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Cotransmission

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After some early hints, cotransmission was proposed in 1976 and the 'chemical coding' gradually established for sympathetic, parasympathetic, sensory-motor, enteric and some invertebrate nerves. More recently, cotransmission has been recognised in the central nervous system. ATP appears to be a primitive signalling molecule that has been retained as a cotransmitter in every nerve type in both peripheral and central nervous systems, although the relative role of ATP varies considerably in different species and pathological conditions. In the past two years, interest has focused on the mechanisms underlying cotransmission, plasticity and differential control of cotransmitter expression.

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Abbreviations

ACh	acetylcholine
BDNF	brain-derived neurotrophic factor
CGRP	calcitonin gene-related peptide
DA	dopamine
GABA	γ-amino butyric acid
LGV	large granular vesicle
NA	noradrenaline
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NPY	neuropeptide Y
SP	substance P
VIP	vasoactive intestinal polypeptide

Introduction

After some early hints, the possibility that some nerve fibres synthesise, store and release more than one nerve transmitter that produces changes in postjunctional activity via specific postjunctional receptors was postulated by Burnstock in 1976 [1], challenging what had become known as Dale's Principle — the concept that nerves utilise only one transmitter [2]. Soon after, Hökfelt *et al.* [3] focused on the colocalisation, vesicular storage and release of peptides from both peripheral and central nerves. Colocalised substances are not necessarily cotransmitters; however, they can (especially peptides) act as pre- and/or postjunctional neuromodulators of the release and actions of the principal cotransmitters. For example, neuropeptide Y (NPY) synthesised, costored and released from sympathetic nerves in several preparations does not have a direct action on postjunctional cells, but rather acts as a pre- and postjunctional modulator of both the release and the actions of noradrenaline (NA) and ATP [4,5] (Figure 1j).

Several reviews of the early literature on cotransmission are available [6–10]. Most of the early studies were carried out on vertebrate autonomic and invertebrate nervous systems [6]. ATP and NPY were established as cotransmitters with NA in sympathetic nerves supplying both visceral and cardiovascular systems. Vasoactive intestinal polypeptide (VIP) and ATP were shown to be cotransmitters with acetylcholine (ACh) in parasympathetic nerves. The 'chemical coding' of neurotransmitters in the gut were described [11], including non-adrenergic non-cholinergic inhibitory nerves utilizing a combination of ATP, nitric oxide (NO) and VIP. Calcitonin gene-related peptide (CGRP) and substance P (SP) were identified as cotransmitters in sensory-motor nerves (together with ATP in some fibres), as were ACh and ATP in motor nerve endings. The proportions of these cotransmitters vary considerably between species and organs, and show plasticity of expression during development and in pathological conditions [6]. In general, classical transmitters are contained in small synaptic vesicles, whereas peptides are stored in large granular (dense-cored) vesicles (LGVs), although small molecule transmitters are sometimes stored together with peptides in LGVs [12,13•].

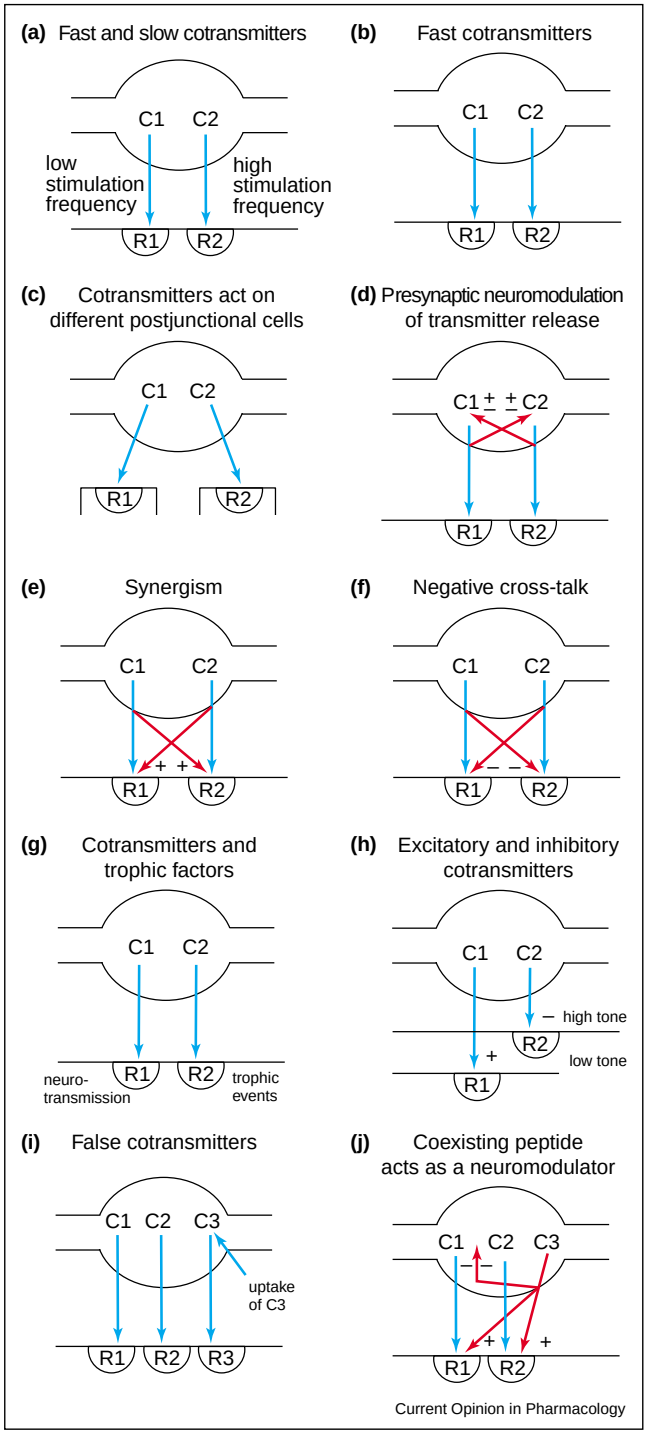
Recent interest has focused on the mechanisms that underlie cotransmission and its physiological significance [13•,14,15]; these issues are discussed in this review.

