BIOLOGY REPORTS

Published: 09 June 2009
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Purinergic cotransmission

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F1000 Biology Reports 2009, **1**:46 (doi:10.3410/B1-46)

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Abstract

ATP is a cotransmitter with classical transmitters in most nerves in the peripheral nervous system and central nervous system, although the proportions vary between species and tissues and in different developmental, physiological and pathophysiological conditions. ATP is released together with noradrenaline and neuropeptide Y from sympathetic nerves. It is released as a cotransmitter with acetylcholine from parasympathetic nerves supplying the bladder, developing skeletal neuromuscular junctions and some neurons in the brain. It is also released with nitric oxide and vasoactive intestinal polypeptide from non-adrenergic inhibitory enteric nerves, with glutamate from primary afferent sensory nerves and in the hypothalamus, and with dopamine and 5-hydroxytryptamine from some neurons in the central nervous system. Cotransmission offers subtle, local variations in neurotransmission and neuromodulation mechanisms.

Introduction and context

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 sensory neuron. However, there were hints in the literature that the one nerve/one transmitter principle might not be universally true and this led to my commentary introducing the cotransmitter hypothesis in 1976 [1].

There is a substantial body of evidence to show that ATP is a cotransmitter with classical transmitters in most nerves in the peripheral nervous system and central nervous system (CNS), although the proportions vary between tissues and species and in different developmental, physiological and pathophysiological circumstances [2-4]. There was early evidence that ATP was released together with acetylcholine (ACh) from cholinergic nerves in various tissues – for example, the electric organ of elasmobranch fish, frog neuromuscular junction and phrenic nerve endings in rat diaphragm [5]. However, 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. Later it was shown that in neuromuscular junction early development, released ATP acted on P2X receptor ion channels as a genuine cotransmitter with ACh acting on nicotinic receptors, while in mature animals ATP no longer acted as a cotransmitter, but rather as a modulator at pre- and postjunctional sites [6].

ATP was first shown to be released from sympathetic nerves supplying the guinea-pig taenia coli [7]. Soon after, the possibility that ATP might be co-released with noradrenaline (NA) from the hypogastric nerve to the guinea-pig seminal vesicle was raised [8], and that the substantial residual non-adrenergic, non-cholinergic (NANC) responses of cat nictitating membrane following depletion of NA by reserpine might be due to the release of ATP from sympathetic nerves [9]. The most extensive evidence for sympathetic cotransmission came from studies of the vas deferens, initially by Dave Westfall and colleagues [10]. Sympathetic purinergic cotransmission has also been clearly demonstrated in many blood vessels, although the proportion of NA:ATP is extremely variable in different vessels [2].

Parasympathetic nerves supplying the urinary bladder utilise ACh and ATP as cotransmitters, in variable proportions in different species [11,12] and, by analogy with sympathetic nerves, ATP acts through P2X ionotropic receptors, whereas the slow component of the response is mediated by a metabotropic muscarinic receptor. There is also evidence to suggest that there is parasympathetic purinergic cotransmission to resistance vessels in the heart and airways.

The early work of Pamela Holton [13] 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. Calcitonin gene-related peptide and substance P are well established as coexisting in sensory-motor nerves and, in some subpopulations, ATP is also likely to be a cotransmitter [14]. Concurrent release of ATP and substance P from guinea-pig trigeminal ganglionic neurons *in vivo* has been described [15].

A subpopulation of intramural enteric nerves provides NANC inhibitory innervation of gut smooth muscle. Three major cotransmitters are released from these nerves: ATP producing fast inhibitory junction potentials; nitric oxide also producing inhibitory junction potentials, but with a slower time course; and vasoactive intestinal polypeptide producing slow tonic relaxations [16]. The proportions of these three transmitters vary considerably in different regions of the gut and in different species [17]. Purinergic inputs of myenteric origin come from neurons utilising ACh and ATP as cotransmitters in presynaptic fibres. In the heart, subpopulations of intrinsic nerves in the atrial and intra-atrial septum have been shown to contain ATP as well as nitric oxide, neuropeptide Y, ACh and 5-hydroxytryptamine [18].

Major recent advances

The spectrum of physiological signalling variations offered by cotransmission has been discussed more recently [19]. For example, ATP released as a cotransmitter can act as a prejunctional modulator of transmitter release or as a postjunctional modulator, synergistically enhancing the action of the cotransmitter.

