

## Purinergetic cotransmission

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Adenosine 5'-triphosphate (ATP) is a cotransmitter with classical transmitters in most nerves in the peripheral and central nervous systems, although the proportions vary between tissues and species and in different developmental and pathophysiological circumstances. There was early evidence that ATP was released together with acetylcholine (ACh) from motor nerves supplying skeletal muscle, although it was considered at the time as a molecule involved in the vesicular uptake and storage of ACh. Later it was shown that in the developing neuromuscular junction, released ATP acted on P2X receptor ion channels as a genuine cotransmitter with ACh. Adenosine triphosphate was shown to be released from sympathetic nerves supplying the guinea-pig taenia coli in 1971. Soon after, the possibility was raised that ATP was coreleased with noradrenaline from sympathetic nerves to guinea-pig seminal vesicle, cat nictitating membrane and guinea-pig vas deferens. Sympathetic purinergetic cotransmission has also been demonstrated in many blood vessels. Parasympathetic nerves supplying the urinary bladder use ACh and ATP as cotransmitters; ATP acts through P2X ionotropic receptors, whereas the slower component of the response is mediated by the metabotropic muscarinic receptor. Adenosine triphosphate and glutamate appear to be cotransmitters in primary afferent sensory neurons. Adenosine triphosphate, calcitonin gene-related peptide and substance P coexist in some sensory-motor nerves. A subpopulation of intramural enteric nerves provides non-adrenergic, non-cholinergic inhibitory innervation of gut smooth muscle. Three cotransmitters are involved, namely ATP, nitric oxide and vasoactive intestinal polypeptide. In recent years, studies have shown that ATP is released with ACh, noradrenaline, glutamate,  $\gamma$ -aminobutyric acid, 5-hydroxytryptamine and dopamine in different subpopulations of neurons in the central nervous system.

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The idea that neurons can synthesize, store and release only a single substance became known as 'Dale's principle', although Dale never explicitly suggested this; rather, he speculated that the same neurotransmitter would be stored and released from all the terminals of a single neuron. However, there were a number of hints in the literature that this might not be universally true, and this, together with the appeal of the general idea that neurons contain genes capable of producing more than one transmitter, but that during development and differentiation certain genes are triggered and others suppressed, led to a commentary by Burnstock introducing the cotransmitter hypothesis in 1976 (Burnstock, 1976). There is now a substantial body of evidence to show that ATP is a cotransmitter with classical transmitters in most nerves in both the peripheral and the central nervous systems (CNS), although the

proportions vary between tissues and species as well as in different developmental and pathophysiological circumstances (see Burnstock, 1990; Kupfermann, 1991; Lundberg, 1996). The spectrum of physiological signalling variations offered by cotransmission has been discussed (Burnstock, 2004). Adenosine triphosphate released as a cotransmitter can act both as a postjunctional modulator, enhancing the responses of their cotransmitter, and as a prejunctional modulator of transmitter release (see Burnstock, 2007). Regarding prejunctional modulation, there was early recognition that ATP and its breakdown product, adenosine, modulated prejunctional inhibition of acetylcholine (ACh) release from the skeletal neuromuscular junction and of noradrenaline (NA) release from peripheral sympathetic nerves in a wide variety of tissues.

## Peripheral motor nerves

There was early evidence that ATP was released together with ACh from cholinergic nerves in various tissues, including phrenic nerve endings in rat diaphragm (see Silinsky & Hubbard, 1973; Dowdall *et al.* 1974). Although it was accepted that ATP was stored in and released together with ACh from motor nerve terminals, it was not recognized at the time as a cotransmitter, but was considered rather as a molecule involved in the vesicular uptake and storage of the neurotransmitter, ACh. Adenosine triphosphate, via adenosine, inhibits the release of ACh and ATP was also shown to act postsynaptically to facilitate the action of ACh. In early development of the neuromuscular junction, released ATP acts on P2X receptor ion channels as a genuine cotransmitter with ACh acting on nicotinic receptors, while in mature animals, ATP no longer acts as a cotransmitter, but rather as a modulator at both prejunctional and postjunctional sites.

