible for the transport of ATP. It has been postulated for some time that there must be a transporter for this purine, given that it is concentrated in vesicles at ~100 mM. The recently identified VNUT is a member of the SLC17 type I phosphate transporter family that includes the three different vesicular glutamate transporters. SLC17A9 codes for a 430 amino acid residue protein with 12 putative transmembrane spanning helices (Sawada et al., 2008).

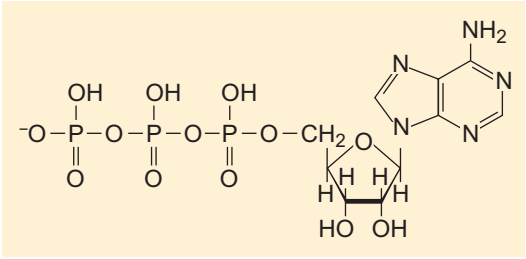

FIGURE 19-1 **Adenosine 5′-triphosphate. Adenosine is a purine nucleoside consisting of adenine and ribose**. The addition of triphosphate generates ATP.

In addition to exocytotic release, it has been shown that there are a variety of pathways for the release of ATP. For example, activity-dependent release of ATP from axons is mediated by volume-activated anion channels (Fields & Ni, 2010).

Numerous studies have demonstrated that purines are critical signals utilized by glia in the central nervous system. Because astrocytes are slower signaling cells than neurons, it is not surprising that there is a diversity of pathways that can contribute to the release of purines from these glial cells. Just as neurons can release purines through a vesicular

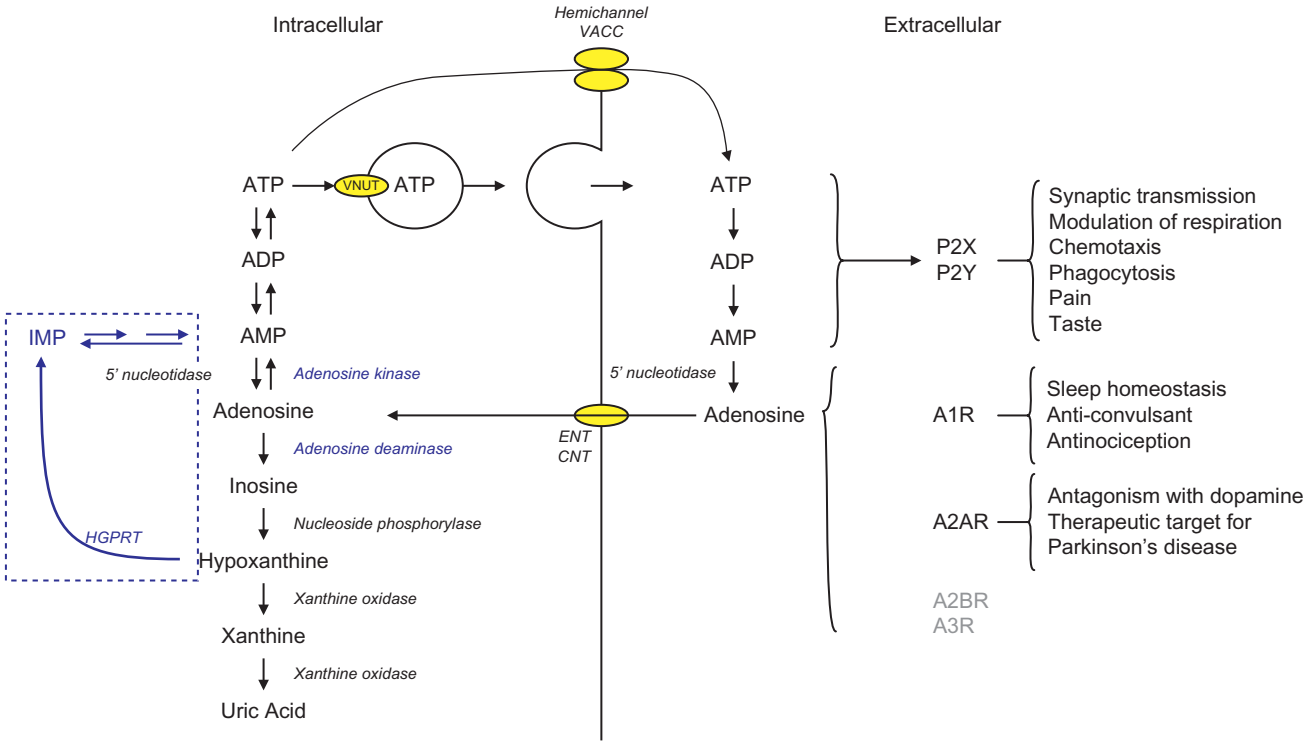

FIGURE 19-2 **Purine release and metabolism**. Although ATP is used as an energy source, it is also released as a signaling molecule. ATP is packaged into vesicles by vesicular nucleotide transporter (VNUT), which provides an exocytotic release pathway. ATP can also be released by channels and transporters. In the extracellular space ATP is rapidly hydrolyzed to adenosine by ectoenzymes. Purines have actions through P_1, P_{2X} and P_{2Y} receptors. Adenosine is taken back up into cells via equilibrative or concentrative nucleoside transporters (ENT, CNT) where it is metabolized to uric acid or via the action of adenosine kinase to AMP and ultimately to ATP. Metabolism through adenosine kinase dominates over adenosine deaminase. Hypoxanthine can be metabolized to IMP and subsequently to AMP via the action of hypoxanthine-guanine phosphoribosyltransferase (HPGRT). Equilibrative transport of adenosine allows bidirectional flow of adenosine and consequently the metabolic state of the cell to regulate extracellular adenosine. Under normoxic conditions, adenosine kinase phosphorylates intracellular adenosine, causing a concentration gradient of adenosine into the cell and its uptake via ENT. However, under hypoxic conditions, or during periods of metabolic demand, intracellular adenosine accumulation leads to its release through ENTs. (Blue). Mutations in adenosine deaminase are associated with changes in sleep, presumably resulting from increased intracellular adenosine leading to release of this purine via ENTs and to extracellular adenosine accumulation (see Fig. 19-3). Increased expression of adenosine kinase in rodent models of epilepsy lead to enhanced clearance of adenosine and removal of this natural anticonvulsant. Lesch-Nyhan Syndrome is an inherited disorder with mutations in HPGRT.

mechanism, evidence is accumulated to support the presence of a similar mode of release in these glia. For example, ATP has been shown to be concentrated in vesicles, and the expression of a dominant negative construct that impairs exocytosis reduces the release of ATP/adenosine from astrocytes (Pascual et al., 2005). Unlike neurons, however, this release is likely to be a more tonic release pathway since astrocytes do not generate action potentials, which would be needed for fast synchronized release. In addition to vesicular release there is accumulating evidence for the release of ATP by alternative pathways. Astrocytes are known to express connexin 43 (Cx43), which is able to form hemichannels as well as gap junctions. Overexpression of Cx43 leads to increased release of ATP (Cotrina et al., 1998). Recent evidence also supports the idea that pannexins can also mediate ATP release (Chekeni et al., 2010). As will be discussed later, the release of these compounds contributes to the control of sleep homeostasis.

