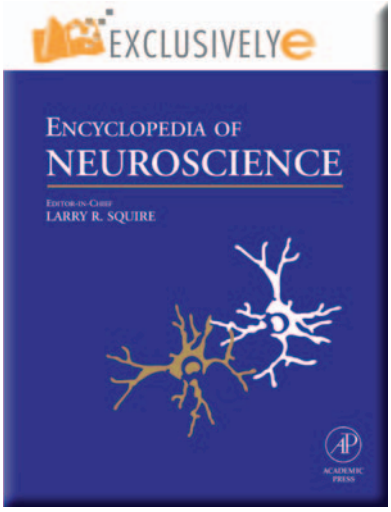


Burnstock G (2009) Adenosine Triphosphate (ATP). In: Squire LR (ed.) Encyclopedia of Neuroscience, volume 1, pp. 105-113. Oxford: Academic Press.

Adenosine Triphosphate (ATP)

G Burnstock, Royal Free and University College School of Medicine, London, UK

Early History

Drury and Szent-Györgyi, in 1929, were the first to demonstrate the potent extracellular actions of adenosine 5′-triphosphate (ATP) and adenosine on the heart and coronary blood vessels. In 1948, Emmelin and Feldberg demonstrated that intravenous injection of ATP into cats caused complex effects that affected both peripheral and central mechanisms. Injection of ATP into the lateral ventricle produced muscular weakness, ataxia, and a tendency of the cat to sleep. Application of ATP to various regions of the brain produced biochemical or electrophysiological changes. Holton presented in 1959 the first hint of a transmitter role for ATP in the nervous system by demonstrating the release of ATP during antidromic stimulation of sensory nerves supplying the ear artery. Buchthal and Folkow recognized a physiological role for ATP at the neuromuscular junction in 1948, finding that acetylcholine (ACh)-evoked contraction of skeletal muscle fibers was potentiated by exposure to ATP. Presynaptic modulation of ACh release from the neuromuscular junction by purines in the rat was also reported.

The existence of nonadrenergic, noncholinergic (NANC) neurotransmission in the gut and bladder was established in the mid-1960s. Several years later, after many experiments, Burnstock and his colleagues published a study that suggested that the NANC transmitter in the guinea pig taenia coli and stomach, rabbit ileum, frog stomach, and turkey gizzard was ATP. The experimental evidence included mimicry of the NANC nerve-mediated response by ATP; measurement of release of ATP during stimulation of NANC nerves with luciferin-luciferase luminometry; histochemical labeling of subpopulations of neurons in the gut with quinacrine, a fluorescent dye known to selectively label high levels of ATP bound to peptides; the later demonstration that the slowly degradable analogue of ATP, α,β-methylene ATP (α,β-meATP), which produces selective desensitization of the ATP receptor, blocked the responses to NANC nerve stimulation. Soon after, evidence was presented for ATP as the neurotransmitter for NANC excitatory nerves in the urinary bladder. The term 'purinergic' was proposed in a short letter to *Nature* in 1971, and the evidence for purinergic transmission in a wide variety of systems was presented in *Pharmacological Reviews* in 1972 (Figure 1). This concept met with considerable resistance for many years. This was partly because it was felt that ATP was established as an intracellular energy source involved in various metabolic cycles and that such a ubiquitous molecule was unlikely to be involved in extracellular signaling. However, ATP was one of the biological molecules to first appear, and therefore it is not really surprising that it should have been utilized for extracellular, as well as intracellular, purposes early in evolution. The fact that potent ectoATPases were described in most tissues in the early literature was also a strong indication for the extracellular actions of ATP. Purinergic neurotransmission is now generally accepted, and a volume of *Seminars in Neuroscience* was devoted to purinergic neurotransmission in 1996.

Purinergic Cotransmission

Another concept that has had a significant influence on our understanding of purinergic transmission was that of cotransmission. Burnstock wrote a commentary in *Neuroscience* in 1976 titled, "Do some nerves release more than one transmitter?" This position challenged the single-neurotransmitter concept, which became known as 'Dale's Principle,' even though Dale himself never defined it as such. The commentary was based on hints about cotransmission in the early literature describing both vertebrate and invertebrate neurotransmission and more specifically, with respect to purinergic cotransmission, on the surprising discovery in 1971 that ATP was released from sympathetic nerves supplying the taenia coli as well as from NANC inhibitory nerves. The excitatory junction potentials (EJPs) recorded in the vas deferens were blocked by α,β-meATP, a selective desensitizer of P2X receptors (Figures 2(a) and 2(b)). This finding clearly supported the earlier demonstration of sympathetic cotransmission in the vas deferens in the laboratory of Dave Westfall, following an earlier report of sympathetic cotransmission in the cat nictitating membrane. Purinergic cotransmission was later described in the rat tail artery and in the rabbit saphenous artery. Noradrenaline (NA) and ATP are now well established as cotransmitters in sympathetic nerves (see Figure 3 (a)), although the proportions vary in different tissues and species, during development and aging, and in different pathophysiological conditions.