More recent papers have added some further details about the mechanisms underlying release of ATP from motor nerve terminals (Burnstock, 2007; Zimmermann, 2008). For example, excitatory adenosine A<sub>2A</sub> receptors probably coexist with inhibitory A<sub>1</sub> receptors at the rat neuromuscular junction, modulating the evoked release of ACh, with the balance of inhibition or facilitation depending on the frequency of motor nerve stimulation. Depression of ACh release via presynaptic A<sub>1</sub> receptors is by inhibition of N-type Ca<sup>2+</sup> channels. A presynaptic facilitating effect of P2X receptor activation on rat phrenic nerve endings has also been recognized. Adenosine triphosphate, via P2Y receptors, inhibits non-quantal spontaneous ACh release at the neuromuscular junction of mice (De Lorenzo *et al.* 2006). Much of the evidence for purinergic involvement in skeletal neuromuscular transmission has come from studies of the fish electric organ and of frog and chick neuromuscular junctions (see Burnstock, 1996).

## Sympathetic nerves

It was recognized early that ATP was co-stored with catecholamines in and coreleased from adrenal medullary chromaffin cells. Adenosine triphosphate was also shown to be contained together with NA in sympathetic nerve terminals in a molar ratio estimated to be from 7:1 to 12:1, NA:ATP (Lagercrantz & Stjärne, 1974). The first evidence for sympathetic cotransmission involving ATP together with NA came from studies showing release of ATP during stimulation of periarterial sympathetic nerves supplying the taenia coli and that release of both ATP and NA was blocked by guanethidine (Su *et al.* 1971). The possibility that ATP might be coreleased with NA in chemical transmission from the hypogastric nerve to the seminal vesicle of the guinea-pig followed, and the substantial residual non-adrenergic, non-cholinergic

(NANC) responses of the cat nictitating membrane following depletion of NA by reserpine were also considered to be due to ATP (Langer & Pinto, 1976). The most extensive evidence for sympathetic cotransmission, however, came from studies of the vas deferens, initially by Westfall and colleagues (Westfall *et al.* 1978; Fedan *et al.* 1981). Although it was not realized at the time, when excitatory junction potentials (EJPs) were first recorded in smooth muscle cells of the vas deferens in response to stimulation of sympathetic nerves (Burnstock & Holman, 1961), they were evoked by ATP rather than NA. Subsequent studies showed that EJPs are blocked by ATP receptor antagonists and also following selective desensitization of the ATP receptor with the stable analogue of ATP,  $\alpha,\beta$ -methylene ATP (Sneddon & Burnstock, 1984a), but not by depletion of NA with reserpine (Kirkpatrick & Burnstock, 1987). Furthermore, local injection of ATP mimicked the EJP, whereas NA did not. Adenosine triphosphate has recently been shown to be a cotransmitter with NA in sympathetic nerves supplying the human vas deferens (Banks *et al.* 2006). Sympathetic cotransmission to the seminal vesicles, epididymis and prostate has also been described. Evidence that soluble ectonucleotidases were released together with ATP and NA in the vas deferens was presented (Todorov *et al.* 1997). The mechanisms underlying the synergistic postjunctional actions of NA and ATP on smooth muscle of the vas deferens have been explored (Smith & Burnstock, 2004). Neuropeptide Y (NPY) is also colocalized in sympathetic nerve varicosities, but when released acts largely as a postjunctional neuromodulator, potentiating the responses to both NA and ATP in rat vas deferens and most blood vessels, as well as acting as a prejunctional modulator of release of NA and ATP (Ellis & Burnstock, 1990a).

Sympathetic purinergic cotransmission has also been clearly demonstrated in many different blood vessels, although the relative sizes of the adrenergic and purinergic components are extremely variable (see Sneddon & Burnstock, 1984b; Burnstock, 1990). The purinergic component is relatively minor in rabbit ear and rat tail arteries, is more pronounced in the rabbit saphenous artery and has been claimed to be the sole transmitter in sympathetic nerves supplying arterioles in the mesentery and the submucosal plexus of the intestine, whereas NA released from these nerves acts as a modulator of ATP release (Evans & Suprenant, 1992). Adenosine triphosphate is a prominent sympathetic cotransmitter in guinea-pig mesenteric vein, but not in artery. Sympathetic purinergic vasoconstriction of canine cutaneous veins is involved in thermoregulation (Koganezawa *et al.* 2006). Sympathetic cotransmission involves activation of vasoconstrictive P2X<sub>1</sub> and P2Y<sub>6</sub>-like receptors in mouse perfused kidney.  $\beta$ -Nicotinamide adenine dinucleotide was shown recently to be released from sympathetic nerve

terminals in canine mesenteric artery and proposed as a putative neurotransmitter or neuromodulator (Smyth *et al.* 2006).