Extracellular nucleotides are regulated by ectoenzymes

Neurotransmitters are characteristically inactivated once they have been released in order to terminate their signaling activity. This occurs by two mechanisms. First, transmitters such as serotonin, glutamate and γ-amino butyric acid (GABA) are taken up by transporters to terminate their action (Chs. 15, 17 and 18). Pharmacological inhibition of these transporters leads to an extended lifetime of the neurotransmitter in the extracellular space, which is the mechanism of action of serotonin selective reuptake inhibitor antidepressants (Ch. 15). Second, once released, extracellular enzymes can metabolize the transmitter into inactive products, which are then transported back into the neuron to provide a renewable source of transmitter. A classic example is the metabolism of acetylcholine (Ch. 13). Cholinesterase inhibitors are targeted to these enzymes to increase the lifetime of acetylcholine, which is a strategy that has been developed to enhance cholinergic transmission in Alzheimer's disease. In contrast to how classical transmitters are inactivated, ATP is metabolized to distinct compounds, each of which can have bioactivity. Ectoenzymes are involved in the rapid metabolism of ATP and other nucleotides in the extracellular space (Zimmermann & Braun, 1996). ATP applied to rat brain hippocampal slices is mostly converted to adenosine in less than a second. Some of the enzymes involved in ATP, UTP and nucleoside metabolism are depicted in Fig. 19-2. The hydrolysis of ATP to adenosine, for example, provides unique spatiotemporal opportunities for this signaling system: one can envision that a brief focal release of ATP would lead to local actions on ATP sensitive receptors, whereas following its metabolism to adenosine, a delayed spatially diffuse effect could be mediated by adenosine-sensitive receptors.

Several classes of ectoenzymes are found, including ecto-ATPase, which catalyzes the hydrolysis of ATP, and ecto-ADPase, which hydrolyzes ADP. Ecto-ATP diphosphohydrolase (ADPase or apyrase) is a plasmalemma-bound enzyme that dephosphorylates extracellular ATP and ADP to produce AMP. This enzyme is identical to CD39, an activation marker found on B-lymphocytes. A selective inhibitor of ecto-ADPase is ARL67156. This compound potentiates the effect of endogenously released as well as endogenously added ATP. Extracellular AMP is converted to adenosine by ecto-5′-nucleotidase, an enzyme that is attached to the cell surface by a glycosyl phosphatidylinositol linker (Ch. 5). 5′-Nucleotidases catalyze the conversion of purine and pyrimidine nucleoside monophosphates to the corresponding nucleosides. CD73 is a 5′-nucleotidase that is found on T and B lymphocytes (Resta & Thompson, 1997). Ecto-5′-nucleotidase can be blocked by α,β-methylene-adenosine diphosphate (AOPCP). In histochemical studies, ecto-5′-nucleotidase has been found to be associated with plasma membranes of glial cells and astrocytes. Soluble cytosolic 5′-nucleotidases also exist and are involved in the formation of adenosine during increased metabolic activity. In rat brain, ischemia results in an upregulation of 5′-nucleotidase on activated astrocytes. This is thought to increase the capacity of damaged tissue to form neuroprotective adenosine. Extracellular adenosine also can be derived from the metabolism of extracellular cyclic AMP by an ectocyclic AMP phosphodiesterase.

There are several sources of extracellular adenosine

Adenosine is not a classical neurotransmitter because it is not stored in neuronal synaptic granules nor is it released in quanta. It is generally thought of as a neuromodulator that gains access to the extracellular space in part from the breakdown of extracellular adenine nucleotides and in part by translocation from the cytoplasm of cells by nucleoside transport proteins, particularly in stressed or ischemic tissues (Figs. 19-2 and 19-3). Extracellular adenosine is rapidly removed in part by re-uptake into cells and conversion to AMP by adenosine kinase and in part, by degradation to inosine by adenosine deaminases.

Adenosine and homocysteine are formed from the hydrolysis of S-adenosylhomocysteine (SAH) by the enzyme SAH hydrolase. Attempts to measure intracellular adenosine are complicated by the fact that over 90% of intracellular adenosine may be weakly bound to this enzyme. SAH is formed from S-adenosylmethionine (SAM), which is a cofactor in transmethylation reactions. SAH is the precursor of a sizable fraction of adenosine under resting conditions, but most adenosine is derived from the 5′-nucleotidase pathway during conditions of hypoxia, ischemia or metabolic stress. The accumulation of high concentrations of adenosine under these conditions also leads to a large increase in inosine resulting from adenosine deamination. Intracellular adenosine can be reincorporated into the nucleotide pool upon phosphorylation by the cytosolic enzyme adenosine kinase. In normoxic resting tissues, most adenosine is rephosphorylated, since the Km of adenosine kinase is 10–100 times lower than the Km of adenosine deaminase. Deamination, which leads to a large accumulation of inosine, becomes the major pathway of adenosine metabolism when adenosine levels are elevated. Human genetic variations in adenosine deaminase do result in changes in the duration and intensity of sleep, a process that is known to be modulated by adenosine. The concentrations of adenosine and inosine in the interstitial

fluid of brain and other tissues are increased when oxygen demand exceeds oxygen supply. The effect of adenosine is to increase oxygen delivery by dilating most vascular beds and generally to decrease oxygen demand by reducing cellular energy utilization. In the brain this is usually manifested as a decrease in neuronal firing and decreased release of excitatory neurotransmitters.

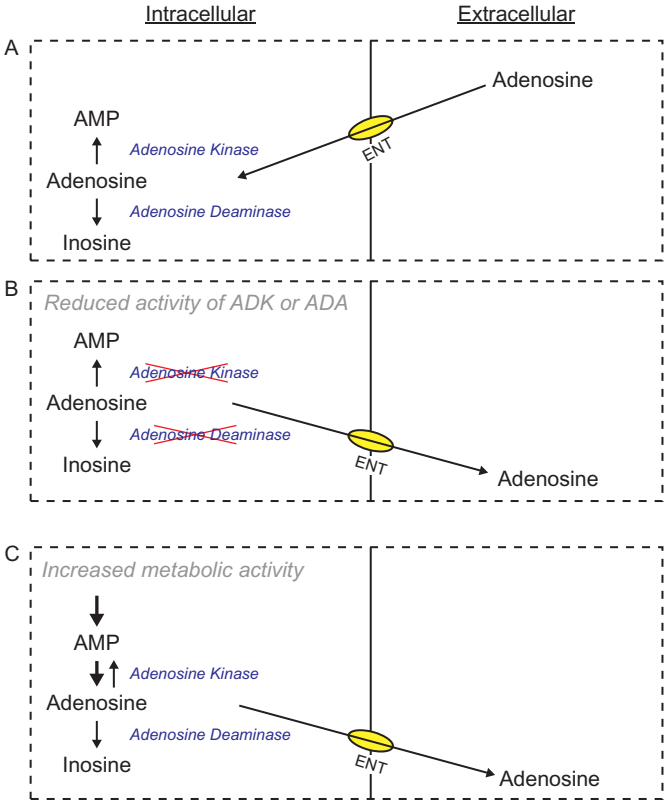

FIGURE 19-3 Equilibrative transporters (ENT) allow bi-directional transport of adenosine. (A) Intracellular adenosine is normally phosphorylated to AMP or converted to inosine. This resulting reduction in intracellular adenosine causes an inward concentration gradient promoting uptake of adenosine. **(B)** Mutations in adenosine deaminase (ADA) or inhibition of either ADA or adenosine kinase lead to reduced metabolism of adenosine causing an accumulation of intracellular adenosine. The elevated level of adenosine reverses the concentration gradient, resulting in a net efflux of adenosine. **(C)** Under periods of increased metabolic activity, or in hypoxic conditions, intracellularly accumulated adenosine drives the concentration-dependent release of adenosine through the ENT.