ACh and ATP are cotransmitters in parasympathetic nerves supplying the urinary bladder. Subpopulations of sensory nerves have been shown to utilize ATP in addition to substance P and calcitonin gene-related peptide; it seems likely that ATP cooperates

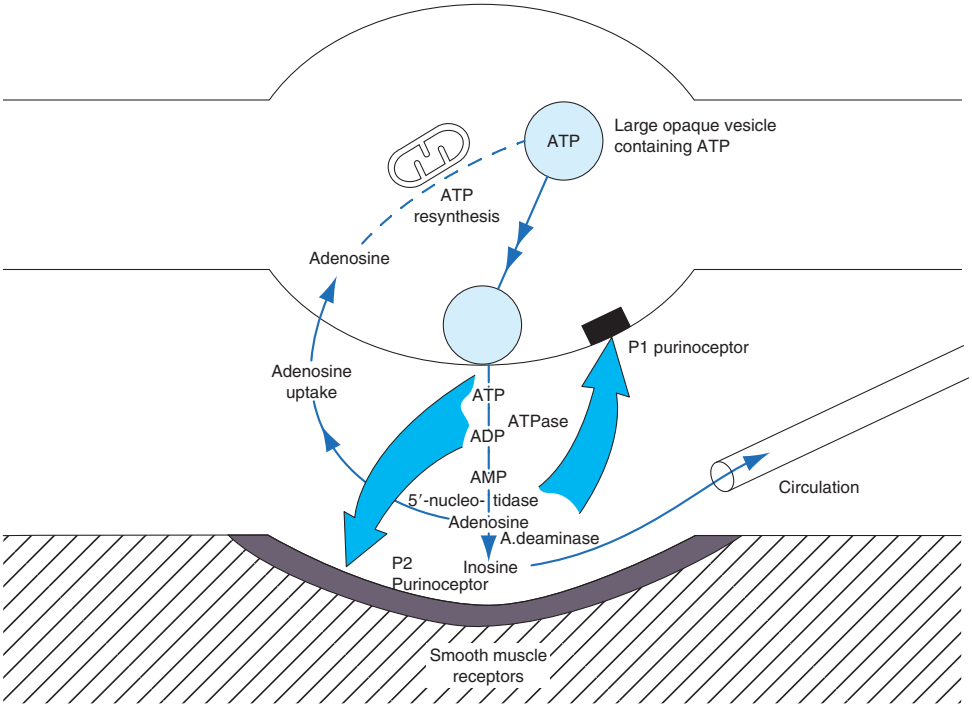


Figure 1 Purinergic neuromuscular transmission depicting the synthesis, storage, release, and inactivation of adenosine 5′-triphosphate (ATP). ATP, stored in vesicles in nerve varicosities, is released by exocytosis to act on postjunctional P2 purinoceptors on smooth muscle. ATP is broken down extracellularly by ATPases and 5′-nucleotidase to adenosine, which is taken up by varicosities to be resynthesized and restored in vesicles. Adenosine acts prejunctionally on P1 purinoceptors to modulate transmitter release. If adenosine is broken down further by adenosine deaminase to inosine, it is removed by the circulation. Adapted from Burnstock G (1972) Purinergic nerves. *Pharmacological Reviews* 24: 509–581, with permission from the American Society for Pharmacology and Experimental Therapeutics.

with these peptides in axon reflex activity. ATP, vasoactive intestinal polypeptide and nitric oxide (NO) are cotransmitters in NANC inhibitory nerves, but that they vary considerably in proportion in different regions of the gut. More recently, ATP has been shown to be a cotransmitter with NA, 5-hydroxytryptamine, glutamate, dopamine and γ-aminobutyric acid (GABA) in the central nervous system (CNS) (see **Figure 3(b)**). ATP and NA act synergistically to release vasopressin and oxytocin, which is consistent with ATP cotransmission in the hypothalamus. ATP, in addition to glutamate, is involved in long-term potentiation in hippocampal CA1 neurons associated with learning and memory.

Receptors for Purines and Pyrimidines

Implicit in the purinergic neurotransmission hypothesis was the presence of purinoceptors. A basis for distinguishing two types of purinoceptor, identified as P1 and P2 for adenosine and ATP/adenosine diphosphate (ADP), respectively, was recognized in 1978. This helped resolve some of the ambiguities in earlier reports, which were complicated by the breakdown of ATP to adenosine by ectoenzymes so that some of the actions of ATP were directly on P2 receptors, whereas others were due to indirect action via P1 receptors.

At about the same time, two subtypes of P1 (adenosine) receptor were recognized, but it was not until 1985 that a pharmacological basis for distinguishing two types of P2 receptors (P2X and P2Y) was proposed. A year later, two further P2 receptor subtypes were named, a P2T receptor selective for ADP on platelets and a P2Z receptor on macrophages. Further subtypes followed, perhaps the most important being the P2U receptor, which could recognize pyrimidines such as uridine 5′ triphosphate (UTP) in addition to ATP. However, to provide a more manageable framework for newly identified nucleotide receptors, Abbracchio and Burnstock proposed in 1994 that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G-protein-coupled receptors. This was based on studies of transduction mechanisms and the cloning of nucleotide receptors: P2Y receptors were cloned first, in 1993, and a year later P2X receptors were cloned. This nomenclature has been widely adopted, and currently seven P2X subtypes and eight P2Y