Recent reports of cotransmission

Central nervous system

Evidence for ATP being a cotransmitter with established neurotransmitters in the nervous system has recently been reviewed [16••]. In preparations of affinity-purified cholinergic nerve terminals from the rat cuneate nucleus, ATP and ACh are co-released. Co-release of ATP with catecholamines from neurons in the hypothalamus and locus ceruleus has been reported, and there is recent evidence for co-release of ATP with γ-amino butyric acid (GABA) in dorsal horn and lateral hypothalamic neurons [17,18•], and for ATP with glutamate in the hippocampus. Colocalisation of functional nicotinic and ionotropic nucleotide receptors has been identified in isolated

Figure 1



Spectrum of signalling variations offered by cotransmission (blue arrows = neurotransmission; red arrows = pre- or post-junctional neuromodulation). **(a)** Fast transmission is usually produced by small molecules (C1) released at low frequency nerve stimulation acting on ionotropic receptors (R1), whereas slow transmission is usually produced by release of peptides (C2) or other molecules at high frequency stimulation acting on G-protein-coupled receptors (R2). **(b)** Cotransmitters C1 and C2 can both be fast messengers acting via ionotropic receptors (R1 and R2). **(c)** Cotransmitters C1 and C2 act on receptors (R1 and R2) localised on different postjunctional cells. **(d)** Cotransmitters C1 and C2 not only act postjunctionally via R1 and R2 receptors but can also act as prejunctional modulators to either inhibit (–) or enhance (+) the release of C1 and/or C2. **(e)** Cotransmitters C1 and C2 act synergistically to enhance the combined responses produced via R1 and R2 receptors. **(f)** Cotransmitters C1 and C2 act to inhibit the responses evoked via R1 and/or R2 receptors. **(g)** Cotransmitter C1 evokes neurotransmission via R1 receptors, while C2 evokes long-term (trophic) responses of postjunctional cells via R2 receptors. **(h)** Cotransmitter C1 produces excitation via R1 receptors when the postjunctional smooth muscle target has low tone, with C2 having little influence; however, when the smooth muscle tone is high, the dominant response might be relaxation produced by C2 via R2 receptors. **(i)** Substance C3 is taken up by nerve terminals, rather than being synthesised and stored as is true for the cotransmitters C1 and C2. C3 can then be released on nerve stimulation to act on postunctional R3 receptors. In these circumstances, C3 would be known as a 'false transmitter'. **(j)** A coexisting substance C3 (often a peptide) can be sythesised and stored in a nerve, but not act directly via a postjunctional receptor to produce changes in postjunctional cell activity. It could, however, act as a prejunctional inhibitor (–) of the release of the cotransmitters C1 and C2, or as a postjunctional enhancer (+) of the responses mediated by R1 and R2.

cholinergic synaptic terminals in the midbrain [19]; ATP and dopamine (DA) are probably co-released from the terminals of ventral tegmental neurons in the nucleus accumbens.