Adenosine and inosine can be transported across cell membranes in either direction, facilitated by a membrane-associated nucleoside transport protein. Concentrative transporters have also been identified. Messenger RNA for a pyrimidine-selective Na^+-nucleoside co-transporter (rCNT1) and a purine-selective Na^+-nucleoside cotransporter (rCNT2) are found throughout the rat brain. In addition to concentrative transporters, equilibrative nucleoside transporters have been discovered. These provide cells with the potential to facilitate bidirectional transport of adenosine across the cell membrane. Under normoxic conditions, the metabolic activity of adenosine kinase promotes a concentration gradient from the extracellular to the intracellular milieu. However, with hypoxia, metabolic activity leads to an accumulation of intracellular adenosine, which now exits the cell via the same transporter (Fig. 19-3) and can promote neuroprotective actions. Most degradation of adenosine is intracellular, as evidenced by the fact that inhibitors of adenosine transport, such as dipyridamole, increase interstitial levels of adenosine. Dipyridamole is used clinically to elevate adenosine in coronary arteries and produce coronary vasodilation. In high doses, dipyridamole can accentuate adenosine receptor-mediated actions in the CNS, resulting in sedation and sleep, anticonvulsant effects, decreased locomotor activity and decreased neuronal activity.

Hypoxanthine is derived from inosine by the enzyme nucleoside phosphorylase. Hypoxanthine can be converted to IMP by hypoxanthine-guanine phosphoribosyl transferase (HGPRT), one of the enzymes of the purine salvage pathway (see Fig. 19-4 for chemical structures). Lesch–Nyhan syndrome is a severe neurological disorder caused by a deficiency of HGPRT (see Fig. 19-2 and Box 19-1). This deficiency leads to a build up of uric acid that contributes to gout and kidney disorders, as well as to cognitive impairments. HGPRT normally catalyzes the formation of IMP from hypoxanthine, and AMP is produced as a result of the insertion of an amino group at C-6 in place of the carbonyl oxygen. This is a two-step reaction involving the formation of adenylosuccinate as an intermediate. Unsalvaged hypoxanthine is oxidized to xanthine, which is further oxidized to uric acid by xanthine oxidase. Molecular oxygen, the oxidant in both reactions, is reduced to H_2O_2 and other reactive oxygen species. In humans, uric acid is the final product of purine degradation and is excreted in the urine.

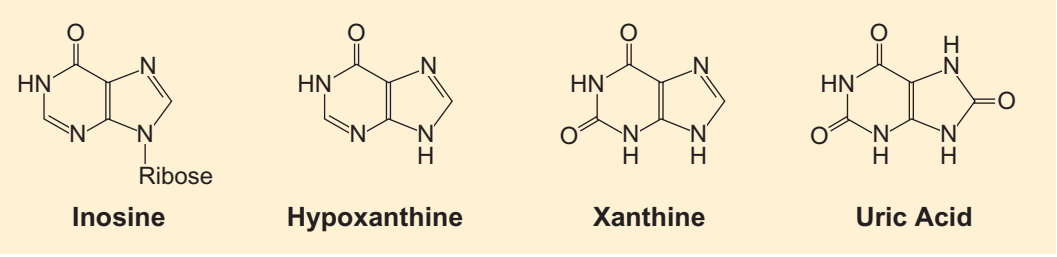

FIGURE 19-4 Adenosine metabolites. Adenosine is converted to inosine by adenosine deaminase. Removal of the ribose by nucleoside phosphorylase produces hypoxanthine, which is sequentially oxidized to xanthine and uric acid by xanthine oxidase.

INHERITED DISEASES OF PURINE METABOLISM

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Lesch–Nyhan syndrome (LNS, MIM 300322) is an X-linked recessive inherited disorder usually evident at 6–10 months of age with choreiform movements, compulsive self-mutilation, spasticity, mental retardation, hyperuricemia and gout. LNS is associated with many types of mutations in the gene for the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (Nyhan, 1997; Jinnah et al., 2004). This enzyme normally catalyzes the recycling of purines from degraded DNA and RNA. There is an almost complete deficiency of this enzyme in patients with the full syndrome. However, individuals with a partial deficiency in HGPRT generally have hyperuricemia and gout but not the full neurologic manifestations (Puig et al., 2001). Treatment with allopurinol, which inhibits xanthine oxidase, reduces the levels of uric acid and the attendant symptoms of hyperuricemia but does not ameliorate the neurologic phenomena. Rasburicase, a urate oxidase enzyme, reduced urate to normal in one neonatal male (Roche et al., 2009). Positron emission tomography with [^{18}F]-fluorodopa has demonstrated abnormally few dopaminergic nerve terminals and cell bodies in the basal ganglia and frontal cortex of LNS patients, suggesting pervasive developmental abnormalities in dopaminergic systems (Visser et al., 2000). A study of human neural stem cells from a fetal LNS brain demonstrated aberrant expression of several transcription factors and DA markers while HPRT-deficient DA neurons showed striking deficits in neurite outgrowth (Cristini et al., 2010). Developmental dopaminergic neuronal system abnormalities may be related to the genetic alteration that occur in the context of an aberrant multigene regulation pattern (Smith et al., 2000; Jinnah et al., 1999).

Mutations in the gene for adenylosuccinate lyase (ASL), inherited as an autosomal recessive disorder in purine metabolism, are associated with severe mental retardation, seizures and autistic behavior, but apparently not self-mutilation (Stone et al., 1992; Sivendran et al., 2004). This homotetrameric enzyme catalyzes two distinct reactions in the *de novo* biosynthesis of purines: the cleavages of adenylosuccinate (S-Ado) and succinylaminoimidazole carboxamide ribotide (SAICAR), both of which accumulate in plasma, urine and cerebrospinal fluid of affected individuals. The accumulation of S-Ado in gray and white matter of patients can be detected as a specific signal by high-resolution proton MRS, which is a reliable noninvasive diagnostic tool (Henneke et al., 2010). Phenotypic severity may vary from neonatal fatality to severe or moderate childhood forms. Nineteen ADSL mutant proteins have been identified in 16 patients representing clinically distinct subgroups that could be correlated with biochemical properties of the mutant proteins (Zikanova et al., 2010). Mutations may involve the active site and/or cooperativity of subunit interactions that affect enzyme activity and stability (Ariyananda et al., 2009)

Deficiency of the muscle-specific myoadenylate deaminase (MADA1, MIM 102770) has been frequently associated with exercise-related weakness and has been believed a common cause of metabolic myopathy. MADA1 catalyzes the deamination of AMP to IMP in skeletal muscle and is critical in the purine nucleotide cycle. However, a more recent study has called into question the significance of the MADA1 deficiency, finding no difference in the mutation incidence between symptomatic and asymptomatic carriers (Hanisch et al., 2008).