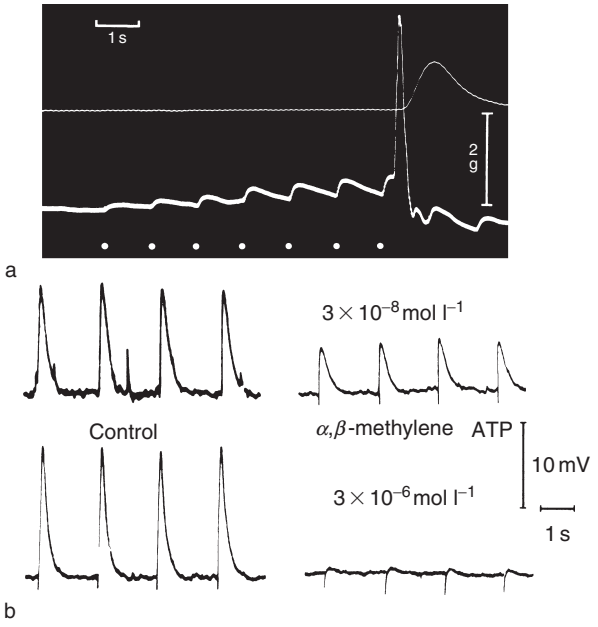


Figure 2 (a) EJPs in response to repetitive stimulation of adrenergic nerves (white dots) in the guinea pig vas deferens. The upper trace records the tension, the lower trace the electrical activity of the muscle recorded extracellularly by the sucrose gap method. Note both summation and facilitation of successive junction potentials. At a critical depolarization threshold, an action potential is initiated which results in contraction. (b) The effect of various concentrations of α,β-methylene ATP (α,β-meATP) on EJPs recorded from guinea pig vas deferens (intracellular recordings). The control responses to stimulation of the motor nerves at 0.5 Hz are shown on the left. After at least 10 min in the continuous presence of the indicated concentration of α,β-meATP, EJPs were recorded using the same stimulation parameters. (a) Reproduced from Burnstock G and Costa M (eds.) (1975) Adrenergic neuroeffector transmission. In *Adrenergic Neurones: Their Organisation, Function and Development in the Peripheral Nervous System*, pp. 51–106. London: Chapman and Hall, with permission from Springer Science and Business Media. (b) Reproduced from Sneddon P and Burnstock G (1984) Inhibition of excitatory junction potentials in guinea-pig vas deferens by α,β-methylene-ATP: Further evidence for ATP and noradrenaline as cotransmitters. *European Journal of Pharmacology* 100: 85–90, with permission from Elsevier.

receptor subtypes are recognized. Four subtypes of P1 receptor have been cloned and characterized.

P2X receptors in general mediate fast neurotransmission but are sometimes located prejunctionally to mediate increase in release of cotransmitters, for example, glutamate in terminals of primary afferent neurons in the spinal cord. $P2X_3$ and $P2X_{2/3}$ receptors are prominent in sensory neurons and are involved in nociception. $P2X_7$ receptors are involved in cell death.

P2Y receptors are particularly involved in prejunctional inhibitory modulation of transmitter release, as well as long-term (trophic) events such as cell pro liferation. $P2Y_1$ receptors are widespread in many regions of the brain, while the $P2Y_2$ receptors have been localized on pyramidal neurons in the hippocampus and prefrontal cortex, on supraoptic magnocellular neurosecretory neurons in the hypothalamus, and on neurons in the dorsal horn of the spinal cord. In addition, mRNA but not protein has been reported for $P2Y_4$ and $P2Y_6$ receptor subtypes in the cerebellum and hippocampus, while $P2Y_{12}$ receptor mRNA has also been described in the cerebellum and $P2Y_{13}$ in the cortex. In the periphery, $P2Y_{1,2,4,6}$ receptors have been described on subpopulations of sympathetic neurons, $P2Y_2$ and $P2Y_4$ receptors in intracardiac ganglia, $P2Y_1$ and $P2Y_2$ receptors on sensory neurons (although $P2Y_4$ and $P2Y_6$ mRNA have also been reported) while $P2Y_1$ receptors appear to be the dominant subtype on enteric neurons. $P2Y_{1,2,4,6}$ functional receptors have been described on astrocytes in the CNS and also on microglia, where functional $P2Y_{12}$ receptors have also been identified. $P2Y_1$ and $P2Y_2$ receptors have been located in Schwann cells and oligodendrocytes, where functional $P2Y_{12}$ receptors also appear to be present. $P2Y_2$ (and/or $P2Y_4$) receptors are expressed on enteric glial cells. There is also emerging evidence for P2Y receptors on stem cells.

ATP Release and Degradation

There is clear evidence for exocytotic vesicular release of ATP from nerves, and the concentration of nucleotides in vesicles is claimed to be up to 1000 mmol l^{-1}. It was generally assumed that the main source of ATP acting on purinoceptors was damaged or dying cells. However, it is now recognized that ATP release from many cells is a physiological or pathophysiological response to mechanical stress, hypoxia, inflammation, and some agonists. There is debate, however, about the ATP transport mechanisms involved. There is compelling evidence for exocytotic release from endothelial and urothelial cells, osteoblasts, astrocytes, mast, and chromaffin cells, but other transport mechanisms have also been proposed, including ATP binding cassette transporters, connexin hemichannels, and plasmalemmal voltage-dependent anion channels.

Much is now known about the ectonucleotidases that break down ATP released from neurons and non-neuronal cells. Several enzyme families are involved: ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases), of which NTPDase1, 2, 3, and 8 are extracellular; ectonucleotide pyrophosphatase of 3 subtypes; alkaline phosphatases; ecto-5′-nucleotidase; and ecto-nucleoside diphosphokinase. NTPDase1 hydrolyzes ATP directly to adenosine monophosphate (AMP) and UTP to uridine diphosphate (UDP), while NTPDase2 hydrolyzes ATP to ADP and 5′-nucleotidase AMP to adenosine.