Evidence for purinergic cotransmission in the CNS has lagged behind that presented for purinergic cotransmission in the periphery. However, in the past few years several such studies have been reported [20].

Release of ATP from synaptosomal preparations and slices from discrete areas of the rat and guinea-pig brain, including cortex, hypothalamus, medulla, and habenula, has been measured. Earlier papers reported that in cortical synaptosomes, a proportion of the ATP appeared to be co-released with ACh, and a smaller proportion with NA. There is also evidence for co-release of ATP with catecholamines from neurons in the hypothalamus and locus coeruleus [21]. Purinergic and adrenergic agonist synergism for vasopressin and oxytocin release from hypothalamic supraoptic neurons is consistent with ATP cotransmission in the hypothalamus [22].

Co-release of ATP with γ-aminobutyric acid (GABA) has been demonstrated in the rabbit retina and in dorsal horn and lateral hypothalamic neurons [23]. P2X and GABA receptors are also colocalised in subpopulations of rat postnatal dorsal root ganglion neurons and their central terminals laminae I-III. The intracellular loop of $GABA_B$ subunits and the carboxy-terminal domain of $P2X_2/P2X_3$ receptors are necessary for cross-talk between ATP and GABA-gated channels [24]. There is evidence for co-release of ATP with glutamate in the hippocampus [25] as well as widespread and pronounced modulatory effects of ATP on glutamatergic mechanisms. A recent study has shown that in central neuronal terminals, ATP is primarily stored and released from a distinct pool of vesicles and that the release of ATP is not synchronized with the cotransmitters GABA or glutamate [26]. Colocalisation of functional nicotinic and ionotropic nucleotide receptors has also been identified in isolated cholinergic synaptic terminals in midbrain, and interactions between $P2X_2$ and both $\alpha_3\beta_4$ and $\alpha_3\beta_2$ nicotinic receptor channels have been shown in oocyte expression studies [27].

There is indirect evidence supporting the possibility that dopamine and ATP are cotransmitters in the CNS [28]. After cerebellar lesions in rats producing axotomy of mossy and climbing fibre systems, nitrergic and purinergic systems were activated with similar time courses on pre-cerebellar stations [29]. It has been speculated that postsynaptic selection of co-released fast transmitters is used in the CNS to increase the diversity of individual neuronal outputs and achieve target-specific signalling in mixed inhibitory networks [30].

There have been significant recent advances in the development of sensitive assays for real-time assessment of ATP release from individual cells (for example, see [31,32]).

There has also been much emphasis recently on neuron-glial interactions, including co-release of transmitters

(glutamate and ATP) from glial cells, as well as from neurons [33,34].

Future directions

One of the questions still to be resolved concerns the physiological roles of the many neuropeptides colocalised with small molecule neurotransmitters in nerves. This is particularly so in the enteric nervous system, where careful immunohistochemical studies of the 'chemical coding' of different populations of enteric nerves showed that some neurons contain up to five neuropeptides. For example, Dogiel type 1 neurons contain bombesin, vasoactive intestinal polypeptide, cholecystokinin, galanin and enkephalin, as well as its primary transmitter ACh [35]. Some of the released peptides appear to play neuromodulatory roles, others long-term (trophic) roles, but for others their roles remain a mystery.

There are well-established examples in the periphery (for example, sympathetic cotransmission to the vas deferens) where cotransmitters have synergistic postjunctional actions [36]. However, few studies of the synergistic actions of cotransmitters in the CNS have been carried out. For example, it would be interesting to see if ATP potentiates the long-term potentiation action of glutamate on hippocampal neurons and the responses of NA on hypothalamic neurons.

Finally, although the drug evaluation authorities have always focused on single therapeutic agents, perhaps the presence of cotransmitters will encourage the development of multiple therapeutic agents for some conditions.

Abbreviations

ACh, acetylcholine; CNS, central nervous system; GABA, gamma-aminobutyric acid; NA, noradrenaline; NANC, non-adrenergic, non-cholinergic.

Competing interests

The author declares that he has no competing interests.

Acknowledgements

The author thanks Dr Gillian E Knight for excellent editorial assistance.

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