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PURINERGIC RECEPTORS

Receptors for both ATP and adenosine are widely distributed in the nervous system, as well as in other tissues. The notion that there are purinergic receptors, i.e., proteins on the surface of cells that bind and respond to purines, was slow to evolve. The first evidence was the observation of cardiovascular effects of purines. Drury & Szent-Gyorgyi (1929) first noted effects of adenine nucleotides on cardiac and vascular tissues in 1929. Then, 34 years later, Berne identified a physiological role for adenosine as a mediator of coronary vasodilation in response to myocardial hypoxia (Berne 1963). In the 1970s, adenosine was found to stimulate cyclic AMP formation in brain slices. Since then, physiological effects of adenosine on almost all tissues have been described. Based on the responses of various tissues to purines, Burnstock proposed that there are distinct receptors that bind adenosine or ATP, designated P_1 and P_2 receptors, respectively (Burnstock, 1978). The existence of adenosine receptors was not widely accepted until the 1980s, when saturable binding sites for radioactive adenosine analogs were demonstrated in brain. The existence of adenosine receptors was proved unequivocally when the first adenosine receptors were cloned in 1990 (Maenhaut et al., 1990). Originally, the 'P' in P_1 and P_2 was meant to designate 'purinergic' receptors. However, it has now been discovered that some of the P_2 receptors bind pyrimidines (UTP or UDP) preferentially over the purine ATP. Hence, the 'P' in P_2 is now used to designate purine or pyrimidine. Despite these exceptions, P_1 and P_2 receptors collectively are still generally referred to as purinergic receptors.

The development of synthetic compounds that activate P_1 or P_2 receptors has been important for elucidating how these receptors function, because some of these compounds are more potent and selective than the parent purines, and most are more stable than the short-lived endogenous compounds adenosine and ATP.

There are four adenosine receptor subtypes

Four subtypes of adenosine (P_1) receptor have been cloned. These are referred to as A_1, A_{2A}, A_{2B} and A_3 (Fig. 19-5). All four of these belong to the superfamily of receptors that signal via guanosine triphosphate (GTP)-binding proteins (G proteins). The adenosine receptor subtypes, their effectors and the identity of some selective agonists and antagonists are listed in Table 19-1.

TABLE 18-1 Subtypes of Adenosine Receptor, Selective Agonists and Antagonists

Receptor (accession #)	Effector	Agonist	Antagonist	Therapeutic potential
A_1 (NM_000674)	Adenylyl cyclase (−)	CPA,	DPCPX,	Epilepsy,
	K channels (+)	CCPA,	CPT,	neuroprotection
	Ca^{2+} channels (−)	S-ENBA,	MRS1754	
	PLC (+)	GR79236,		
		CVT-510		
A_{2A} (NM_000675)	Adenylyl cyclase (+)	ATL146e, CGS21680	ZM241385	Parkinson's disease
		CVT-3146	SCH58261	
A_{2B} (NM_000676)	Adenylyl cyclase (+)	Bay60-6583,	MRS1754,	
	PLC (+)	MRS3997	MRS1706,	
			PSB1115	
A_3 (NM_000677)	Adenylyl cyclase (−)	2-Cl-IB-MECA,	MRS1220,	
	PLC (+)	IB-MECA,	VUF5574,	
		VT160	MRS1523	

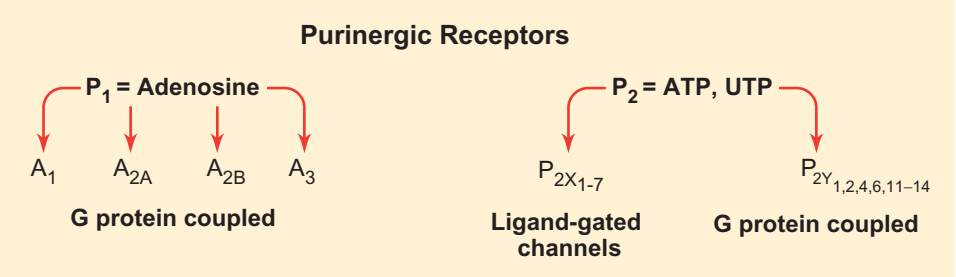

FIGURE 19-5 The purinergic receptor family. The purinergic receptors are divided into two major families, the P1 (or adenosine receptors) and P2 receptors, which principally bind ATP, ADP, UTP or UDP. The P2Y14 receptor binds UTP sugars. P1 and P2Y receptors are coupled to GTP-binding proteins. P2X subunits form trimeric ligand-gated ion channels.

Adenosine A_1 receptors (A_1R)

A_1 receptors were originally characterized on the basis of their ability to inhibit adenylyl cyclase in adipose tissue. A number of other G-protein–mediated effectors of A_1 receptors have subsequently been discovered; these include activation of K^+ channels, extensively characterized in striatal neurons (Trussell & Jackson, 1985), and inhibition of Ca^{2+} channels, extensively characterized in dorsal root ganglion cells (Dolphin et al., 1986). Activation of A_1 receptors has been shown to produce a species-dependent stimulation or inhibition of the phosphatidylinositol pathway in cerebral cortex. In other tissues, activation of A_1 receptors results in synergistic activation of the phosphatidylinositol pathway in concert with Ca^{2+}-mobilizing hormones or neurotransmitters (Linden, 1991).

A_1 adenosine receptors are widely distributed in the central nervous system. In the periphery, adenosine A_1 receptors are found in the heart, where they produce negative inotropic, chronotropic and dromotropic responses; in adipose tissue where they inhibit lipolysis and enhance insulin-stimulated glucose transport; and in kidney where they constrict preglomerular arterioles and produce antidiuresis. At the neuromuscular junction, adenosine inhibits acetylcholine release by a prejunctional effect on A_1 adenosine receptors.

Effects of adenosine that have been attributed to activation of central A_1 receptors include sedation, anticonvulsant activity, analgesia and neuroprotection. Adenosine modulates synaptic plasticity associated with distinct stimulation frequency patterns. Presynaptic A_1 adenosine receptors inhibit neurotransmitter release, especially at high neuronal stimulation frequency. Release of glutamate from nerve terminals in the CA1 region of the hippocampus is highly sensitive to inhibition by adenosine. Adenosine-induced antinociception is mediated mostly by activation of dorsal horn postsynaptic A_1 receptors located in lamina II of the spinal cord, but also by both central A_1 receptors and by presynaptic A_1 receptors that mediate inhibition of the release of substance P and calcitonin gene-related peptide (CGRP) from terminals of sensory afferents.