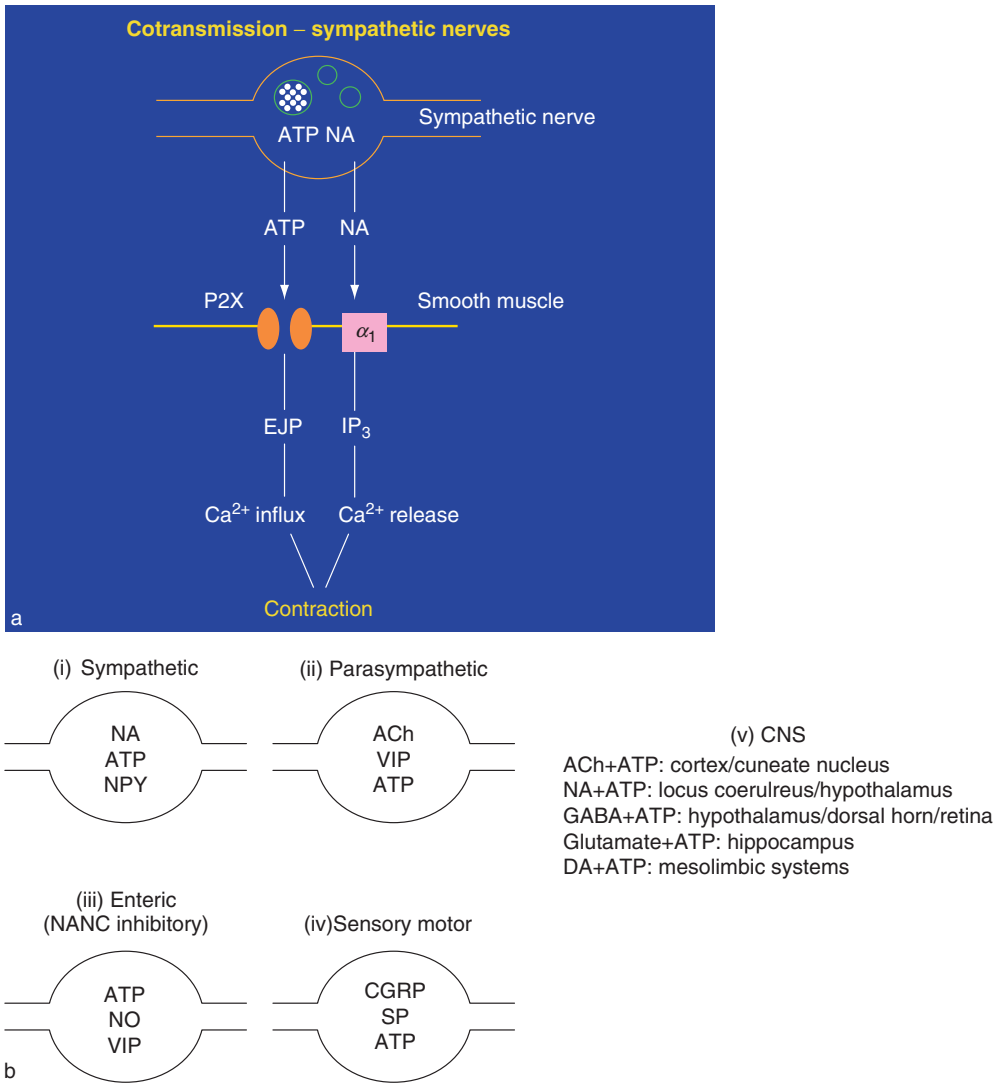


Figure 3 (a) Cotransmission in sympathetic nerves. Adenosine 5′-triphosphate (ATP) and noradrenaline (NA) from terminal varicosities of sympathetic nerves can be released together. With NA acting via the postjunctional α_1-adrenoceptor to release cytosolic Ca^{2+}, and with ATP acting via $P2X_1$-gated ion channels to elicit Ca^{2+} influx, both contribute to the subsequent response (contraction). IP_3 is inositol triphosphate, EJP is excitatory junction potential. (b) Schematic diagram of the principal cotransmitters with ATP in the nervous system. Nerve terminal varicosities of (i) sympathetic, (ii) parasympathetic, (iii) enteric (NANC inhibitory), (iv) sensory-motor neurons, and (v) central nervous system (CNS). (a) Adapted from Kennedy C, McLaren GJ, Westfall TD, and Sneddon P (1996) ATP as a co-transmitter with noradrenaline in sympathetic nerves – Function and fate. In: Chadwick DJ and Goode J (eds.) *P2 Purinoceptors: Localization, Function and Transduction Mechanisms*, pp. 223–235. Chichester: John Wiley and Sons, with permission from John Wiley & Sons. (b) Reproduced from Burnstock G (2007) Physiology and pathophysiology of purinergic neurotransmission. *Physiological Reviews* 87: 659–797, with permission from The American Physiological Society.

Physiology of Purinergic Neurotransmission

Purinergic signaling appears to be a primitive system that is involved in many nonneuronal and neuronal mechanisms, in both short-term and long-term (trophic) events, including exocrine and endocrine secretion, immune responses, inflammation, mechanosensory transduction, platelet aggregation, endothelial-mediated vasodilatation and in cell proliferation, differentiation, migration, and death in development and regeneration.

The first clear evidence for nerve–nerve purinergic synaptic transmission was published in 1992. Synaptic potentials in the coeliac ganglion and in the medial habenula in the brain were reversibly antagonized by the antitrypanosomal agent suramin. Since then, many articles have described either the distribution of various P2 receptor subtypes in the brain and

spinal cord or electrophysiological studies of the effects of purines in brain slices, isolated neurons, and glial cells. Synaptic transmission has also been demonstrated in the myenteric plexus and in various sensory, sympathetic, parasympathetic, and pelvic ganglia. Adenosine produced by the ectoenzymatic breakdown of ATP acts through presynaptic P1 receptors to inhibit the release of excitatory neurotransmitters in both the peripheral and the central nervous systems. Purinergic signaling is also implicated in higher order cognitive functions, including learning and memory in the prefrontal cortex.