Co-release and interaction of two fast inhibitory cotransmitters, GABA and glycine, in synaptic bouton preparations of the sacral dorsal commissural nucleus of the sacral spinal cord have been described [20••]. Earlier papers showed that glycine/GABA cotransmission occurred in brain stem motor neurones and spinal interneurones. Co-release of NA and DA from neurones in the cerebral cortex has also been reported. Neurons in the tuberomammillary nucleus in the posterior hypothalamus contain histamine, GABA, galanin, enkephalin and SP as cotransmitters [21]. GABA/somatostatin cotransmission has been reported at synapses in a subpopulation of amygdala projection neurons to the nucleus tractus solitarius, which might inhibit cardiovascular reflex responses to fear or emotion-related stimuli [22].

Cholecystokinin is colocalised with DA in rat mesencephalic neurons, and with glutamate in cortico-striatal neurones; released CCK appears to be involved in locomotor behaviour [23]. Synthesis and storage of glutamate, ACh and GABA in basal forebrain neurones projecting to the entorhinal cortex have been reported. Galanin is co-stored with enkephalin, and often NPY, in some neurones of the substantia gelatinosa, whereas tachykinins and enkephalin, galanin and SP are co-stored in neurones in deeper layers of the dorsal horn. Co-storage of galanin and neurotensin, as well as CGRP and SP, has been shown using postembedding immunocytochemistry to be present in LGVs [13•]. RT–PCR and *in situ* hybridisation studies have shown co-storage of oxytocin and vasopressin mRNA in LGVs in hypothalamic neurosecretory neurones.

Sympathetic neuromuscular cotransmission

Early experiments establishing sympathetic cotransmission have been reviewed [24]. Excitatory junction potentials evoked by sympathetic nerve stimulation of the smooth muscle of visceral organs and blood vessels are caused by ATP, whereas NA activates second messenger systems that produce little change in membrane potential. Studies of the vas deferens have been extended by experiments showing that enzymes responsible for degrading ATP are released together with the cotransmitters ATP and NA. In a recent paper, ATP diphosphohydrolase and AMPase were identified as the enzymes likely to be involved [25]. In contrast, clearance of NA occurs through reuptake into nerves. Ectonucleotidase in cardiac sympathetic nerve endings modulates ATP-mediated feedback of NA release [26]. In a study examining why sympathetic cotransmission to prostatic and epididymal ends of the vas deferens differs markedly, it was concluded that most varicosities located in the epididymal end of the vas deferens release an insufficient amount of ATP to evoke excitatory junctional currents [27]. Nerve terminals on sympathetic neurones controlling the rat tarsal muscle show diverse chemical coding [28].

Enteric nervous system

In recent papers, evidence has suggested that ACh and ATP are fast excitatory cotransmitters to myenteric neurones [29•] and that there may be colocalisation of ACh, ATP and serotonin in enteric S neurones [30].

Invertebrate cotransmitters

The neuropeptide proctolin is a cotransmitter with SchistoFLRFamide, octopamine and probably glutamate in nerves supplying the oviduct of the locust [31]. GABA and a catecholamine (probably DA) are colocalised in a subpopulation of interneurones within the central pattern generator currents that control feeding-related behaviours in Aplysia [32•]. Neuropeptides are colocalised with classical transmitters in the crayfish stomatogastric nervous system [33]. Various neuropeptides, including periviscerokinin-1 and -2, pyrokino-5, YLSamide, VEA acid and SKN acid have been shown to be synthesised and colocalised in clear vesicles in median neurosecretory cells in abdominal ganglia of the cockroach [34]. Serotonin and NO appear to function as presynaptic cotransmitters to serotinergic neurones in the nervous system of the snail [35].

Physiological significance of cotransmission — synaptic integration

Several major themes have emerged about the physiology of cotransmission; these are described below.

Fast and slow cotransmitters: different firing patterns

Although single presynaptic action potentials release small molecule neurotransmitters, trains of impulses are needed to release neuropeptides (see Figure 1a,b for different firing patterns of cotransmitters). ACh is released at lower firing rates than those needed for the two peptide cotransmitters from motor neurones that innervate the Aplysia accessory radula closer muscle. For sympathetic and parasympathetic cotransmission, release of ATP is favoured at low frequency stimulation, whereas NA and ACh are released at higher frequencies [5,36]. There are instances where more than one fast cotransmitter is released (e.g. glutamate and ATP) together with one or more peptides.

Different cotransmitters act at different targets

Neurones using multiple transmitters may project to two or more targets (Figure 1c). For example, in frog sympathetic ganglia, ACh and neuropeptide luteinising hormone-releasing hormone both influence postganglionic C cells, but only the neuropeptide influences B cells. An early paper by Lundberg [9] reported that ACh released at low frequency stimulation from parasympathetic nerves supplying salivary glands acts on acinus cells to produce secretion and a minor dilatation of vessels whereas, at higher frequency stimulation, its cotransmitter VIP causes powerful vasodilatation of vessels in the glands and postjunctional enhancement of ACh-induced saliva secretion.