A_{2A} adenosine receptors are highly expressed in the basal ganglia

A_2 receptors were originally classified on the basis of their ability to stimulate cyclic AMP accumulation in neuronal tissues. Based on substantial differences in binding affinity for adenosine, these were divided into A_{2A} and A_{2B} subtypes, a subdivision that has subsequently been confirmed by molecular cloning. Caffeine, the most widely used psychoactive drug, has some selectivity as an antagonist of A_{2A} receptors. In transgenic mice that lack the A_{2A} receptors, caffeine reduces exploratory activity, an effect opposite to its usual one of stimulating exploratory activity. Potent and selective antagonists of A_{2A} receptors have been developed that are caffeine-like xanthines, such as CSC and KW-6002 and nonxanthine antagonists ZM241385 and SCH58261.

In the CNS, high-density expression of A_{2A} receptors is restricted to the striatum, nucleus accumbens and olfactory tubercle (Fig. 19-6). In striatum, A_{2A} receptors are coexpressed in striatopallidal neurons with enkephalin and dopamine D2 receptors. Blockade of A_{2A} receptors mimics the action of dopamine D2 receptor agonists. Activation of A_{2A} adenosine receptors enhances cyclic AMP formation, whereas activation of D2 dopamine receptors inhibits cyclic AMP formation. The administration of drugs that stimulate dopamine receptors, such as apomorphine and L-DOPA, to rodents with a unilateral lesion of the nigrostriatal pathway induces a turning behavior contralateral to the lesioned side, an effect due to the development of supersensitivity of dopamine receptors in the denervated striatum (Ch. 14). Compounds that block adenosine receptors also produce such turning behavior and potentiate the effects of dopamine agonists. On the other hand, adenosine A_{2A} agonists inhibit D2-mediated behaviors. Modulation by adenosine of striatal dopaminergic systems may contribute to the psychomotor depressant effects of adenosine agonists and to the psychomotor stimulatory effects of methylxanthines.

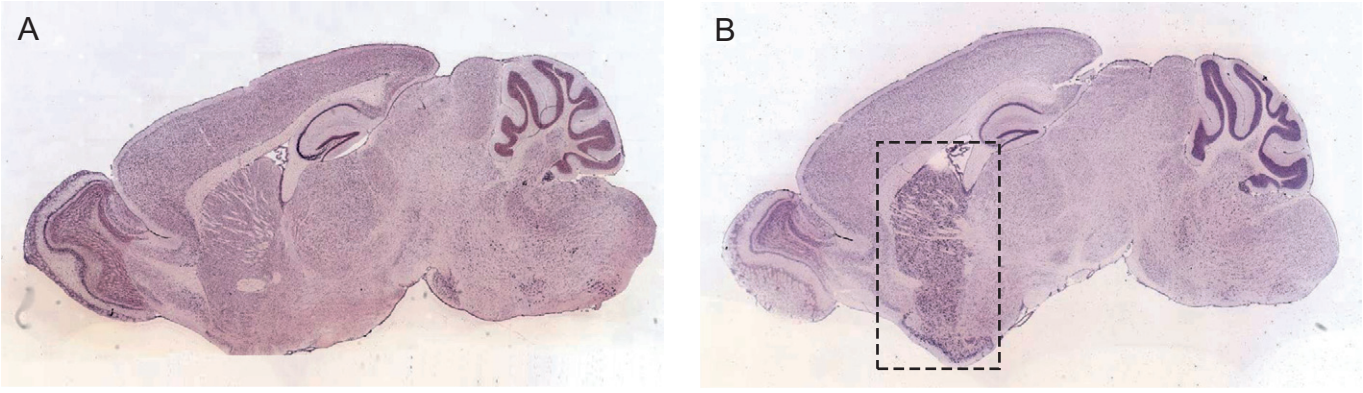

FIGURE 19-6 **Distribution of A_1 and A_{2A} receptor mRNA in mouse brain. (A)** In situ hybridization of A_1R and $A_{2A}R$ in sagittal sections form mouse brain. Note the widespread distribution of A_1 receptors and the particularly high density of receptors in the hippocampus. **(B)** Note the restricted distribution of $A_{2A}R$ with particular enrichment in the striatum (dashed box). Images from Allen Mouse Brain Atlas [Internet]. Seattle (WA): Allen Institute for Brain Science. c.2011. Available from: http://mouse.brain-map.org.

A_{2B} adenosine receptors regulate vascular permeability

A_{2B} receptors are found on endothelial cells, where they regulate vascular permeability, and on epithelial cells, where they regulate water secretion. These receptors are upregulated in the hippocampus following cerebral ischemic preconditioning and are thought to play a protective role. Both A_{2A} and A_{2B} receptors contribute to dilation of cerebral microvessels in response to adenosine.

A_3 adenosine receptors are few in number in the central nervous system

In the rat, abundant A_3 transcript is found exclusively in testes. However, the transcript for similar sheep and human receptors is modestly expressed throughout the brain and heavily expressed in pineal gland, lung and spleen.

P_2 receptors are subdivided into ionotropic P_{2X} receptors and metabotropic P_{2Y} receptors

P_{2X} receptors are ionotropic ligand-gated ion channels ($P_{2X1\text{-}7}$). They have two transmembrane domains with intracellular N and C termini separated by a large extracellular loop. P_{2X} receptors are heteromultimeric proteins that combine several subunits to form homomeric or heteromeric functional ion channels (Khakh et al., 2001). P_{2X} receptors serve essential functions in mediating fast ATP-dependent synaptic transmission. Homomeric and/or multimeric P_{2X2} and P_{2X3} receptors serve important functions in the transduction of taste (Ch. 52). Several P_{2X} receptors are expressed in cells of the immune system including mast cells, B and T lymphocytes, macrophages, microglia and monocytes.

There are eight subtypes of metabotropic G protein-coupled P_{2Y} receptors. The subtypes that have been cloned and identified to date as P_{2Y} receptors are P_{2Y1}, P_{2Y2}, P_{2Y4}, P_{2Y6} and $P_{2Y11\text{-}14}$. The nomenclature is not sequential because of receptors that were either cloned from non-mammalian vertebrates or misidentified as purinergic receptors. The P_{2Y} receptors can be subdivided into four categories based on their pharmacology: (a) P_{2Y1} and $P_{2Y11\text{-}13}$ receptors bind adenine-nucleotides, preferentially ATP and ADP; (b) P_{2Y6} receptors bind uracil-nucleotides, preferentially UTP and UDP; (c) P_{2Y4} and P_{2Y6} receptors bind ligands from both categories; and (d) P_{2Y14} receptors bind UDP-glucose, UDP-galactose or UDP-N-acetylglucosamine. They can also be grouped according to their G-protein coupling, either to Gq (P_{2Y1}, P_{2Y2}, P_{2Y4}, P_{2Y6}, P_{2Y11}) with activation of phospholipase C and an inositol trisphosphate (IP_3)-dependent mobilization of intracellular Ca^{2+} (Ch. 23) or to G_i ($P_{2Y12\text{-}14}$) with inhibition of adenylyl cyclase (Ch. 22). The subset of receptors that respond to UTP was previously referred to as P_{2U} receptors. The P_{2Y1} receptor is selectively activated by ADP and has the unusual feature of being blocked by ATP. The P_{2Y2} receptor is activated by UTP and ATP with similar potency and is not activated by nucleoside diphosphates. Diadenosine tetraphosphate is a potent agonist at this receptor. The P_{2Y4} receptor is highly selective for UTP over ATP and is not activated by nucleoside diphosphates. The P_{2Y6} receptor is activated most potently by UDP and weakly or not at all by UTP, ADP or ATP.