CNS Control of Autonomic Function

Functional interactions seem likely to occur between purinergic and nitrergic neurotransmitter systems; these interactions might be important for the regulation of hormone secretion and body temperature at the hypothalamic level and for cardiovascular and respiratory control at the level of the brain stem. The nucleus tractus solitarius (NTS) is a major integrative center of the brain stem involved in reflex control of the cardiovascular system; stimulation of P2X receptors in the NTS evokes hypotension. P2X receptors expressed in neurons in the trigeminal mesencephalic nucleus might be involved in the processing of proprioceptive information.

Neuron–Glia Interactions

ATP is an extracellular signaling molecule between neurons and glial cells. ATP released from astrocytes might be important in triggering cellular responses to trauma and ischemia by initiating and maintaining reactive astrogliosis, which involves striking changes in the proliferation and morphology of astrocytes and microglia. Some of the responses to ATP released during brain injury are neuroprotective, but at higher concentrations, ATP contributes to the pathophysiology initiated after trauma. Multiple P2X and P2Y receptor subtypes are expressed by astrocytes, oligodendrocytes, and microglia. ATP and basic fibroblast growth factor (bFGF) signals merge at the mitogen-activated protein kinase cascade, which underlies the synergistic interactions of ATP and bFGF in astrocytes. ATP can activate $P2X_7$ receptors in astrocytes to release glutamate, GABA, and ATP, which regulate the excitability of neurons.

Microglia, immune cells of the CNS, are also activated by purines and pyrimidines to release inflammatory cytokines such as interleukins 1β (IL-1β) and IL-6 and tumor necrosis factor α. Thus, although microglia might play an important role against infection in the CNS, overstimulation of this immune reaction might accelerate the neuronal damage caused by ischemia, trauma, or neurodegenerative diseases. $P2X_4$ receptors induced in spinal microglia gate tactile allodynia after nerve injury. $P2X_7$ receptors mediate superoxide production in primary microglia and are upregulated in a transgenic mouse model of Alzheimer's disease, particularly around β-amyloid plaques.

Purine Transmitter and Receptor Plasticity

The purinergic neurotransmission field is expanding rapidly; there is increasing interest in the physiology and pathophysiology of this neurosignaling system, and therapeutic interventions are being explored. The autonomic nervous system shows marked plasticity: that is, the expression of cotransmitters and receptors shows dramatic changes during development and aging, in nerves that remain after trauma or surgery, and in disease conditions. There are several examples where the purinergic component of cotransmission is increased in pathological conditions. The parasympathetic purinergic nerve-mediated component of contraction of the human bladder is increased to 40% in pathophysiological conditions such as interstitial cystitis, outflow obstruction, idiopathic instability, and also some types of neurogenic bladder. ATP also has a significantly greater cotransmitter role in sympathetic nerves supplying hypertensive compared to normotensive blood vessels. Upregulation of $P2X_1$ and $P2Y_2$ receptor mRNA in hearts of rats with congestive heart failure has been reported, and there is a dramatic increase in expression of $P2X_7$ receptors in the kidney glomerulus in diabetes and hypertension.

Neuroprotection

In the brain, purinergic signaling is involved in nervous tissue remodeling following trauma, stroke, ischemia, or neurodegenerative disorders. The hippocampus of chronic epileptic rats shows abnormal responses to ATP associated with increased expression of $P2X_7$ receptors. Neuronal injury releases fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor. In combination with these growth factors, ATP can stimulate astrocyte proliferation, contributing to the process of reactive astrogliosis and to hypertrophic and hyperplasic responses. P2Y receptor antagonists have been proposed as potential neuroprotective agents in the cortex, hippocampus, and cerebellum. Blockade of

A_{2A} (P1) receptors antagonizes tremor in Parkinson's disease. ATP–$MgCl_2$ is being explored for the treatment of spinal cord injuries.

Dual Purinergic Neural and Endothelial Control of Vascular Tone and Angiogenesis

ATP and adenosine are much involved in the mechanisms underlying local control of vessel tone in addition to cell migration, proliferation, and death during angiogenesis, atherosclerosis, and restenosis following angioplasty. ATP, released as a cotransmitter from sympathetic nerves, constricts vascular smooth muscle via P2X receptors, whereas ATP released from sensory-motor nerves during 'axon reflex' activity dilates vessels via P2Y receptors. Furthermore, ATP released from endothelial cells during changes in flow (shear stress) or hypoxia acts on P2Y receptors in endothelial cells to release NO, resulting in relaxation (Figure 4). Adenosine, following breakdown of extracellular ATP, produces vasodilatation via smooth muscle P1 receptors.