The relative contributions of NA and ATP to postjunctional responses depend on the parameters of nerve discharge. For example, in the central ear artery, short pulse bursts (1 s) at low frequency (2–5 Hz) favour the purinergic component of the response, while long stimulation bursts at higher frequencies favour the noradrenergic component. In the pithed rat, there is selective blockade by nifedipine of the purinergic rather than the adrenergic component of nerve-

mediated vasopressor responses (Bulloch & McGrath, 1988). The different prejunctional effects of agents such as prostaglandin E<sub>2</sub>, angiotensin II and calcitonin gene-related peptide on the release of ATP and NA suggest that they are not stored in the same vesicles in the sympathetic nerve terminals (Ellis & Burnstock, 1990*b*).

### Parasympathetic nerves

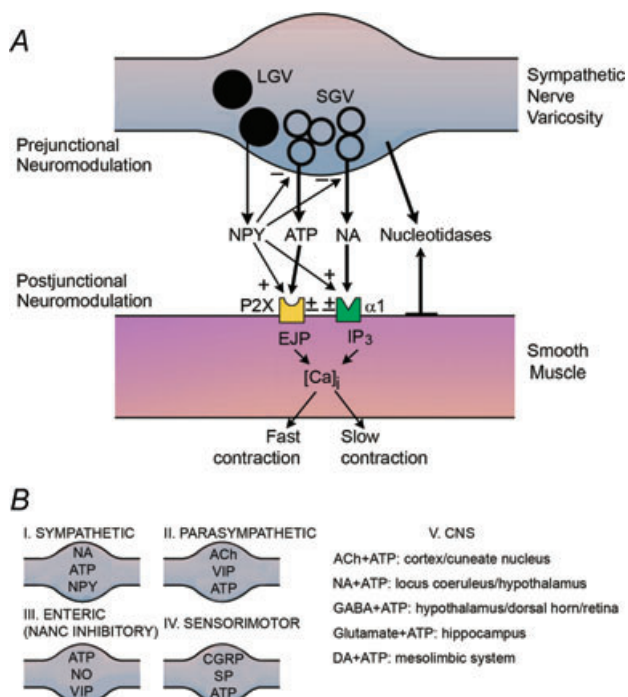
Parasympathetic nerves supplying the urinary bladder use ACh and ATP as cotransmitters, in variable proportions in different species (Burnstock *et al.* 1978; Burnstock, 2001*a*) and, by analogy with sympathetic nerves, ATP again acts through P2X ionotropic receptors, whereas the slow component of the response is mediated by a metabotropic receptor, in this case muscarinic. There is also evidence to suggest that there is parasympathetic, purinergic cotransmission to resistance vessels in the heart and airways. Colocalization of P2X and nicotinic ACh receptors has been shown in rat vagal preganglionic nerves.

### Sensory-motor nerves

It has been well established since the seminal studies of Lewis in 1927 that transmitters released following the passage of antidromic impulses down sensory nerve collaterals during 'axon reflex' activity produce vasodilatation of skin vessels (Lewis, 1927). The early work of Holton (1959) showing ATP release during antidromic stimulation of sensory collaterals, taken together with the evidence for glutamate in primary afferent sensory neurons, suggests that ATP and glutamate may be cotransmitters in these nerves. We know now that 'axon reflex' activity is widespread in autonomic effector systems and forms an important physiological component of autonomic control. Calcitonin gene-related peptide and substance P (SP) are well established as coexisting in sensory-motor nerves and, in some subpopulations, ATP is also likely to be a cotransmitter (Burnstock, 1993). Concurrent release of ATP and SP from guinea-pig trigeminal ganglionic neurons *in vivo* has been described.

### Intrinsic nerves in the gut and heart

Intrinsic neurons exist in most of the major organs of the body. Many of these are part of the parasympathetic nervous system, but certainly in the gut and perhaps also in the heart and airways, some of these intrinsic neurons are derived from neural crest tissue that differs from those that form the sympathetic and parasympathetic systems and appears to represent an independent local control system. A subpopulation of intramural enteric nerves provides NANC inhibitory innervation of gastrointestinal smooth muscle. Three major cotransmitters are released