EFFECTS OF PURINES IN THE NERVOUS SYSTEM

ATP-adenosine is an important glial signal

Studies over the past decade have shown that glial cells make extensive use of purines as a signal to enable communication between one another and with neighboring neurons. All of the major classes of glial cells, including Schwann cells, oligodendrocytes, astrocytes, microglia and $NG2^+$ glia, have been demonstrated to incorporate this signaling system into their repertoire.

On the one hand, purinergic signaling is known to act on glia to activate second-messenger signaling such as phospholipase dependent IP_3/Ca^{2+} signals. However, an exciting additional discovery is the importance of the release of ATP from glial cells as a signal that is used either in a paracrine manner, or to signal to neighboring cells, neurons (see also Ch. 12).

For glial cells, a seminal discovery concerning astrocytes was the observation that astrocytes can signal to one another through the release of ATP (Guthrie et al., 1999). This discovery was based on an initially curious observation: Ca^{2+} elevations in one astrocyte could lead to delayed Ca^{2+} elevations in neighboring, but non-contacting astrocytes. The distance separating these cells was of the order of tens of micrometers, indicating that a diffusible extracellular messenger was likely mediating intercellular signaling.

In support of the importance of a diffusible extracellular message was the demonstration that it was possible to induce a Ca^{2+} signal in a distant astrocyte by application of the contents of a small pipette that had previously been used to capture the extracellular saline surrounding an astrocyte that was exhibiting a Ca^{2+} signal. That this signal was mediated by ATP was subsequently discovered by using a combination of purinergic receptor pharmacology, as well as using the demonstration that the addition of apyrase, an enzyme that metabolizes ATP, impaired the ability of the extracellular message to be transmitted to neighboring cells. Final demonstration of the nature of the purinergic nature of the signal was provided by the ability of luciferase/luciferin imaging to detect ATP released from astrocytes. In this assay, the firefly luciferase assay is used to optically detect ATP. When the enzyme and substrate were included in the cell culture saline, the presence of ATP that was derived from stimulated astrocytes resulted in a propagating burst of photons (Wang et al., 2000).

According to this mechanism, it was demonstrated that an astrocyte releases ATP, which signals to a neighboring astrocyte to cause a P_{2Y} receptor-dependent Ca^{2+} signal, and the subsequent release of ATP from that cell. As a consequence, at least in cell culture, ATP-induced ATP release occurs, which, in Ca^{2+} imaging studies, is seen as a radially propagating Ca^{2+} wave.

As elegant as these observations were, it is still unclear when ATP-induced ATP release is used *in vivo*. While it is not being questioned whether astrocytes release ATP, the spatial constraints of cell-cell contact *in vivo* enable extracellular enzymes that hydrolyze ATP to rapidly modify the nature of

the extracellular purinergic signal. This was beautifully demonstrated by the observation that puffer application of ATP onto a hippocampal slice induced an adenosine-mediated A_1 receptor–dependent activation of neurons within 200 ms of the onset of application of ATP (Dunwiddie et al., 1997). Further support to the idea that spatial constraints of the nervous system modify the nature of purinergic signaling is provided by the observation that when studying co-cultured astrocytes and neurons, these glial cells modulate neurons through P_2 receptors, whereas in brain slices similar signaling pathways are dependent on the metabolite, adenosine, and P_1 receptor signaling. However, this is not to say that ATP is not and cannot be used as a signal between astrocytes and neurons. Indeed in the hypothalamus, Ca^{2+} signals in astrocytes induce ATP-dependent modulation of postsynaptic signaling (Gordon et al., 2005). Whether the ability of the astrocyte to signal through ATP, rather than adenosine, is dependent on differential expression of ectonucleotidases or occurs because of differing spatial constraints is unknown.

Myelination and importance of the axonal release of ATP

Myelination in both the peripheral and central nervous system is essential for the support of effective axonal propagation. It is perhaps not surprising therefore that there is an activity-dependent regulation of myelination. Neural activity can lead to a non-synaptic release of ATP (Fields & Ni, 2010). Moreover, this purinergic signal regulates the differentiation of Schwann cells and oligodendrocytes, the peripheral and central sources of myelin. Interestingly, the types of purinergic receptors expressed by Schwann cells and oligodendrocytes are different. Thus although Schwann cells express P_2 receptors, they do not express P_1 receptors, whereas in contrast, oligodendrocytes express all four P_1 receptors. Concordant with this differential receptor expression, these cell types express differential responses to axonal activity. Activity-dependent release of ATP causes a P_2 receptor-dependent inhibition of Schwann cell proliferation, differentiation and myelination (Stevens & Fields 2000). In contrast, the activity-dependent release of ATP stimulates a differentiation of oligodendrocytes that is mediated by P_1 receptors (Stevens et al., 2002).

Astrocyte-mediated, adenosine-dependent heterosynaptic depression

Contrast enhancement is an important and well-known biological process that the nervous system uses to enhance feature detection. While synaptic interactions can mediate this process, it has recently been demonstrated that glial cells can mediate spatial contrast control of synaptic signaling. In the early 1990s it was demonstrated that brief high-frequency activation of a synaptic pathway in the hippocampus would lead to a suppression of neighboring unstimulated synapses (Manzoni et al., 1994). Moreover, this suppression was mediated by the A_1R. Because ATP can be co-released with synaptic transmitter, it was naturally postulated that synaptically released ATP was hydrolyzed in the extracellular space to adenosine, which in turn acted at a distance to cause an A_1R-mediated presynaptic inhibition of neighboring synapses.

The discovery that synaptic activity could induce astrocytic Ca^{2+} signals, which in turn were shown to be able to cause ATP release, led to the insight that astrocytes may be the source of purines that mediated heterosynaptic depression. Two general strategies were used to test this possibility: in the first, a compound that binds Ca^{2+} with high affinity was dialyzed into astrocytes to inhibit their Ca^{2+} signals (Serrano et al., 2006), and in the second, molecular genetic inhibition of ATP release from astrocytes was utilized to study this process (Pascual et al., 2005). The results of both types of studies were in agreement: when the astrocytic purinergic signaling pathway is impaired, the ability of presynaptic stimulation to induce a distance A_1 receptor–dependent presynaptic inhibition was prevented. These observations provided some of the first compelling evidence that glial cells can act as intermediates in multicellular signaling networks to coordinate synaptic activity.