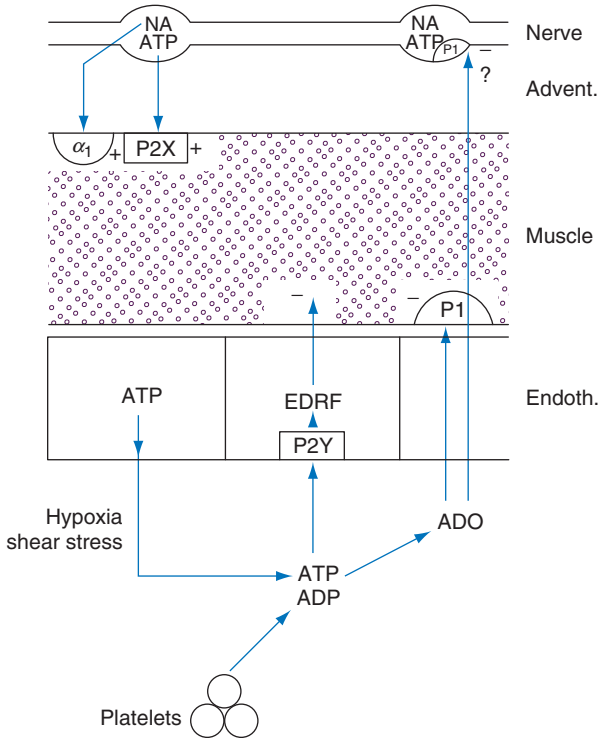


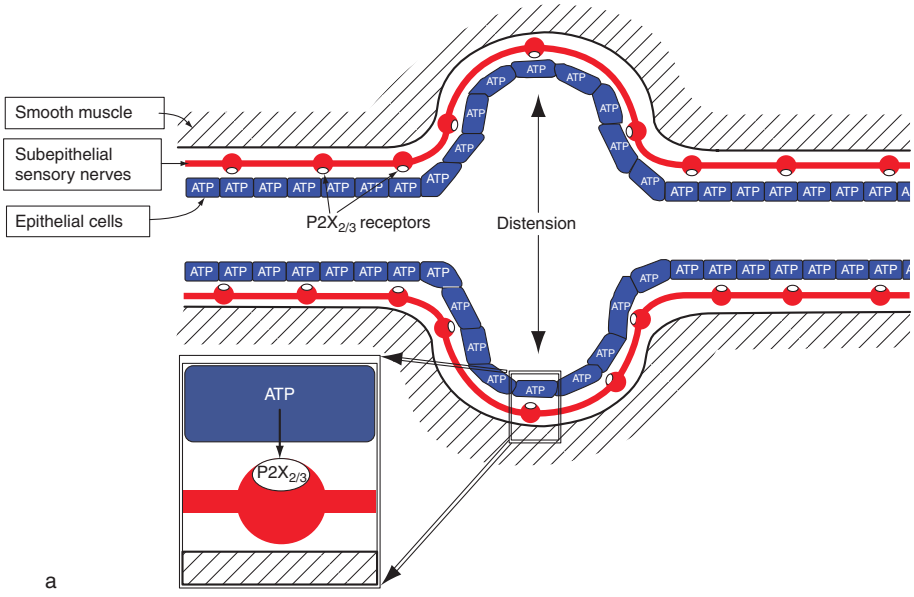
Figure 4 A schematic representation of the interactions of adenosine 5′-triphosphate (ATP) released from perivascular nerves and from the endothelium (Endoth.). ATP is released from endothelial cells during hypoxia to act on endothelial P2Y receptors, leading to the production of endothelium-derived relaxing factor (EDRF), nitric oxide (NO), and subsequent vasodilation (–). In contrast, ATP released as a cotransmitter with noradrenaline (NA) from perivascular sympathetic nerves at the adventitia (Advent.)–muscle border produces vasoconstriction (+) via P2X receptors on the muscle cells. Adenosine (ADO), resulting from rapid breakdown of ATP by ectoenzymes, produces vasodilation by direct action on the muscle via P1 receptors and acts on the perivascular nerve terminal varicosities to inhibit transmitter release. Reproduced from Burnstock G (1987) Local control of blood pressure by purines. *Blood Vessels* 24: 156–160, with permission from S. Karger AG, Basel.

Pain and Purinergic Mechanosensory Transduction

The involvement of ATP in the initiation of pain was recognized first in 1966 and later in 1977 using human skin blisters. A major advance was made when the $P2X_3$ ionotropic receptor was cloned in 1995 and shown later to be predominantly localized in the subpopulation of small nociceptive sensory nerves that label with isolectin B4 in dorsal root ganglia whose central projections terminate in inner lamina II of the dorsal horn. A unifying purinergic hypothesis for the initiation of pain was proposed in 1996 with ATP acting via $P2X_3$ and $P2X_{2/3}$ receptors associated with causalgia, reflex sympathetic dystrophy, angina, migraine, and pelvic and cancer pain. This has been followed by an increasing number of published reports expanding on this concept for acute, inflammatory, neuropathic, and visceral pain. $P2Y_1$ receptors have also been demonstrated in a subpopulation of sensory neurons that colocalized with $P2X_3$ receptors.

A hypothesis was proposed that purinergic mechanosensory transduction occurred in visceral tubes and sacs, including ureter, bladder, and gut, where ATP, released from epithelial cells during distension, acted on $P2X_3$ homomultimeric and $P2X_{2/3}$ heteromultimeric receptors on subepithelial sensory nerves, initiating impulses in sensory pathways to pain centers in the CNS (Figure 5(a)). Subsequent studies of bladder, ureter, gut, tongue, and tooth pulp have produced evidence in support of this hypothesis. $P2X_3$ knockout mice were used to show that ATP released from urothelial cells during distension of the bladder act on $P2X_3$ receptors on subepithelial sensory nerves to initiate both nociceptive and bladder voiding reflex activities. In the distal colon, ATP released during moderate distension acts on $P2X_3$ receptors on low-threshold intrinsic subepithelial sensory neurons to influence peristalsis, whereas high-threshold extrinsic subepithelial sensory fibers respond to severe distension to initiate pain (see Figure 5(b)).