**Figure 1. Schematic diagrams of cotransmission in both peripheral and central nervous systems**

**A**, schematic diagram of sympathetic cotransmission. Adenosine triphosphate and NA released from small granular vesicles (SGV) act on P2X and  $\alpha_1$ -adrenoceptors on smooth muscle, respectively. Adenosine triphosphate acting on ionotropic P2X receptors evokes excitatory junction potentials (EJPs), increase in intracellular calcium ( $[Ca^{2+}]_i$ ) and fast contraction, while occupation of metabotropic  $\alpha_1$ -adrenoceptors leads to production of inositol trisphosphate (IP<sub>3</sub>), increase in  $[Ca^{2+}]_i$  and slow contraction. Neuropeptide Y (NPY) stored in large granular vesicles (LGV) acts after release both as a prejunctional inhibitory modulator of release of ATP and NA and as a postjunctional modulatory potentiator of the actions of ATP and NA. Soluble nucleotidases are released from nerve varicosities and are also present as ectonucleotidases. **B**, schematic diagram of the principal cotransmitters with ATP in the nervous system. Nerve terminal varicosities of sympathetic, parasympathetic, enteric (NANC inhibitory), sensory-motor neurons and central nervous system (CNS) are illustrated. Abbreviations: CGRP, calcitonin gene-related peptide; DA, dopamine; GABA,  $\gamma$ -aminobutyric acid; NO, nitric oxide; SP, substance P; and VIP, vasoactive intestinal polypeptide. [From Burnstock (2007), with permission of the American Physiological Society.]

from these nerves: (1) ATP, producing fast inhibitory junction potentials (IJPs); (2) nitric oxide (NO), also producing IJPs, but with a slower time course; and (3) vasoactive intestinal polypeptide (VIP), producing slow tonic relaxations (Burnstock, 2001*b*, 2008). The proportions of these three transmitters vary considerably in different regions of the gut and in different species. For example, in some sphincters, the NANC inhibitory nerves primarily use VIP, in others they use NO, and in non-sphincteric regions of the intestine, ATP is more prominent. It seems likely that purinergetic synaptic neurotransmission in the myenteric plexus is due to presynaptic fibres that use ACh and ATP as cotransmitters (Nurgali *et al.* 2003). In guinea-pig submucosal and myenteric neurons, activation of 5-hydroxytryptamine (5-HT) and P2X receptors are interdependent (Boué-Grabot *et al.* 2003), raising the possibility that ATP and 5-HT are cotransmitters in some presynaptic nerve terminals.

In the heart, subpopulations of intrinsic nerves in the atrial and intra-atrial septum have been shown to contain ATP as well as NO, NPY, ACh and 5-HT. Many of these nerves project to the coronary microvasculature and produce potent vasomotor actions (Saffrey *et al.* 1992).

### Nerves in the brain and spinal cord

Evidence for purinergetic cotransmission in the CNS has lagged behind that presented for purinergetic cotransmission in the periphery. However, in the last few years a number of such studies have been reported (see Burnstock, 2007). In cortical synaptosomes, ATP appears to be coreleased with ACh, and a smaller proportion with NA. There is also evidence for corelease of ATP with catecholamines from neurons in the locus coeruleus and hypothalamus. Purinergetic and adrenergic agonist synergism for vasopressin and oxytocin release from hypothalamic supraoptic neurons is consistent with ATP cotransmission in the hypothalamus. Corelease of ATP with  $\gamma$ -aminobutyric acid (GABA) has been demonstrated in the rabbit retina and in dorsal horn and lateral hypothalamic neurons. There is evidence for corelease of ATP with glutamate in the hippocampus, as well as widespread and pronounced modulatory effects of ATP on glutamatergic mechanisms (Illes *et al.* 2001). In central neuron terminals, ATP is primarily stored and released from a distinct pool of vesicles, and release of ATP is not synchronized with either of the cotransmitters, GABA or glutamate (Pankratov *et al.* 2006). Co-operativity between extracellular ATP and *N*-methyl-D-aspartate receptors in the induction of long-term potentiation in hippocampal CA1 neurons is consistent with ATP–glutamate cotransmission. Colocalization of functional nicotinic and ionotropic nucleotide receptors has also been identified in isolated cholinergic synaptic terminals in

midbrain. Interactions between P2X<sub>2</sub> and both  $\alpha_3\beta_4$  and  $\alpha_3\beta_2$  nicotinic receptor channels has been shown in oocyte expression studies (Khakh *et al.* 2005). There is indirect evidence to support the possibility that dopamine and ATP are cotransmitters in the CNS. After cerebellar lesions in rats, producing axotomy of mossy and climbing fibre systems, nitrergic and purinergetic systems were activated with similar time courses on precerebellar stations. This raises the possibility that, as in a subpopulation of neurons in the gut, NO and ATP are cotransmitters.

It is speculated that postsynaptic selection of coreleased fast transmitters is used in the CNS to increase the diversity of individual neuronal outputs and achieve target-specific signalling in mixed inhibitory networks (Dugué *et al.* 2005).

Figure 1 summarizes current knowledge of purinergetic cotransmission in the peripheral and central nervous systems.

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