Behavioral roles for glial-derived ATP and adenosine: respiration and sleep

The control of sleep is a complex process involving many signaling pathways. Our personal experiences of sleep demonstrate the importance of two regulatory pathways, the circadian oscillator and the sleep homeostat. When we travel across time zones we wake at inappropriate times of day, a problem termed jet lag, which results from the slow resetting of the phase of the circadian oscillator. A second process of sleep homeostasis also regulates sleep on a daily basis. When we stay up late at night, the well-known drowsiness that results is due to the sleep homeostat, which integrates the period of prior wakefulness and provides the drive to sleep. Sleep homeostasis critically relies on purinergic signaling (Ch. 57).

Many of us will have a drink in the morning that contains the xanthine caffeine, which is an adenosine receptor antagonist. The stimulatory effects of caffeine are well known and indicate the importance of adenosine signaling in mediating some aspect of sleep. If one provides mice with either caffeine or selective A_{2A} or A_1 receptor antagonists at the beginning of the light phase (the onset of subjective nighttime for rodents) the stimulatory effect of these compounds promotes wakefulness. In $A_1R^{-/-}$ and $A_2R^{-/-}$ mice such antagonists do not promote wakefulness providing compelling evidence of the importance of purines in the control of sleep and wakefulness (Porkka-Heiskanen et al., 1997).

Further evidence for a purinergic control of sleep homeostasis is provided by the results of experiments in which polysomnographic recordings (EEG/EMG) were made from rodents while delivering antagonists, or in mice subjected to molecular genetic modifications. The pressure or drive to sleep is reflected by the power of slow wave activity during the non–rapid eye movement (NREM) component of sleep. A brief period of sleep deprivation (akin to staying up late at night) leads to an increase in the power of slow wave activity. Evidence indicating the importance of purinergic signaling in this process comes from several fronts: microdialysis studies show an increase in adenosine levels following

sleep deprivation, whereas in conditional $A_1R^{-/-}$ mice or in control animals, following the administration of A_1R antagonists blunted EEG responses to sleep deprivation are observed.

What is the source of adenosine? Recent evidence suggests that the adenosine is derived from an astrocytic source. The molecular genetic inhibition of ATP/adenosine release from astrocytes induces a similar sleep-deprivation phenotype as that observed following antagonism of A_1R receptors. Thus the astrocytic regulation of neuronal A_1R signaling is key for the modulation of sleep homeostasis and thus the pressure to sleep (Halassa et al., 2009).

pH-dependent release of purines from astrocytes controls breathing

There are numerous physiological pathways under homeostatic control. In addition to sleep, respiration is under continuous feedback regulation. Do purines play more widespread roles in homeostatic control in the brain? It is well known that changes in pH lead to feedback alterations in respiration, but the cell types and mechanisms are still being elucidated. The introduction of optogenetics to the neurosciences has allowed the discovery that astrocyte-derived ATP plays a key role in the pH-dependent excitation of neurons and the consequent respiratory response. In elegant studies by the Kasparov laboratory, researchers were able to demonstrate that pH changes as small as 0.2 pH units cause Ca^{2+} responses in astrocytes in the chemoreceptor regions of the brainstem. Associated with this astrocyte Ca^{2+} signal, they demonstrated that ATP is released and that there is a P_2 receptor–dependent depolarization of associated neurons. Viral transduction of astrocytes with channel rhodopsin allowed experimentally induced optical activation of the astrocyte. In response to a pulse of light, the astrocyte was shown to cause an ATP-dependent excitation of then neurons and to cause a robust respiratory response (Gourine et al., 2010).

Microglia and their response to injury

The microglial cells of the nervous system exhibit a variety of response to purines. Of particular interest are responses to injury that are mediated by P_2 receptors. In mice expressing EGFP in microglia it has been possible to perform two-photon microscopy to image microglia going about their daily business *in vivo*. In response to high-intensity laser irradiation to elicit local and focal damage, microglia rapidly extend their processes to the site of injury (Fig. 19-7)(Davalos et al., 2005), where they presumably utilize their phagocytotic machinery to clear the local debris. The induction of this directed process extension relies on purinergic signaling mediated by ATP and P_2 receptors presumably the source of ATP is leakage of intracellular ATP from damaged cells.

In addition to local process extension, microglia can read a chemical gradient of ATP and exhibit chemotaxis to the source of ATP. The diversity of P_2 receptors facilitates differential cellular behaviors: P_{2Y12} receptors mediate chemotaxis, while P_{2Y6} receptors stimulate phagocytosis (Koizumi et al., 2007).

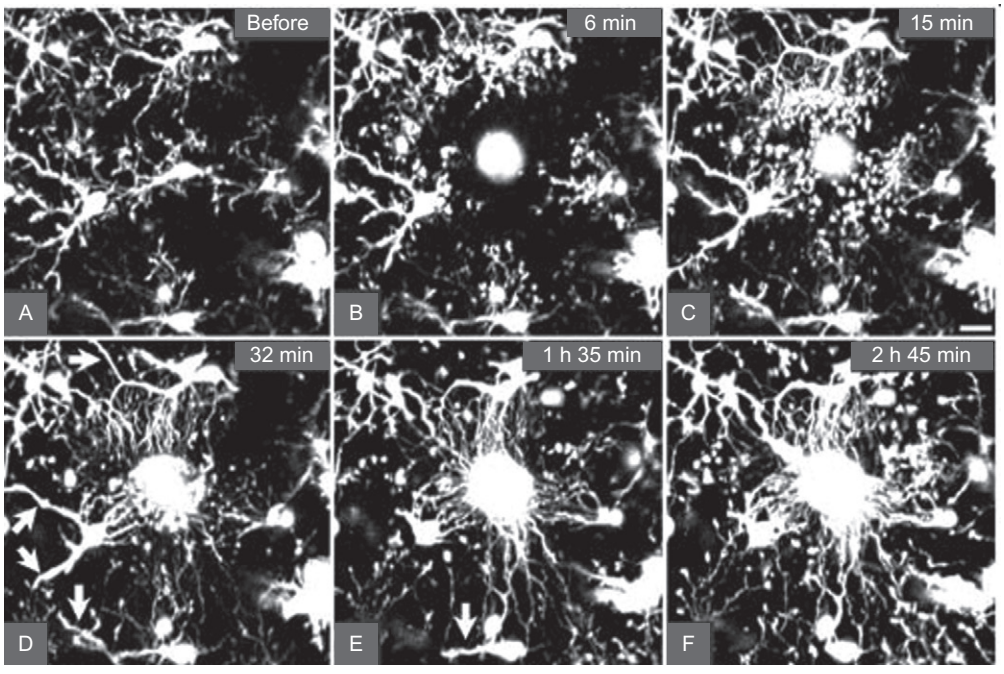

FIGURE 19-7 **Cell damage activates microglia**. *In vivo* two-photon microscopy of GFP-labeled microglia was performed. Photon-induced ablation (between image a and b) induced the extension of microglia processes over time (panels b through f). Process extension requires ATP signaling, because it is inhibited by purinergic antagonists. *(From Davalos et al., 2005).*

Adenosine and the effects of alcohol

It is without question that alcohol has numerous actions in the nervous system. However, molecular genetic studies have shown that one mode of action is mediated by alcohol acting on the control of extracellular adenosine. Acute alcohol exposure inhibits the equilibrative nucleoside transporter 1 (ENT1), which is responsible for considerable uptake of adenosine from the extracellular space. Mice lacking ENT1 show reduced behavioral sensitivity to alcohol and an increased voluntary consumption. Addition of an A_1R agonist, CPA to $ENT1^{-/-}$ mice, decreases consumption in these mice (Choi et al., 2004; Coull et al., 2005).