ATP is also a neurotransmitter released from the spinal cord terminals of primary afferent sensory nerves to act at synapses in the central pain pathway. Using transverse spinal cord slices from postnatal rats, excitatory postsynaptic currents have been shown to be



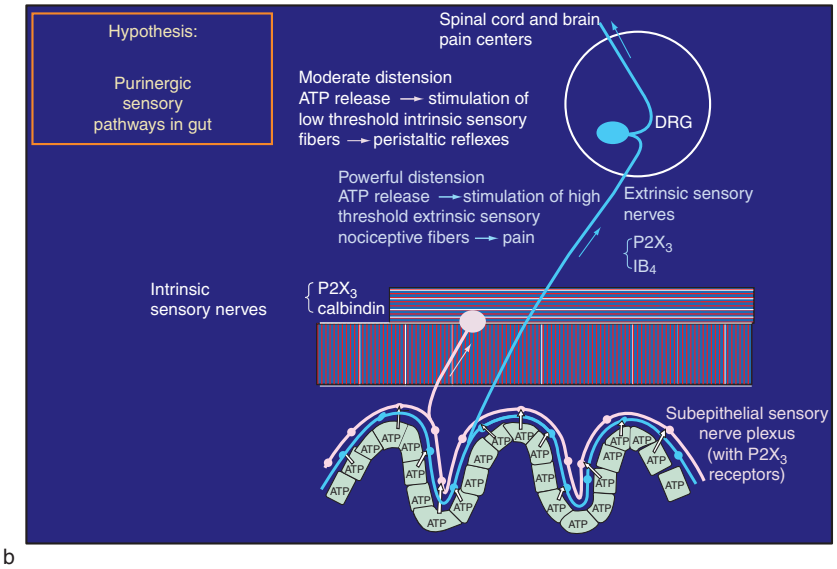


Figure 5 (a) Schematic representation of the hypothesis for purinergic mechanosensory transduction in tubes (e.g., ureter, vagina, salivary, and bile ducts and gut) and sacs (e.g., urinary and gall bladders and lung). It is proposed that distension leads to the release of adenosine 5′ triphosphate (ATP) from the epithelium lining the tube or sac, which then acts on $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey sensory (nociceptive) information to the central nervous system (CNS). (b) Schematic of a novel hypothesis about purinergic mechanosensory transduction in the gut. It is proposed that ATP released from mucosal epithelial cells during moderate distension acts preferentially on $P2X_3$ receptors on low-threshold subepithelial intrinsic sensory nerve fibers (labeled with calbindin), contributing to peristaltic reflexes. ATP released during extreme distension also acts on $P2X_3$ receptors on high-threshold extrinsic sensory nerve fibers (labeled with isolectin B_4 (IB_4)) that send messages via the dorsal root ganglia (DRG) to pain centers in the CNS. (a) Adapted from Burnstock G (1999) Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. *Journal of Anatomy* 194: 335–342, with permission from Blackwell Publishing. (b) Adapted from Burnstock G (2001) Expanding field of purinergic signaling. *Drug Development Research* 52: 1–10, with permission of Wiley-Liss, Inc.

mediated by P2X receptors activated by synaptically released ATP, in a subpopulation of less than 5% of the neurons in lamina II, a region known to receive major input from nociceptive primary afferents.

There is an urgent need for selective $P2X_3$ and $P2X_{2/3}$ receptor antagonists that do not degrade *in vivo*. Pyridoxal-phosphate-6-azophenyl-2′, 4′-disulphonic acid is a nonselective P2 receptor antagonist but has the advantage that it dissociates about 100 to 10 000 times more slowly than other known antagonists. The trinitrophenyl-substituted nucleotide TNP-ATP is a selective and very potent antagonist at both

$P2X_3$ and $P2X_{2/3}$ receptors. 5-((3-Phenoxybenzyl) [(1*S*)-1,2,3,4-tetrahydro-1-naphthalenyl]amino carbonyl)-1,2,4-benzenetricarboxylic acid (A-317491) is a potent and selective nonnucleotide antagonist of $P2X_3$ and $P2X_{2/3}$ receptors, and it reduces chronic inflammatory and neuropathic pain in the rat. Antisense oligonucleotides have been used to downregulate the $P2X_3$ receptor, and in models of neuropathic (partial sciatic nerve ligation) and inflammatory (complete Freund's adjuvant) pain, inhibition of the development of mechanical hyperalgesia was observed within 2 days of treatment. $P2X_3$ double-stranded-short interfering RNA also relieves chronic neuropathic pain and opens up new avenues for therapeutic pain strategies in humans. Tetramethylpyrazine, a traditional Chinese medicine used as an analgesic for dysmenorrhoea, is claimed to be a P2X receptor antagonist, and it inhibited significantly the first phase of nociceptive behavior induced by 5% formalin and attenuated slightly the second phase in the rat hindpaw pain model. Antagonists to P2 receptors are also beginning to be explored in relation to cancer pain.

Special Senses

Eye

$P2X_2$ and $P2X_3$ receptor mRNA is present in the retina and receptor protein expressed in retinal ganglion cells. $P2X_3$ receptors are also present on Müller cells, which release ATP during Ca^{2+} wave propagation. ATP, acting via both P2X and P2Y receptors, modulates retinal neurotransmission, affecting retinal blood flow and intraocular pressure. Topical application of diadenosine tetraphosphate has been proposed for the lowering of intraocular pressure in glaucoma. The formation of $P2X_7$ receptor pores and apoptosis is enhanced in retinal microvessels early in the course of experimental diabetes, suggesting that purinergic vasotoxicity might have a role in microvascular cell death, a feature of diabetic retinopathy. The possibility has been raised that alterations in sympathetic nerves might underlie some of the complications observed in diabetic retinopathy; ATP is well established as a cotransmitter in sympathetic nerves, raising the potential for P2 receptor antagonists in glaucoma. $P2Y_2$ receptor activation increases salt, water, and mucus secretion and thus represents a potential treatment for dry eye conditions.