Presynaptic modulation of cotransmitter release

A cotransmitter can feed back on presynaptic receptors that increase or decrease its own release or that of its cotransmitter(s) (Figure 1d). For example, tachykinins presynaptically stimulate the release of DA and NA in the striatum and the locus coerulus, respectively [13•]. ATP released as a cotransmitter with glutamate from primary afferent fibres in lamina II of the spinal cord can act on prejunctional $P2X_3$ receptors to facilitate the release of its cotransmitter, glutamate, whereas adenosine resulting from ectoenzymatic breakdown of ATP acts on presynaptic P1 receptors to inhibit glutamate release. At hypothalamic synapses, ACh can differentially modulate the release of cotransmitters: activation of presynaptic muscarinic receptors enhances the release of ATP, while concomitantly depressing GABA transmission [37••]. Neuropeptides co-released with classical transmitters from nerves of Drosophila can act as modulators of the actions of the classical transmitters [38•]. The modulation of cotransmitter release and presynaptic action by other agents might provide a new level of synaptic flexibility, in which individual neurones utilise more than one transmitter but retain independent control over their synaptic activity [37••].

Synergistic co-operativity

There are an increasing number of reports of the synergistic actions of cotransmitters (Figure 1e). It has long been established that ATP and NA released from sympathetic nerves have synergistic actions on smooth muscle of vas deferens and blood vessels, and that ATP released from motorneurones facilitates the nicotinic actions of

ACh at the skeletal neuromuscular junction [6]. In recent reports, co-operativity between receptors for ATP and N-methyl-D-aspartate (NMDA) in induction of long-term potentiation in hippocampal CA1 neurons has been demonstrated [39•]. GABA and ATP appear to act in concert on primary afferent nerve terminals to regulate transmitter release [18•]. Thyrotropin-releasing hormone and serotonin have been reported to have synergistic actions in spinal cord neurons [10].

In view of the evidence for cotransmitter synergy, the reports that known nucleotide P2 receptor antagonists, such as suramin, have actions on non-purinergic receptors needs to be questioned. For example, the claims that suramin and reactive blue-2 have antagonistic actions on NMDA and GABA receptor channels in hippocampal neurones are probably explained by blockade of the P2 receptor-mediated responses of the cotransmitter ATP, thereby removing its synergistic potentiating effect.

The mechanisms underlying cotransmitter synergism are not well understood. However, it has been suggested that postjunctional synergism between the responses of vas deferens to NA and ATP is caused by the ability of NA to potentiate the contractile responses to ATP by sensitising smooth muscle cells to Ca^{2+} via an inhibitory action on myosin light chain phosphatase, an action mediated by protein kinase C (Smith and Burnstock, unpublished).

Negative crosstalk

Co-application of nicotinic and P2X receptor agonists produces less than the additive responses predicted by independent receptor activation (Figure 1f). A recent study of the interactions between nicotinic $\alpha3\beta4$ and nucleotide $P2X_2$ channels expressed in Xenopus oocytes showed that the activation of one channel type affects distinct kinetic and conductance states of the other; co-activation resulted in non-additive responses because of inhibition of both channel types [40]. In synaptically coupled myenteric neurones, nicotinic fast excitatory postsynaptic currents were occluded during activation of endogenously coexpressed P2X channels. Inhibitory interactions between 5-hydroxytryptamine $(5\text{-HT})_3$ and P2X receptors have been described in submucosal [41] and myenteric [42•] neurones. Cross-inhibition of $GABA_A$ and glycine receptors has been demonstrated in rat sacral dorsal commissural neurones that co-release GABA and glycine [20••]. Negative cross-talk has also been described between anionic $GABA_A$ and cationic P2X ionotropic receptors of rat dorsal root ganglion cells. Occlusion was absent in the presence of the antagonists 2′,3′-O-(2,4,6-trinitrophenyl)-ATP or picrotoxin.