DISORDERS OF THE NERVOUS SYSTEM—PURINES AND PAIN: A_1R, P_{2X} AND P_{2Y} RECEPTORS

Pain is an explosive area of research in which purinergic receptors are being targeted to determine their potential for therapeutic opportunities (Ch. 54). This intense investigation has arisen because of the recognized importance of ATP during inflammatory and neuropathic pain. Following injury it is thought that there is a release of ATP, which stimulates a cacophony of events. Initially, ATP acts directly on nerve terminals and also acts through P_{2Y12} receptors to stimulate migration and chemotaxis of microglia and macrophages to the injured regions. Activation of P_{2Y6} receptors stimulates phagocytosis in order to clear debris. However, with time and as microglia become activated, there is an increase in expression of the P_{2X4} receptor, whose activation leads to a sequence of downstream effects including the release of brain-derived neurotrophic factor (BDNF), which leads to a reversal of the chloride gradient. As a consequence, normally hyperpolarizing inhibitory inputs become depolarizing, leading to activation of circuits in the spinal cord, a mechanism that is thought to mediate pain (Coull et al., 2005).

In addition to interest in ATP, there is considerable interest in the therapeutic activation of A_1 receptors for the treatment of pain. As discussed previously, A_1R activation can lead to a presynaptic inhibition of excitatory synaptic transmission. Adenosine and A_1R agonists are anti-nociceptive (inhibit painful behaviors), whereas, as is often the case, A_2R activation exerts the opposite action and is pro-nociceptive (promotes pain). Consequently, there has been considerable interest in the activation of A_1R to relieve pain. Since it has been shown that A_1R activation mediates nociceptive threshold there is great interest in developing adenosinergic signaling compounds for the treatment of neuropathic pain.

DISORDERS OF THE NERVOUS SYSTEM: ADENOSINE KINASE AND THE ADENOSINE HYPOTHESIS OF EPILEPSY

In the epileptic brain a variety of changes in neuronal circuitry and biochemistry are associated with the development of a chronic seizure disorder (Ch. 40). Adenosine, through the activation of A_1R, has been known to be an endogenous anticonvulsant. Interestingly, the metabolic control of adenosine is modified in rodent models of epilepsy: the astrocytic expression of adenosine kinase is elevated. Given that the majority of adenosine uptake is mediated by equilibrative nucleoside transporters, control of extracellular adenosine is regulated by the concentration gradient from the extracellular to intracellular compartment. Consequently, enhanced intracellular adenosine kinase expression leads to an increase in phosphorylation of intracellular adenosine, and as a consequence, to an increased clearance of this nucleoside. This in turn leads to an increased concentration gradient of adenosine into the cell, driving further uptake and thus to a depletion of extracellular adenosine. Therefore, the logical hypothesis is that increased adenosine kinase expression will, by reducing the extracellular adenosine, lead to reduced anticonvulsive actions that are normally mediated by A_1R activation. Mice generated to overexpress adenosine kinase exhibited increased seizure susceptibility.

As a consequence of this work, novel potential adenosine related targets for treatment of epilepsy are being investigated. Such targets could include inhibition of adenosine kinase or of equilibrative transporters, as well as treatment with A_1R agonists. However, systemic manipulations of adenosine can have significant side effects because of the widespread nature of purinergic signaling. Indeed there are numerous failed trials with adenosine A_1R agonists since they have potent effects on the cardiovascular system. One intriguing idea, however, is the use of biopolymers to release adenosine locally within the CNS (Szybala et al., 2009). Potentially, such approaches could be used in intractable epilepsy to assist with seizure control.

DISORDERS OF THE NERVOUS SYSTEM: PARKINSON'S DISEASE AND A_{2A} ANTAGONISTS

The progressive degeneration of nigrostriatal neurons that occurs in Parkinson's disease results in a loss of dopaminergic input to striatal output neurons, enhanced striatopallidal GABAergic signaling and, hence, the motor disturbances that are characteristic of the disease (Ch. 49). Replacement of dopaminergic input by administration of exogenous l-dihydroxy phenylalanine (L-DOPA) has been the primary therapeutic strategy for the treatment of Parkinson's disease for decades, but the complications associated with L-DOPA treatment, particularly the 'wearing off' effects and the development of severe motor disturbances known as dyskinesias, have driven the search for alternative treatments. Adenosine A_{2A} receptors have become a target of therapeutic interest for a number of diseases, including Parkinson's disease (Richardson et al., 1997; Ferre et al., 1994).

Epidemiological studies have consistently shown that consumption of caffeinated beverages is associated with a reduced risk of Parkinson's disease. In animal models of Parkinson's disease, A_{2A} receptor selective antagonists as well as deletion of A_{2A} receptors protect dopaminergic neurons (Carta et al., 2009). Such results have led to intensive

investigation into the potential for A_{2A} receptors as therapeutic targets for Parkinson's disease. Currently several A_{2A} selective antagonists are in Phase III clinical trials for this disorder (Barkhoudarian & Schwarzschild, 2011).

Interestingly, the linkage between purines and Parkinson's disease has been further advanced by studies linking serum and cerebrospinal urate levels with the rate of clinical decline of Parkinson's patients. Patients with elevated levels of urate, the final metabolic product of purine metabolism, displayed a slower progression of Parkinson's disease (Ascherio et al., 2009). In agreement with the possible neuroprotective effect of elevated levels of this antioxidant, a study of diet, urate and Parkinson's disease showed that individuals who consumed a diet that enhances urate levels were at reduced risk of Parkinson's disease (Gao et al., 2008).

CONCLUDING COMMENTS

Once ATP is released into the extracellular space there is a diversity of potential actions. ATP and its metabolites have the potential to signal through 19 distinct receptors – 7 P_{2X}, 8 P_{2Y} and 4 P_1 receptors. Each receptor has preferential ligands as well as specific affinities. For example, some preferentially respond to ATP, others to ADP and yet others to adenosine. This diversity of signaling potential provides a plethora of signaling capabilities of purines. Consequently, it is not surprising that purines play roles in numerous behaviors and that these receptors are receiving considerable attention as therapeutic targets in disorders including Parkinson's disease and neuropathic pain. The widespread use of purinergic signaling by all cell types complicates CNS therapeutic opportunities, however, because of systemic side effects of such drugs.

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