Ear

Both P2X and P2Y receptors have been identified in the vestibular system. ATP regulates fluid homeostasis, cochlear blood flow, hearing sensitivity, and development and thus might be useful in the treatment of Ménières disease, tinnitus, and sensorineural deafness. ATP, acting via P2Y receptors, depresses sound-evoked gross compound action potentials in the auditory nerve and the distortion product otoacoustic emission, the latter being a measure of the active process of the outer hair cells. P2X splice variants are found on the endolymphatic surface of the cochlear endothelium, an area associated with sound transduction. Sustained loud noise produces an upregulation of $P2X_2$ receptors in the cochlear, particularly at the site of outer hair cell sound transduction. $P2X_2$ receptor expression is also increased in spiral ganglion neurons, indicating that extracellular ATP acts as a modulator of auditory neurotransmission that is adaptive and dependent on the noise level. Excessive noise can irreversibly damage hair cell stereocilia, leading to deafness. Data have been presented that release of ATP from damaged hair cells is required for Ca^{2+} wave propagation through the support cells of organ of Corti, involving P2Y receptors, and this might constitute the fundamental mechanism to signal the occurrence of hair cell damage.

Nasal Organs

The olfactory epithelium and vomeronasal organs contain olfactory receptor neurons that express $P2X_2$, $P2X_3$, and $P2X_{2/3}$ receptors. It is suggested that the neighboring epithelial supporting cells or the olfactory neurons themselves can release ATP in response to noxious stimuli, acting on P2X receptors as an endogenous modulator of odor sensitivity. Enhanced sensitivity to odors was observed in the presence of P2 antagonists, suggesting that low-level endogenous ATP normally reduces odor responsiveness. It has been suggested that the predominantly suppressive effect of ATP on odor sensitivity could be involved in reduced odor sensitivity that occurs during acute exposure to noxious fumes and might be a novel neuroprotective mechanism.

See also: Adenosine; Adenosine Triphosphate (ATP) as a Neurotransmitter; Adenosine Receptor Mediated Functions; Cotransmission; Pharmacology of Sleep: Adenosine; Purinergic Receptors; Purines and Purinoceptors: Molecular Biology Overview.

Further Reading

Abbracchio MP and Burnstock G (1994) Purinoceptors: Are there families of P2X and P2Y purinoceptors? *Pharmacology and Therapeutics* 64: 445–475.

Abbracchio MP and Burnstock G (1998) Purinergic signalling: Pathophysiological roles. *Japanese Journal of Pharmacology* 78: 113–145.

Bodin P and Burnstock G (2001) Purinergic signalling: ATP release. *Neurochemical Research* 26: 959–969.

Burnstock G (1972) Purinergic nerves. *Pharmacological Reviews* 24: 509–581.

Burnstock G (1987) Local control of blood pressure by purines. *Blood Vessels* 24: 156–160.

Burnstock G (1999) Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. *Journal of Anatomy* 194: 335–342.

Burnstock G (2001) Expanding field of purinergic signaling. *Drug Development Research* 52: 1–10.

Burnstock G (2001) Purine-mediated signalling in pain and visceral perception. *Trends in Pharmacological Sciences* 22: 182–188.

Burnstock G (2002) Purinergic signalling and vascular cell proliferation and death. *Arteriosclerosis, Thrombosis and Vascular Biology* 22: 364–373.

Burnstock G (2001) Purinergic signalling in gut. In: Abbracchio MP and Williams M (eds.) *Handbook of Experimental Pharmacology, Vol. 151: Purinergic and Pyrimidinergic Signalling II: Cardiovascular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function*, pp. 141–238. Berlin: Springer.

Burnstock G (2001) Purinergic signalling in lower urinary tract. In: Abbracchio MP and Williams M (eds.) *Handbook of Experimental Pharmacology, Vol. 151: Purinergic and Pyrimidinergic Signalling I: Molecular, Nervous and Urinogenitary System Function*, pp. 423–515. Berlin: Springer.

Burnstock G (2003) Purinergic receptors in the nervous system. In: Schwiebert EM (ed.) *Current Topics in Membranes, Vol. 54: Purinergic Receptors and Signalling*, pp. 307–368. San Diego, CA: Academic Press.

Burnstock G (2004) Cotransmission. *Current Opinion in Pharmacology* 4: 47–52.

Burnstock G (2006) Pathophysiology and therapeutic potential of purinergic signalling. *Pharmacological Reviews* 58: 58–86.

Burnstock G (2007) Physiology and pathophysiology of purinergic neurotransmission. *Physiological Reviews* 87: 659–797.

Burnstock G and Costa M (eds.) (1975) Adrenergic neuroeffector transmission. In *Adrenergic Neurones: Their Organisation, Function and Development in the Peripheral Nervous System*, pp. 51–106. London: Chapman and Hall.

Burnstock G and Knight G (2004) Cellular distribution and functions of P2 receptor subtypes in different systems. *International Review of Cytology* 240: 31–304.

Dunn PM, Zhong Y, and Burnstock G (2001) P2X receptors in peripheral neurones. *Progress in Neurobiology* 65: 107–134.

James G and Butt AM (2002) P2Y and P2X purinoceptor mediated Ca^{2+} signalling in glial cell pathology in the central nervous system. *European Journal of Pharmacology* 447: 247–260.

Kennedy C, McLaren GJ, Westfall TD, and Sneddon P (1996) ATP as a co-transmitter with noradrenaline in sympathetic nerves – Function and fate. In: Chadwick DJ and Goode J (eds.) *P2 Purinoceptors: Localization, Function and Transduction Mechanisms*, pp. 223–235.

Sneddon P and Burnstock G (1984) Inhibition of excitatory junction potentials in guinea-pig vas deferens by α, β-methylene-ATP: Further evidence for ATP and noradrenaline as cotransmitters. *European Journal of Pharmacology* 100: 85–90.

Zimmermann H (2001) Ectonucleotidases: Some recent developments and a note on nomenclature. *Drug Development Research* 52: 44–56.