Cotransmitters and trophic factors

Some co-stored and co-released substances can act as long-term (trophic) factors, as well as neurotransmitters (Figure 1g). For example, it is well known that ATP can act on P2 receptors, or P1 (adenosine) receptors after ectoenzymatic breakdown, to promote cell proliferation, motility, differentiation or death [43]. NPY released from sympathetic nerves has cardiovascular trophic effects in end-stage renal disease [44]. NPY also appears to play a role in modulating immunological effects such as differentiation of T helper cells and monocyte mediator release [45]. There is growing evidence that neurotrophic factors might be synthesised, stored and released from nerve terminals together with fast neurotransmitters [13•]. For example, it has been claimed that brain-derived neurotrophic factor (BDNF) is localised in LGVs in neurones of the spinal cord, is released from sensory neurones and can act as a trophic modulator of central neuronal activity, at least in the hippocampus, visual system and spinal cord.

Mixed excitatory and inhibitory effects of cotransmission

Although cotransmitters generally have similar actions on postjunctional cells, there are early examples of cotransmitters having opposite actions on the mammalian uterus, one or other dominating depending on the hormonal and/or tonic status of the postjunctional muscle cells (Figure 1h). The discovery that GABA and ATP are co-released from presynaptic nerves in the spinal cord raised the possibility that a dorsal horn synapse could be excitatory or inhibitory, depending on its postsynaptic receptor or the amount of transmitter released. BDNF increases the release of ACh and reduces NA release from sympathetic nerves to cause a rapid shift from excitatory to inhibitory transmission [46••,47].

Cotransmission transporters and false transmitters

Co-expression of neurotransmitter transporters, which recapture endogenously synthesised and released transmitter, has been demonstrated in the plasma membrane of some central nervous system neurones and, in some instances, this may indicate cotransmission [48]. In other cases, however, the neurones might be taking up transmitters originating from neighbouring structures. For example, it has been known for some time that sympathetic nerves take up serotonin, which can then be released as a 'false transmitter', rather than a genuine 'cotransmitter'. A 'false transmitter' is a substance actively taken up and subsequently released by a neurone that does not synthesise it (Figure 1i).

Cotransmitter plasticity: control of transmitter expression

Cotransmitter plasticity during development and ageing, following trauma or surgery and after chronic exposure to drugs, as well as in disease, has been reviewed by Burnstock [6]. In a recent study using primary cultures of neonatal rat spinal neurones, evidence was presented for the regulation of SP (NK1) receptor expression by CGRP [49•]. There were some outstanding early studies of the factors influencing cotransmitter expression in

sympathetic nerves [50], and a physiological role for neuropoietic cytokines in the control of VIP expression during the development of cholinergic sympathetic neurones was proposed. A recent study presented evidence that cholinergic differentiation in sympathetic neurones is promoted by neurotrophic factors from three different protein families (glial cell line-derived neurotrophic factor, neurotrophin 3 and ciliary neurotrophic factor), whereas noradrenergic differentiation is promoted by nerve growth factor [51]. In another study, BDNF was claimed to switch sympathetic neurotransmission to the heart from an adrenergic excitation to cholinergic inhibition; it was also shown that the action of BDNF was mediated by the P75 neurotrophic receptor [46••,47]. Histamine, galanin and GABA acting as cotransmitters in neurones of the tuberomammillary nucleus (hypothalamus) have independent control mechanisms [21]. Changes in chemical coding of myenteric neurones in ulcerative colitis have been reported, with a shift from cholinergic to more SP-positive cotransmission [52].

Conclusions

To establish that compounds shown to be localised in nerves are actually cotransmitters, several criteria need to be satisfied:

1. The substance is synthesised and stored in the nerve.
2. The substance is released upon nerve stimulation.
3. Specific receptors for the substance need to be identified on postjunctional sites that, when occupied, lead to changes in postjunctional activity.
4. A transport system needs to be present for the substance itself or its breakdown products, uptake of which leads to renewal of messenger storage in nerve terminals.

It has been particularly difficult to establish cotransmitter roles for the many peptides found in nerves, partly because specific receptors and physiological roles have not been established for some of these and partly because of the lack of selective antagonists. In some enteric neurons, up to six neuropeptides have been identified. It is important to distinguish between neuromodulator, neurotransmitter and neurotrophic roles for released peptides.

It is becoming clear that ATP is a primitive signalling molecule that has been retained as a cotransmitter in every nerve type in both the peripheral and central nervous systems [15,16••], although the relative role of ATP varies considerably in different species and pathophysiological conditions. ATP appears to become a more prominent cotransmitter in stress and inflammatory conditions. Most nerves contain and release ATP as a fast cotransmitter together with classical fast transmitters such as ACh, NA, glutamate, GABA and one or more peptides. In view of this, I recommend that we give up using the terms 'adrenergic', 'cholinergic', 'peptidergic', 'purinergic', 'aminergic' and 'nitrergic' when describing nerves, although adrenergic, cholinergic, peptidergic, purinergic, aminergic or nitrergic transmission is still meaningful.

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